STUDIES ON THE EFFECT OF PROBIOTIC AND PHYTOGENIC FEED ADDITIVES ON HEALTH AND GROWTH PERFORMANCE OF BROILER CHICKENS AT HIGH ALTITUDE

Thesis submitted in fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

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DECLARATION BY THE SCHOLAR

I hereby declare that the work reported in the Ph.D. thesis entitled **"Studies on the effect of probiotic and phytogenic feed additives on health and growth performance of broiler chickens at high altitude"** submitted at **Jaypee University of Information Technology, Waknaghat, India,** is an authentic record of my work carried out under the supervision of **Dr. Udayabanu Malairaman** and **Dr. Vijay Kumar Bharti.** I have not submitted this work elsewhere for any other degree or diploma. I am fully responsible for the contents of my Ph.D. thesis.

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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Studies on the effect of probiotic and phytogenic feed additives on health and growth performance of broiler chickens at high altitude** submitted by **Sahil Kalia** at **Jaypee University of Information Technology, Waknaghat, India** is a bonafide record of his original work carried out under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

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(Sahil Kalia)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ADP	Adenosine diphosphate
AGP	Antibiotic growth promoters
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BWG	Body weight gain
CFU	Colony forming units
CHE	Cholesterol esterase
CHO	Cholesterol oxidase
DAP	Dihydroxy-acetone phosphate
DIHAR	Defence Institute of High Altitude Research
dl DMSO	Decilitre Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH EDTA	2, 2-diphenyl-1-picry-hydrazyl Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
FBS	Fetal bovine serum
FCR	Feed conversion ratio
FI	Feed intake
FRAP	Ferric reducing antioxidant capacity
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
H_2O_2	Hydrogen peroxide
HDL	High density lipoprotein
IECs	Intestinal epithelial cells
IL	Interleukin
LBW	Live body weight
LDL	Low density lipoprotein
LPO	Lipid peroxidation
MDA	Malondialdehyde
MTT	3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NF-kB	Nuclear Factor Kappa Beta
NGPs	Natural growth promoters
nmol/ml	Nano mole per millilitre
NSP	Non-starch polysaccharides
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffer saline
PFA	Phytogenic feed additives
PO2	Partial pressure of oxygen
ppm	Parts per million
QE	Quercetin equivalent

RIR	Rhode island Red
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RPM	Rotation per minute
S.E	Standard error
SPSS	Statistical package for social sciences
TAC	Total antioxidant capacity
TBA	Thio barbituric acid
TCA	Trichloroacetic acid
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor α
TPTZ	2,4,6 tripyridyl-s-triazine
Treg	Regulatory T cells
WI	Water intake

ABSTRACT

Unavailability of suitable broiler strains, low growth rate and high mortality in poultry birds at Leh-Ladakh are the major constraints in the development of poultry industry at high altitude Himalayas due to adverse climatic conditions. Hence, identification of suitable broilers breed having more adaptability to hypoxic and cold conditions and development of novel feed additives for better growth are need of the hour. In the current study, first experimental trial was performed to recognize appropriate broiler breed/strain for high altitude. Among the different broiler breeds, the RIR cross-bred chickens had shown better growth rate and survivability. In second experimental trial, probiotic efficacy was evaluated on performance of RIR cross-bred chickens. The results revealed no improvement in body weight gain however probiotic reduces the mortality rate. Hence, these findings are suggesting that RIR cross-bred which is a dual purpose breed are appropriate for the purpose of rearing in high altitude region and commercially available probiotic supplementation has no beneficial effects on production performance of broilers at high altitude although it reduces the loss from mortality.

Thereafter, hydro-ethanolic extract of *Rhodiola imbricata* roots, and aqueous extract of *Hippophae rhamnoides* fruits, *Salix alba* leaves and *Prunus armeniaca* seeds were phytochemically characterized for their utilization in development of new feed additives in poultry. These extracts are rich in phenolics, flavonoids, and carotenoids contents which has beneficial role on various physiological functions. Further, *in-vitro* efficacy evaluation indicated the cytoprotective activity of the phytoextracts in chicken peripheral blood lymphocytes. Furthermore, the efficacy of all phytoextracts was evaluated for different physio-biochemical indices, growth performance, and survivability of broiler chicken and thereafter cost economics of their rearing at high altitude were also computed. These studies indicated significant effect of these phytoextracts on weight gain and physio-biochemical indices at different dose concentrations. Among the different dose level of these four phytoextracts, 400 mg/kg body weight of chicken, 200 mg/kg body weight of chicken, 300 mg/kg body weight of chicken, and 200 mg/kg body weight of chicken of *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca*, respectively has shown better effect on weight gain and favorable changes in physio-biochemical parameters in chickens at high altitude.

At the last, these effective dose concentrations of all phytoextracts were taken for formulation of three feed additives. The freshly prepared formulations were F1 (contains, *H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg and *P. armeniaca* @ 100mg/kg), F2 (*H. rhamnoides* @ 100mg/kg, *S. alba* @ 300mg/kg, and *P. armeniaca* @ 100mg/kg), and F3 (*H. rhamnoides* @ 100mg, *S. alba* @ 200mg/kg, and *P. armeniaca* @ 200mg/kg). Thereafter, all these feed additives were compared with commercially available feed additives for their efficacy on body weight gain and health. Among the three formulations, the formulation F1 has shown better effects and therefore, selected for the preparation of phytogenic feed additive product. Hence, the current study provides the first report that demonstrating the feed additive potential of *H. rhamnoides*, *S. alba* and *P. armeniaca* extract in broiler chickens at high altitude. Prepared feed additive formulation ameliorates the hypobaric hypoxia-induced reduced growth performance in broiler chickens through attenuating the effect of oxidative stress. As a whole, it can be concluded that the prepared feed additive formulation could be an aid for poultry birds in the stressful environment of high altitude where there is marked deficiency of nutritious poultry feed and fodder.

CHAPTER 1

INTRODUCTION

1.1 High altitude climatic conditions

Ladakh sector is ranged from 3000 to 5000 meters above mean sea level (MSL) and comes under the trans-Himalayan region of India. It covers Leh and Kargil districts of Jammu and Kashmir. High altitude is a region which lies in between 1500 to 3500 meters above MSL, and where the climate is extreme and hostile to livestock rearing. While the proportion of oxygen in the air is constant at a different altitude, but a decrease in the barometric pressure with an increase in the altitude lead to decreases in the partial pressure of oxygen (PO_2) [1]. The high altitude trans-Himalayan region of India is distinctive in the world and is marked by intense temperature variations (from +35°C to -35°C), low precipitation mostly in the form of snowfall and frozen ice, high UV- radiation, little accessibility of oxygen, high wind velocity, low humidity, spare plant density, fragile ecosystem, short growing season for vegetable cultivation etc. and all of these adverse climatic conditions makes agriculture and animal husbandry practices very difficult in this terrain [2]. These unfavourable environmental conditions cause variation in physiological functions which could results in metabolic disorders and deficiency in essential nutrients [3]. Low PO_2 (hypobaric hypoxia) at high altitude alters the electron transport mechanisms of mitochondria which leads to the excessive formation of free radicals causing oxidative stress which results in the more oxidative damage to macromolecules and impaired immune status in livestock animals [4].

Ladakh sector is a strategic place for India and large number of armed force are deployed in this region which required continues supply of animal origin fresh food for health and operational requirements. Livestock plays significant part in fulfilling the nutritional requirements of Indian troops by providing nutritional security (meat and milk). Livestock also help in the socio-economic upliftment of local people of Leh-Ladakh. However, the harsh climatic conditions of trans-Himalayan Ladakh lead to poor productive and poor reproductive performance of livestock animals in Leh-Ladakh and this led to the poor availability of fresh animal origin food to locals and troops deployed in this region. A major part of the land in Ladakh sector is un-cultivated and therefore the available feed resources for livestock are very limited [5]. Due to such harsh climatic conditions, the survivability and productivity of the livestock animal population is a major challenge in this part of the world.

1.2 Status of poultry industry at high altitude

A number of avian species viz. chickens, turkey, duck, geese, quail, and ostrich are included in the term Poultry and have been reared by the farmers for economic benefits. Among the avian species chicken contributes more than 90% in poultry population [6] and in the last twenty years, broiler poultry industry attained a stunning progress in India. However, the same is not true for Leh-Ladakh region wherein poultry industry is virtually nonexistence. Both productive and reproductive performance of broiler chickens is very poor at high altitude as compared to their performance at low altitudes. This variability in the performance could be because of prevalent harsh climatic conditions at high altitude Himalayas which contributes to oxidative stress in broiler chickens and hinders their growth rate and optimum production [5]. The low temperature at high altitude often leads to increase in the basal metabolic rate of birds which requires more oxygen, however, low availability of oxygen at high altitude conflict with higher oxygen demand which inevitably results in mortality in birds due to compromised immune status, ascites and pulmonary hypertension [7]. Moreover, birds are also infected with coccidiosis and other microbial diseases at high altitude due to falling in the immune system [8]. A further aspect which affects the poultry production is the unavailability of suitable poultry feed. Still, so for no special feed has been developed which can meet the nutritional requirements of poultry birds at high altitude.

Furthermore, the condition of poultry rearing in Leh-Ladakh is not too impressive due to unavailability of day old chicks and the total number of poultry birds restricted to barely a few thousand. Hypobaric hypoxia at high altitude limits the hatchability percentage of chicks and which is the main reason for the absence of commercial hatcheries in this region. Other factors such as lack of economic sustenance in the form of subsidies from the government, unavailability of appropriate germplasm, and restricted availability of poultry feed components also contributed to poor poultry farming [2]. Furthermore, maintaining the required temperature inside the poultry houses during the winter season makes poultry housing very tough in this area. Besides, the region is cut off from rest of the country for up to 6-7 months in a year and therefore it is very hard for the poultry farmers to acquire day old chicks as well as commercial feed for poultry chickens from the other parts of the country during the winter season. Due to all these reasons, the poultry industry is not more successful in Ladakh and has been an underdeveloped venture [5].

1.3 Availability of broiler strains/breeds at high altitude

At high altitude, there is a scarcity of appropriate high-yielding variety of layers and broiler breeds those are adapted to regional harsh climatic conditions. There are no available poultry breeds/strains which are suitable to this high altitude region. Therefore, our institute (DIHAR DRDO) is working on development of new strains/breeds suitable for this region. So for, White Rock, Red Cornish, RIR cross-bred, Black Rock etc. are found suitable. However, detailed studies are going on which may reveal their suitability for cross breeding, pure breeding for economic weight gain, survivability etc.

1.4 Ameliorative measures for improvement in growth performance of poultry chickens at high altitude

1.4.1 Identification of suitable broiler strains

Severe climatic conditions of Leh-Ladakh are not appropriate for the growth of local poultry breed as well as for plain area breeds. Still, so far there is a no breed of chicken which is well defined for this region and therefore there is a need to find suitable broiler breeds which should be adaptable to high altitude climatic conditions. New broiler breeds can be identifying by carrying out experimental field trials at high altitude. Broiler strains which are adaptable to hypoxic conditions might have better growth rate with less susceptibility to high altitude stress.

1.4.2 By developing suitable feed additives for poultry birds

Achieving an excessive performance through effective utilisation of feed and retaining the maximum survivability is the basic principle of poultry production. In a farm profit feeding management play very important role because in the production of food from animal, feed is a relatively expensive cost factor (usually 50 to 70% of the cost), and development to any extent in the potential of the animal to transform feed into food products or betterment in growth rate can eventually improve the net-profit of a food producer [9]. Therefore, in the poultry industry, the feed is an important factor that decides the economy. However, at high altitude nutritional need of poultry chickens are not fulfilled due to unavailability of any feed industries which are manufacturing poultry feed on the commercial basis specific for high altitude chickens. Therefore, there is a need to develop suitable feed or feed additives which might contain stress-ameliorating properties and may have an advantageous effect on the health and survivability of poultry birds at high altitude.

1.5 Thrust Areas

Since the harsh environmental conditions of Leh-Ladakh are not appropriate for the growth and survivability of existing local poultry breeds as well as for plain area breeds. Therefore, recognition of suitable breeds of broiler birds, having more versatility towards hypoxic climatic conditions is essential to endorse poultry farming in this region. Specific breeds can be identified by carrying out experimental trials in different broiler breeds at high altitude which could be beneficial to find out the most suitable broiler strains having more adaptability for hypoxic conditions.

Moreover, commercial broiler feed that is used in the plain area is not suitable for high altitude poultry birds. Therefore, there is a need to develop suitable feed additives or feed formulations having stress-ameliorating properties and which could improve the quality of available feed at high altitude. Furthermore, the utility of probiotics and phytogenic on the performance of poultry birds still remain un-explored at high altitude. Development of suitable feed formulation using different feed additives through exploring their efficacy in poultry nutrition could be a better alternative for improvement in the performance of poultry birds at high altitude. Therefore, considering the above-mentioned problems, the present study has been conducted with the following objectives:

1. To study the effect of probiotic on growth performance and survivability of different broiler chickens at high altitude.
2. To standardize extraction and identification of flavonoids and total phenolic contents in *Rhodiola imbricata* roots, *Hippophae rhamnoides* fruits, *Salix alba leaves, and Prunus armeniaca* seeds and their *in-vitro* evaluation of antioxidant and cytoprotective properties.
3. To evaluate the efficacy of phytoextracts on physio-biochemical indices, growth performance, survivability of broiler chicken and cost economics of their rearing at high altitude.
4. To formulate the phytogenic feed additive for broiler chicken and a comparative study on their efficacy with other commercially available feed additives.

CHAPTER 2

REVIEW OF LITERATURE

2.1 What are feed additives?

Feed additives are the feed supplements which are used in a small quantity to upgrade the feed quality and to boost the performance of livestock animals [10]. The purpose of supplementation of feed additives is to enhance livestock production through increase digestibility of feed ingredients [11]. The development of novel feed additives is a practical solution to improve the efficiency of livestock production, to maintain animal health and to ensure that food is safe and of good quality.

2.1.1 Types of feed additives

Feed additives are classified into three categories

2.1.1.1 Nutritive feed additives

Nutritive feed additives are well off in certain nutrients which are necessary for the growth of livestock animal and poultry birds and any scarcity of these nutrients creates a number of abnormalities such as reduced growth rate, lesser egg production, and lower resilience to health disorders. These feed additives are added to poultry feed only when the formulated feed does not contain essential nutrients in required quantity [10].

Nutritive feed additives are classified into following

2.1.1.1.1 Minerals supplements

Minerals supplements are further classified into following

2.1.1.1.1.1 Macrominerals

These include minerals such as calcium, chlorine, phosphorus, potassium, and sodium which are required in relatively large amounts to poultry birds. Calcium and phosphorus take part in skeletal development while sodium, potassium and chlorine are designated as homeostatic elements [10].

2.1.1.1.1.2 Microminerals

These include minerals such as zinc, copper, iron, manganese and iodine which are required in a small amount to poultry birds.

2.1.1.1.1.3 Trace elements

This includes elements such as selenium, chromium, cobalt and molybdenum which are required only in a trace amount and plays a very important part in immunity and growth of poultry birds.

2.1.1.1.2 Vitamins supplements

Vitamins are essential nutrients having a large effect on bird performance and are usually provided in the form of vitamin premixes. Vitamin supplements contain both fat-soluble vitamins and water-soluble vitamins [10].

2.1.1.2 Non-nutritive feed additives

Non-nutritive feed additives are supplement to the livestock feed to upgrade feed effectiveness by effective digestion, absorption and utilisation of nutrients, and to improve standard and shelf life of the feed without having any exact nutritional effect. It also reduces the mortality in livestock animals caused by multiple stress factors [12].

Non-nutritive feed additives are classified into followings:-

2.1.1.2.1 Antibiotic growth promoters (AGP)

Since 2006, AGP had been used to disrupt the bacterial growth and to boost the livestock performance. In case of the poultry industry, AGP had also been very helpful in the improvement in the growth performance of poultry chickens [13, 14]. Examples of AGP are tetracycline, tiamutin, tylosin, flavomycin, lincomycin, enrofloxacin, and neomycin sulphate [15]. But, enhanced production of antibiotic resistant bacterial strains with the excessive utilization of AGP in livestock diet imposed negative effects on the health of animal and humans via transfer through food chain [16]. Therefore, due to this unfavourable outcome, the use of antibiotics in livestock farming was completely banned by the European Union since 2006 [17]. After the ban, there was a demand to find the replacement of antibiotics with some other additives that on acceptable way increased production performance, maintain poultry health and did not create harmful residues in food [16]. One of the possible alternatives to replace AGP in poultry production was the use of probiotics [18].

2.1.1.2.2 Probiotic

Probiotics are the living microbial feed supplements that improve the host intestinal microbial balance through competitive exclusion mechanism [19, 20]. It provides protection to poultry birds against gastrointestinal pathogens, enhances their immune response which subsequently

improves body weight gain, feed conversion ratio (FCR) and reduces mortality [21, 22]. Probiotics generally contain single or a mixture of harmless microbes such as *Lactobacillus acidophilus, Lactobacillus bulgaricus, Bacillus coagulans, Bacillus licheniformis, Enterococcus faecium, Saccharomyces cerevisiae, Aspergillus oryzae* etc. [23]. Probiotics maintain a host gastrointestinal microbial balance, generate antibacterial substances, competes with pathogenic bacteria for available nutrients and make nutrients unavailable to them. Further, it also helps in the improve digestion and utilization of nutrients [24, 25].

Probiotic can also release anti-enterotoxins such as lactin and acidophilin to neutralize toxins released by pathogens [26]. Supplementation of probiotic in poultry feed inhibits a number of pathogens such as *Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, Clostridium perfringens, Candida albicans,* and *Yersinia enterocolitica* etc. [27]. Probiotic has been considered as a good alternative for AGP and its efficacy must be studied in target species/animal categories under different rearing conditions for evaluating the beneficial effects [28]. Moreover, probiotics effect on the performance and survivability of broiler chickens still remains unexplored at high altitude.

2.1.1.2.3 Prebiotics

Prebiotics are food constituents (mainly small fragments of carbohydrates such as oligosaccharides) that favourably benefit the host animal by promoting the growth of useful bacteria in their gastrointestinal tract. These oligosaccharides acting as a feeding substrate for non-pathogenic bacteria which fermented these prebiotics into short chain fatty acids and inhibits the colonization of harmful bacteria from gastrointestinal tract through lactic acid production [29]. Prebiotic products which have been used in poultry are glacto-oligosaccharides, fructooligosaccharides, and mannan-oligosaccharides [30, 31]. Prebiotics provides a substrate for bacterial fermentation in the gastrointestinal tract for the production of antioxidant and vitamins which also boost the performance of host animal by providing essential micronutrients and energy [29].

2.1.1.2.4 Exogenous enzymes

This includes non-starch polysaccharides (NSP) degrading enzymes such as beta-glucanases, protease, and phytase etc. that help in the better digestion of feed. In poultry feeding, NSP such as beta-glucans and arabinoxylans are anti-nutritive factors and have a negative effect on broiler performance through reduced feed intake [32]. Beta-glucans are degraded with the supplementation of beta-glucanases in the poultry feed [33]. Similarly, the addition of phytase

enzyme in poultry feed degrades insoluble phytase into the soluble phosphorus and increase the availability of phosphorus to poultry birds [34].

2.1.1.3 Phytogenic feed additives

Phytogenic feed additives (PFA) are acquired from herbs, spices or other plant parts and they are also called natural growth promoters [35]. These feed additives are assimilated into the feed of the livestock animals to improve the efficiency through amelioration of feed properties, improvement in nutrient digestibility, removal of gastrointestinal pathogens, and upgrade the standard of food obtain from these animals [36]. PFA comprise mainly of different plant extracts which are usually less toxic, and ideal feed additives for livestock and poultry birds as compared to synthetic antibiotics or inorganic chemicals [37, 38]. They have antimicrobial, antioxidant, antifungal, antiparasitic, antiviral, and insecticidal properties [37, 39]. In the poultry diet, herbal plant extracts are integrated to substitute the inorganic products in order to trigger the valuable utilization of feed nutrients which may consequently result in effective body weight gain and better feed efficiency. Furthermore, herbal plant extract stimulates the immune function in poultry and have coccdiostatic activity [40].

Plant biodiversity has always been the source of food, medicine, and other necessities of life since time immemorial. 'Himalayan' region has been famous for its diverse ecosystem, rich floristic and faunal wealth throughout the world. Plant species of medicinal importance are spread all over the temperate zone of Asia and Europe and also along with cold arid zones of high altitudes of Asia [41]. In India, these plant herbs are widely distributed in central, north-west, western, and trans-Himalayan regions of the Himalayas. Ladakh region of trans-Himalaya is furnished with highly peculiar climate and providing a wide range of medicinal, herbal and important plants [41]. Plants growing in high altitude are enriched with a large number of phytomolecules which are important for the medicinal usefulness of plants and by supplementing plant-derived products into the feeding diet of livestock animals their survivability might be enhanced at high altitude. Moreover, in ameliorating the adverse effect of high altitude, beneficial effect of herbal plant supplements being persistently investigated [41, 42].

In traditional Tibetan system of medicine, the plants found in the high-altitude Himalayas are widely used for curing high-altitude infirmities as preventative and remedial agent [42]. Usually plant parts viz. flowers, fruits, leaves, seeds, root and rhizome of herbs are the source of various bioactive phytomolecules leading for discovery of novel biologically

active compounds and herbal formulations, which have been widely used as drug, nutrient beverage, cosmetics, and other uses related to human health throughout the world due to non-side effects and cost-effectiveness.

Supplementation of phytomolecule rich herbal plant extracts in poultry diet in the form of feed additives would have been promising benefits on health and nutritional status of the chickens. These plant-derived feed additives would be less toxic, increase production performance, maintain poultry health, improve feed efficiency, and does not create harmful residues in feed and thus, ideal to replace AGPs from broiler diet. These diverse phytomolecules show protecting effect against acute disease while proceeding synergistically instead of individually [43]. Some of the major plants of high altitude Himalayas that are rich in stress-ameliorating properties and having a feeding potential in livestock rearing are described below:-

2.1.1.3.1 *Rhodiola imbricata*

Rhodiola imbricata (R. imbricata) is a significant medicinal plant found in the trans-Himalayan cold desert at an altitude of 2700-5000 meters above MSL (Figure 2.1) and belongs to *Crassulaceae* family [44]. It has been used in a local medicinal system for centuries as human work performance enhancer [45, 46]. The root extract of *R. imbricata* is enriched with a large number of phytomolecules due to which it showed its pharmacological activities [47]. Major phytomolecules in the root extract of this plant that exhibited pharmacological activities are salidrosides, ρ-tyrosol, rosavins, and flavonoids [48].

It has been reported that supplementation of *Rhodiola crenulata* root mix in broiler diet minimize the consequences of the hypoxic environment and reduced the mortality rate in chickens in high altitude areas of Tibet [49]. Tulsawani et al. [50] supplemented higher doses of *R. imbricata* in rats for 3 months and reported a remarkable improvement in the body weight of rats without any toxic effect of *Rhodiola*. Moreover, the hepatoprotective activity of *R. imbricata* extract in rat liver cells was also reported by Senthilkumar et al. [51].

Figure 2.1: *Rhodiola imbricata* plant from trans-Himalayan cold desert of Ladakh region, India

Furthermore, both adaptogenic and anti-oxidative activities of *R. imbricata* had been reported in rats by previous coworkers [52, 53]. Kanupriya et al. [54] reported a cytoprotective activity of an alcoholic and aqueous extract of *R. imbricata* in U-937 human macrophages cells [54]. Mishra et al. [55] reported that aqueous extract of *R. imbricata* rhizome at 100 and 200 µg/mL dose concentrations exhibits anticancer activities against human T cell lymphoma cell line *in vitro*. Similarly, the anti-proliferative activity of *R. imbricata* extract had also been reported by Senthilkumar et al. [56] in HT-29 human colon cancer cells. Moreover, the radioprotective activity of a hydro-alcoholic extract of *R. imbricata* was also reported in mice by Arora et al. [57]. However, the effect of *R. imbricata* roots extract on the performance of broiler chickens in Leh-Ladakh sector is still remains unexplored.

2.1.1.3.2 *Hippophae rhamnoides* (Seabuckthorn)

Hipppophae rhamnoides also called sea buckthorn (SBT) and comes under the family *Elaegnaceae.* It is a significant herbal medicinal plant of the trans-Himalayan cold desert and commonly situated at an altitude of 3000 to 4500 meters above MSL [58] (Figure 2.2). In the traditional Ayurvedic system of medicine, the extract of *H. rhamnoides* fruits has been used

as an immunomodulator and anti-stress agent [58]. *H. rhamnoides* is a hardy plant which can tolerate extreme temperature from -43°C to 40°C and it bears barriers of different colours. Every part of the *H. rhamnoides* plant is a good source of a large number of phytomolecules such as polyphenols, flavonoids, vitamins, carotenoids, organic acid, polyunsaturated fatty acids, and amino acids [58-60].

Figure 2.2: *Hippophae rhamnoides* plant from trans-Himalayan cold desert of Ladakh region, India

Many bioactive phytomolecules such as hippophae cerebroside, gallic acid, kaempferol 3-O-sophoroside-7-O-rhamnoside, quercetin etc. were identified in berries of *H. rhamnoides* which are responsible for its pharmacological properties [61-63]. This plant is also extensively used in the oriental traditional system of medicine for treatment of various kinds of health disorders. Previous studies demonstrated that extracts of *H. rhamnoides* plant possess immunomodulatory and antioxidant activity [64]. It has been suggested by Biswas et al. [5] that *H. rhamnoides* seeds, leaves, and fruit residues are ideal feeding material for livestock and poultry birds in high altitude region of trans-Himalayan. Ma et al. [65] reported significant improvement in the performance of broiler birds after supplementation of flavonoids of *H. rhamnoides* fruits in broilers diet. It has also been reported by Solcan et al. [66] that oil from *H. rhamnoides* barriers showed potent hepatoprotective activity in broiler

chickens against aflatoxin B1 (AFB1). Similarly, supplementation of *H. rhamnoides* barriers in broiler chickens at different treatment concentration improves their immunological profile against the adverse effects originated by T-2 toxin [67, 68].

Saggu et al. [58] reported an effective adaptogenic activity of aqueous extract of *H. rhamnoides* leaves in rats without any dose toxicity. A cytoprotective activity of *H. rhamnoides* extract against oxidative stress in rat lymphocytes was also reported by Geetha et al. [64]. Furthermore, the radioprotective activity of *H. rhamnoides* extract in mice was also reported by previous co-workers [69, 70]. Moreover, a protective effect of *H. rhamnoides* extract against toxic effects of mustard gas has also been reported by Arora et al. [71]. These medicinal effects of *H. rhamnoides* have been attributed to the occurrence of greater antioxidant content in this plant [59, 72]. However, the effect of *H. rhamnoides* fruit extract on the performance of broiler chickens still remains unexplored at high altitude cold desert.

2.1.1.3.3 *Salix alba* (Willow)

Willow (*Salix* spp.) belongs to genus *Salix* and there are about 350 to 500 species are recognized worldwide, whereas about 24 willow species are reported in Indian Himalayan region [73]. Among willow spp. *Salix alba* L. and *Salix fragilis* L. are widely cultivated in cold desert high altitude Himalayas [74] (Figure 2.3). Extract of *Salix* leaves has been used for the treatment of pain, fever, inflammation, and osteoarthritis in the traditional medicinal system [75].

The major constituent of aqueous extract of *Salix* leaves is alcoholic glycosides i.e. salicin, which showed marked anti-inflammatory effects due to its ability to inhibit prostaglandin synthesis [76]. This constituent is also reported to possess antipyretic, antirheumatic, uricosuria, antiuricosuric, hyperglycemic and hypoglycemic activities [77]. In addition to salicin, a number of polyphenolics compounds, flavonoids, and condensed tannins are also present in *Salix* extract which also contributes to its anti-inflammatory and antioxidant activities [77]. It has been reported that aqueous extract of *S. alba* leaves has marked antileukemic activity [78].

Figure 2.3: *Salix alba* plant from trans-Himalayan cold desert of Ladakh region, India

Previous studies by Abdalla et al. [79] and Kader et al. [80] reported a decrease in oxidative stress marker MDA in brain and spleen tissues of rats respectively, after administration of salicin extracted from *S. alba* leaves. Jadhav et al. [81] reported a beneficial effect of *S. alba* leaves as feeding material on lambs performance in Leh-Ladakh. However, the effect of *S. alba* extract on the performance of broiler chickens at high altitude is still remains unexplored.

2.1.1.3.4 *Prunus armeniaca* (Apricot)

Prunus armeniaca widely known as apricot is an eatable fruit that comes under the family *Rosaceae* and is habituated to grown in climatic surroundings with chilly winters and hot summers [82]. The major cultivated areas of *P. armeniaca* in India are the hilly areas of Himachal Pradesh, Jammu and Kashmir and some North Eastern regions (Figure 2.4) and in Jammu and Kashmir, the major growing area for *P. armeniaca* cultivation is Leh-Ladakh region [83]. A huge number of diverse bioactive phytomolecules such as polyphenols, flavonoids, and carotenoids has been found in *P. armeniaca* fruits and due to by which this plant exhibited its pharmacological antioxidant properties [84-86].

Figure 2.4: *Prunus armeniaca* plant from trans-Himalayan cold desert of Ladakh region, India

The seeds of *P. armeniaca* are widely used in aromatherapy and cosmetic industries [87, 88] and are a vital source of nutritive protein along with substantial amount of oil and fibres [89, 90]. A wide spectrum of pharmacological effect of *P. armeniaca* has been reported including antimicrobial, antioxidant, antitumor, immunomodulatory, anti-inflammatory, hepatoprotective, radioprotective, and cardio-protective [91-96].

Jadhav et al. [81] described that nourishing of *P. armeniaca* cake to lambs under the high altitude climatic condition of Leh-Ladakh provided the adequate nutrition to support normal body growth and had no adverse effect on their performance. Positive effect on the performance and intestinal microbiota of broiler chicken had been reported by Samli et al. [97] after the supplementation of *P. armeniaca* kernel in broiler diet. Furthermore, the improved fatty acid composition in broiler meat was reported by Takeli [98] after administration of *P. armeniaca*, without any deleterious result on the performance of chickens. However, the effect of the *P. armeniaca* extract on the performance of broiler chickens at high altitude is still remains unexplored.

CHAPTER 3

TO STUDY THE EFFECT OF PROBIOTIC ON GROWTH PERFORMANCE AND SURVIVABILITY OF DIFFERENT BROILER CHICKENS AT HIGH ALTITUDE

Abstract

Unavailability of suitable broiler strains, low growth rate and high mortality in birds are the major constraints in the success of poultry industry at high altitude. Hence, identification of suitable broilers breed and development of novel feed additives are the need of the hour. Therefore, the experimental trial was performed first to recognize appropriate breeds of broilers specific for high altitude. 50 birds of each broiler breed i.e. Vencobb, RIR cross-bred, and Hubbard was randomly distributed into three groups as per randomized design. Same basal diet was given to all the birds. RIR cross-bred birds had higher (P<0.05) body weight gain, improved FCR and lower mortality as compared to Vencobb and Hubbard breeds. Thereafter, we determine the effect of probiotic on the performance of RIR cross-bred in next experimental trial. Total 150 number of one week old RIR cross-bred chicks were randomly distributed into 3 groups viz. Control; basal diet, T1; basal diet + probiotic @ 9 gm/kg feed, and T2; basal diet + probiotic @ 18 gm/kg feed, respectively. All the groups were not different in term of weight gain, feed intake, FCR, and water intake, however; mortality from intestinal coccidiosis and ascites was reduced in T1 and T2 group as compared to Control. In conclusion, at high altitude, RIR cross-bred is appropriate for the purpose of rearing and the dietary addition of probiotic in broiler feed at high altitude does not have any favourable effects on the performance although it reduces the loss from mortality in probiotic-supplemented groups. Therefore, more investigation is required to develop new probiotics using native microflora prevalent in poultry gut at high altitude.

3.1 Introduction

In the last two-decade broiler poultry industry in India ‘attains very stunning’ progress but in high altitude Himalayas, the growth picture is very different. Tense climatic conditions of high altitude Himalayas which are characterized by extreme temperature variations, low availability of oxygen, intense solar radiations, high wind velocity and unavailability of suitable broiler feed are not suitable for the normal growth of broiler birds [2]. Moreover, no

breed of chicken is well defined for this region and therefore there is a need to find suitable broiler breeds which should be adaptable to high altitude climatic conditions. Adaptive broiler strains might have better growth rate with less susceptibility to high altitude stress.

In the poultry industry, the feed is an important factor that decides the profit margin. However, at high altitude, the maintenance and productive requirements of birds is not fulfilled due to non-availability of any feed industry that is producing commercial feed specific for high altitude chickens. Therefore, there is a need to develop a suitable feed formulation for broiler strains. For better growth and survivability, the feed of broilers is frequently supplemented with several types of available feed additives [24, 99]. Improved growth performance in livestock has been recorded with supplementation of AGP [14]. However, development of antibiotic resistant bacterial strains with the immoderate utilization of AGP in the diet imposes adverse effects on the health of animals and humans [16].

Probiotics are the living microorganisms that improve the host intestinal microbial balance through the competitive exclusion of gastrointestinal pathogens [8, 19, 20, 100, 101]. It increases the intestinal mucosal immunity and reduces the number of pathogenic microbial population from the gastrointestinal tract of the animal which gives rise to increase in the utilization of feed and more nutrient absorption [102, 103]. For evaluating the beneficial effects of probiotics their efficacy needs to be studied in different animal species under dissimilar rearing environments [28]. However, so far no study has been conducted under high altitude rearing conditions which explore the probiotic effect on production performance parameters of broiler chickens. Therefore, we first examined the performance of three different broiler breeds at high altitude and thereafter we evaluated the dietary effect of probiotic (BiobloomT, M/S Zydus Animal Health Limited, India) on the performance of most adaptable broiler breed. The used probiotic is commercially available and contains *Bacillus coagulans* and *Saccharomyces cerevisiae* as a source and due to the property of competitive exclusion of these microbes this probiotic has been widely used in the rearing of livestock animals [104-109].

3.2 Material and Methods

3.2.1 Experimental site

The experiment was performed in Defence Institute of High Altitude Research (DIHAR), situated at 3500 meters above MSL in Ladakh after taking approval from Institutional Animal

Ethics Committee (Protocol No: DIHAR/IAEC/27/2012). The experiment was performed in solar poultry house having a stacking density of 0.80 square feet per bird in 4 × 2 feet of pen size (10 birds/pen) and the temperature of the house was maintained at 25-30°C by using a local heating system called bukhari (Figure 3.1).

Figure 3.1: Solar poultry house (a) Front view (b) Rear view

3.2.2 First experiment

We distributed this study into two experimental trials. In the first experimental trial, we studied the growth performance of different broiler breed to recognize the most appropriate breed for high altitude region. 150 numbers of seven day old chicks were randomly selected from three commercially available broiler strains of plain area i.e. Vencobb, RIR cross-bred, and Hubbard (50 chicks from each breed) and divided equally into 3 groups. There were 5 replications per group with 10 chickens per replicate pen (Figure 3.2). Basal diet for all the groups remains the same which included a starter diet from day 1 to day 21 and a finisher diet from day 22 to day 35. Basal diet used in this study was formulated to meet the entire nutrient requirements of the three breeds [110-112]. Ingredients and analysed composition of basal diet are presented in Table 3.1. The experiment period was 35 days. Each chicken was individually weighed at 7, 14, 21, 28, and 35 days. Water and feed intake were measured throughout the experiment (Figure 3.2). FCR was calculated from the ratio between feed intake and body weight gain of chick.

Table 3.1: Different feed ingredients and composition of basal diet

Ingredients (% diet)	Starter diet (1-21 day)	Finisher diet (22-42 day)
Maize	59.00	58.00
Soybean (Solvent extracted)	33.18	21.12
Soybean (Full fat)	-	9.58
Soybean oil	2.00	2.55
Fish Meal	2.15	-
Wheat bran	-	5.08
Salt (NaCl)	0.15	0.15
Limestone	1.50	1.50
Dicalcium phosphate	1.50	1.50
Lysine	0.13	0.13
Methionine	0.19	0.19
Vitamin and Mineral premix*	0.20	0.20
Total	100	100
Analysed composition		
Protein (%)	21.56	19.31
ME (Kcal/Kg)	3100	3200
Calcium (%)	1.02	0.94
Phosphorus (%)	0.48	0.42

*Nutrition value per Kg of vitamin and mineral premix contains 14000 IU of vitamin A, 70 mg of vitamin E, 3000 IU of vitamin D_3, 4 mg of vitamin K, 3 mg of thiamine, 10 mg of vitaminB_2, 8 mg of vitamin B_6, 0.04 mg of vitamin B_{12}, 48 mg of niacin, 20 mg of calcium d-pantothenate, 500 mg of choline chloride, 0.20 mg of biotin, 1.8 mg of folic acid, 80 mg of manganese, 70 mg of zinc, 50 mg of iron, 10 mg of copper, 3 mg of iodine, 0.4 mg of selenium, and 0.2 mg of cobalt.

Figure 3.2: Random distribution of birds in different experimental groups (a) collection of experimental data (b)

3.2.3 Second experiment

In the second experimental trial, we supplemented the probiotic (BiobloomT, M/S Zydus Animal Health Limited, India) mixed basal diet to the broiler breed which performed best among the previously-described three broiler breeds. A total of 150 numbers of seven day old chicks were randomly distributed into 3 experimental groups viz. Control; basal diet, T1; basal diet + probiotic @ 9 gm/kg feed (0.45 x 10^9 CFU of *Bacillus coagulans* and 13.5 ×10^9 CFU of *Saccharomyces cerevisiae)*, and T2; basal diet + probiotic @ 18 gm/kg feed (0.9 x 10^9 CFU of *Bacillus coagulans* and 27×10^9 CFU of *Saccharomyces cerevisiae*), respectively. There were 5 replications per group with 10 chickens per replicate pen. Duration of the study was 35 days of broiler age. A basal diet that was supplemented during the second experimental trial had a similar composition that was supplemented during experiment 1. During each experimental trial post-mortem examination of dead birds was done to find out the cause of death (Figure 3.3).

3.2.4 Statistical analysis

All statistical analysis was performed with SPSS statistical software package version 17.0 (SPSS, Chicago). Data were analyzed by one-way analysis of variance (ANOVA) using completely randomized design. Values were expressed as a mean ± standard error. $P<0.05$ was assumed to be statistically significant.

Figure 3.3: (a) Post mortem examination of birds showing (b) Ascites (c) Intestinal coccidiosis

3.3 Results

3.3.1 Growth performance of broilers in experimental trial I

The mean values of body weight gain (BWG), feed intake, FCR, and water intake are presented in Table 3.2. Among the three broiler strains, BWG was increased (P<0.05) in RIR cross-bred birds as compared to other two strains whereas, no differences were observed in BWG among Vencobb and Hubbard strain. Feed intake was higher (P<0.05) in Hubbard birds followed by RIR cross-bred and Vencobb. Moreover, FCR value was improved significantly (P<0.05) in RIR cross-bred followed by Vencobb and Hubbard. There was no difference in the water intake among the three groups. In between the three broiler strains mortality rate

was lowest in RIR cross-bred birds (16%) and was recorded highest in Hubbard breed (30%). Vencobb breed represents 22% mortality (Table 3.2). Post-mortem examination of birds revealed 14%, 10%, and 6% mortality induced from ascites and 10%, 8%, and 4% mortality induced from coccidiosis in Hubbard, Vencobb, and RIR cross-bred, respectively.

Table 3.2: Growth performance of different strain of broiler chickens in the experimental trial I

	Vencobb	RIR cross-bred	Hubbard
Body weight at 7th day (gm/bird)	40.31 ± 0.44	41.09 ± 0.46	40.03 ± 0.36
BWG (gm/bird)			
7-21 days of age	$90.19^{a} \pm 2.00$	$106.13^{b} \pm 2.38$	$93.65^{a} \pm 2.30$
22-35 days of age	$164.20^{a} \pm 3.75$	$182.09^{b} \pm 3.39$	$156.82^{a} \pm 3.25$
7-35 days of age	$254.39^{a} \pm 3.81$	$288.22^{b} \pm 3.55$	$250.47^{a} \pm 3.63$
Feed intake (gm/bird)			
7-21 days of age	$422.40^{a} \pm 4.75$	$429.52^{a} \pm 4.19$	$449.71^{b} \pm 4.23$
22-35 days of age	$581.05^{a} \pm 6.68$	$607.82^{b} \pm 6.58$	$641.62^{c} \pm 6.45$
7-35 days of age	$1003.45^{a} \pm 6.90$	$1037.34^{b} \pm 6.43$	$1091.33^{c} \pm 6.13$
Feed: gain (gm:gm)			
7-21 days of age	$4.68^{b} \pm 0.03$	$4.05^{a} \pm 0.02$	$4.80^{b} \pm 0.03$
22-35 days of age	$3.54^{b} \pm 0.04$	$3.34^{a} \pm 0.04$	$4.09^{c} \pm 0.06$
7-35 days of age	$3.94^{b} \pm 0.03$	$3.60^{a} \pm 0.05$	$4.36^{c} \pm 0.04$
Water intake (mL/bird)			
7-21 days of age	834.58 ± 5.71	825.83 ± 5.37	840.25 ± 5.59
22-35 days of age	950.48 ± 6.46	954.20 ± 6.43	970.59 ± 6.61
7-35 days of age	1785.06 ± 6.53	1780.03 ± 6.35	1810.84 ± 6.11
Total mortality (%)	22.00	16.00	30.00
Mortality due to ascites (%)	10.00	06.00	14.00
Mortality due to coccidiosis (%)	08.00	04.00	10.00
Mortality due to other reasons (%)	04.00	06.00	06.00

Means bearing the different superscripts $^{(a, b, c)}$ in a row differ significantly ($P<0.05$)

3.3.2 Growth performance of RIR cross-bred chickens in experimental trial II

In the second experimental trial, we evaluated the effect of dietary supplementation of probiotics on growth performance of RIR cross-bred (a best performing broiler strain among the three strains) birds. Supplementation of probiotics did not show any beneficial effect on growth performance of broilers as there was no significant (*P>0.05*) improvement in BWG was recorded in T1 and T2 groups as compared to Control group (Table 3.3). Moreover, there was no difference in the feed and water intake among the Control and probiotic treated groups. Consistently, there was no difference in the FCR value among the three groups. This indicates that probiotic addition in broiler diet did not have any effect on their growth performance at high altitude.

3.3.3 Economics and mortality in RIR cross-bred chickens in experimental trial II

A number of mortalities seen in RIR cross-bred under experimental trial II is presented in table 3.3. In between the three groups, the highest mortality rate was noticed in Control group birds (30%), and the probiotic-treated T1 and T2 groups represent 20% and 16% mortality, respectively. Post-mortem examination of birds revealed 10%, 8%, and 6% mortality from ascites and 16%, 6%, and 4% mortality from coccidiosis in Control, T1, and T2 groups, respectively. On the basis of rearing cost of 50 no. of birds, we also calculated the economics at the end of the experiment. For the experiment, the cost of probiotic supplementation was included along with the feed cost whereas other expenditure remained constant. Probiotic supplementation increased the return due to the reduction in mortality as compared to Control group (Table 3.3).

3.4 Discussion

RIR cross-bred strain has the capability for adapting to stressful environmental conditions [6, 113] and in this study, we evaluated its growth performance along with fast-growing Vencobb and Hubbard broiler strains. The terrible outcome of high altitude stress might contribute to the low growth rate that was observed in this study in all three broiler strains. However, a significant increase in the growth rate and improved FCR was noticed in these three strains when reared at low altitude [112, 114, 115]. High altitude coupled with hypoxia affects the body metabolism, which causes negative energy balance due to a reduction in energy intake and an increase in energy expenditure, so overall growth rate is decreased. This disturbance in energy balance leads to decrease in body mass by intestinal malabsorption and by an increase in the catabolism of muscle protein and body fats [116]. Moreover, as compared to Vencobb and Hubbard strains the RIR cross-bred was performed better in this study and this might be because of the high metabolic rate of Vencobb and Hubbard strains which require more oxygen [117]. However, due to hypoxic conditions, there is an imbalance between oxygen intake and oxygen requirement at high altitude which leads them to ascites and reduced growth rate [7, 118].

Table 3.3: Effect of dietary probiotic on growth performance and mortality rate of RIR crossbred chickens in experimental trial II

	Control	T1	T2
Body weight at 7th day (gm/bird)	39.06 ± 0.39	38.86 ± 0.50	40.53 ± 0.38
BWG (gm/bird)			
7-21 days of age	109.50 ± 2.16	108.03 ± 2.41	106.72 ± 2.29
22-35 days of age	60.17 ± 1.69	57.91 ± 2.03	60.85 ± 1.83
7-35 days of age	169.67 ± 2.35	165.94 ± 2.61	167.57 ± 2.39
Feed intake (gm/bird)			
7-21 days of age	453.67 ± 5.14	451.44 ± 4.89	466.07 ± 5.07
22-35 days of age	605.11 ± 6.27	601.44 ± 6.31	596.16 ± 6.19
7-35 days of age	1058.78 ± 6.55	1052.88 ± 6.17	1062.23 ± 6.31
Feed: gain (gm:gm)			
7-21 days of age	4.14 ± 0.04	4.18 ± 0.03	4.37 ± 0.04
22-35 days of age	10.05 ± 0.07	10.39 ± 0.06	9.80 ± 0.08
7-35 days of age	6.24 ± 0.03	6.34 ± 0.03	6.34 ± 0.04
Water intake (mL/bird)			
7-21 days of age	794.58 ± 4.97	791.51 ± 5.16	787.78 ± 5.11
22-35 days of age	845.32 ± 5.59	864.58 ± 5.81	866.61 ± 5.63
7-35 days of age	1639.90 ± 5.81	1656.09 ± 6.03	1654.39 ± 5.85
Total mortality (%)	30	20	16
Mortality due to ascites (%)	10	8	6
Mortality due to coccidiosis (%)	16	6	4
Mortality due to other reasons (%)	4	6	6
Cost of probiotic / bird (Rs.)	Nil	2	4
Cost of feed / bird (@ Rs. 25/kg) (Rs.)	26.47	26.32	26.56
Total feed cost/bird (Rs.)	26.47	28.32	30.56
Sale of per bird at 35 days (Rs.) (@ Rs. 200/kg live weight *	41.75	40.96	41.62
Loss due to mortality (Rs.)**	626.25	409.60	332.96
Total benefits per group (Rs.) #	-	216.65	293.29

Means bearing no superscript in a row did not differ significantly ($P<0.05$). Control = Basal diet, T1 = Basal diet + 9 gm/kg Probiotic, T2 = Basal diet + 18 gm/kg Probiotic

* Rate of fresh chicken meat is very high at high altitude due to limited availability

**Loss due to mortality: sale cost of per bird x total number of mortality

Total benefits per group: Loss due to mortality (Control) - Loss due to mortality (Treatment)

Moreover, a higher rate of mortality with coccidiosis and ascites in Hubbard and Vencobb strains also indicated their low adaptability at high altitude due to falling in the immunity under hypoxic conditions [8, 20, 116]. However, might be due to its slow metabolic rate and its hardiness nature, the RIR cross-bred strain revealed less mortality from coccidiosis and ascites in this study as compared to other two fast-growing broiler strains [116]. Therefore, RIR cross-bred of chickens may be helpful at high altitude for rearing

purpose.

Furthermore, under experimental trial II RIR cross-bred (a best-performing broiler strain among the three strains) birds were supplemented with probiotics and its effect was evaluated on their growth performance and survivability. Interestingly, no favourable result of probiotic was observed on the growth performance of chickens. Opposite to our findings, remarkable betterment in broilers growth and FCR was noticed by previous co-workers [104, 105] with the dietary addition of *Bacillus coagulans* and *Saccharomyces cerevisiae* as a probiotic. Non-improvement in the broiler growth performance in the present study might be due to either inadequate amount of *Bacillus coagulans* and *Saccharomyces cerevisiae* strain that was used or inappropriate type of microbes in the probiotic [119]. However, the dose of microbes in the used probiotic was much excessive than the dose of plain areas i.e. 0.6×10^8 CFU/gram for *Bacillus coagulans* per Kg feed [104] and 2×10^6 CFU/gram for *Saccharomyces cerevisiae* [105], respectively. Therefore, at high altitude different sort of microbes might be appropriate for broilers. Although an absolute improvement from the terrible effect of high altitude stress could not be attained by probiotics however it may exhibit some betterment in broiler growth and nutrient utilization.

The occurrence of mucus-binding proteins on the surface of probiotic bacteria mediate its attachment with birds IECs and this attachment between probiotic bacteria and IECs may inhibit the colonization of pathogenic bacteria in intestinal mucosa through competitive exclusion mechanism of probiotic and that leads to decrease in the mortality rate in probiotic-treated birds from coccidiosis [120-122]. The *Eimeria* parasite which is a causative agent for coccidiosis in birds needs to adhere IECs to penetrate within the host cell for its replication. However, in this study, the *Bacillus*-based probiotics might compete for adhesions sites on epithelial cells with *Eimeria* oocysts and restrict its penetration inside the host cell and this eventually inhibits the replication of parasites and improves the resistance in chickens [123]. Moreover, probiotics improve the intestinal mucosal barrier functions by inducing the secretion of mucins [124] and β-defensins [125] from epithelial cells and also secrete bacteriocins which have antimicrobial activity against gastrointestinal pathogens [126]. Further, in poultry birds intestinal mucosal immunity are also modulated by probiotic bacteria [127-130] and it has been reported by previous co-workers Bai et al. [105] and Panda et al. [131] that probiotic microbes i.e. *Saccharomyces cerevisiae* and *Bacillus coagulans* have immunomodulatory properties in broilers. Administration of *Bacillus*-based probiotics in

broilers diet also enhanced the phagocytic activity of macrophages and raised the oxidative burst activity of avian heterophils [132-134].

Probiotics bacteria regulate the intestinal immune response by inducing the expression of anti-inflammatory cytokines and downregulates the expression of pro-inflammatory mediators [135-139]. Therefore, it is possible that modification of broilers gastrointestinal microflora with *Bacillus*-based probiotics in this study might stimulate mucosal innate and adaptive immune response against *Eimeria* parasites and other gastrointestinal pathogens. Our finding is in agreement with the report of Dalloul et al. [123]. Furthermore, due to the hardiness nature of RIR cross-bred birds and due to the favourable results of probiotic on body metabolism, there was a reduced mortality in birds from ascites [24]. Our results are in agreement with the report of Saffar and Khajali [140]. Moreover, in spite of no beneficial effect of probiotics on body weight gain in broilers, it increased the net return of income due to the reduction in mortality rate. The efficacy of probiotic depend upon the types of microbes present, a dose of the probiotic, type of microbial population in the gastrointestinal tract, feed ingredients in the basal diet and predominant stress conditions, etc. [28, 141]. Since at high altitude, stresses are much high and kinds of the gut microbial population are also unknown. Hence, further investigation on the utility of different microbial strains at high altitude would be helpful to identify suitable probiotic strains and their dose optimization. This will help in comprehensive knowledge on probiotic usefulness on improving growth, nutrient utilization, and survivability at high altitude as comparable to lowland or plain areas.

3.5 Conclusion

In conclusion, by developing suitable broiler strains having more adaptability under hypoxic conditions at high altitude would be beneficial to overcome unsatisfactory growth performance at high altitude. Among the three broiler strains, RIR cross-bred has shown better growth and survivability, and hence would be suitable for high altitude. Due to less mortality in probiotic-supplemented group, net returns revealed a higher profit. Therefore, to save the loss due to high mortality, dietary supplementation of probiotic as a feed additive in broilers diet could be a valuable source. However, before the selection of probiotics, extra knowledge is required on dose, gut microflora and probiotic efficacies of microfloral species in poultry a high altitude.

CHAPTER 4

TO STANDARDIZE EXTRACTION AND IDENTIFICATION OF FLAVONOIDS AND TOTAL PHENOLIC CONTENTS IN *RHODIOLA IMBRICATA* ROOTS, *HIPPOPHAE RHAMNOIDES* FRUITS, *SALIX ALBA* LEAVES, AND *PRUNUS ARMENIACA* SEEDS AND THEIR *IN-VITRO* EVALUATION OF ANTIOXIDANT AND CYTOPROTECTIVE PROPERTIES

Abstract

The present study was carried out where parts of different herbal plants of high altitude Himalaya's viz. *Rhodiola imbricata* roots, *Hippophae rhamnoides* fruits, *Salix alba* leaves and *Prunus armeniaca* seeds were soxhlet extracted with different extraction solvents. Thereafter, all the extracts with maximum yield were phytochemically characterized for the presence of different phytomolecules. *In-vitro* studies was conducted to evaluate the efficacy of extracts in chicken peripheral blood lymphocytes (PBL). Maximum yield of extract was achieved in a hydro-ethanolic solvent of *R. imbricata*, and aqueous solvent of *H. rhamnoides*, *S. alba* and *P. armeniaca.* Phytochemical characterization of these phyto-extracts revealed the presence of different phenolics, flavonoids, and carotenoids contents in high concentration. HPLC analysis of phytoextracts confirmed the presence of salidroside and p-tyrosol in *R. imbricata*, gallic acid and kaempferol in *H. rhamnoides*, caffeic acid and salicin in *S. alba* and (+)-catechins in *P. armeniaca*. *In-vitro* efficacy evaluation indicated the significant proliferative and cytoprotective activity of all the phytoextracts in chicken peripheral blood lymphocytes. The present study demonstrated the suitable solvent for potential extraction of selected plant parts that are rich in certain bioactive phytomolecules with enhanced antioxidative and cytoprotective activities on chicken peripheral blood lymphocytes.

4.1 Introduction

Ladakh region of trans-Himalaya is characterized by typical and unique flora having medicinal and aromatic importance [41]. Plants growing in high altitude Himalayas are enriched with a large number of phytomolecules which are important for the medicinal applications. Usually plant parts viz. flowers, fruits, leaves, seeds, root and rhizome of herbs are the source of various bioactive phytomolecules leading to discovery of novel therapeutics

and herbal formulations, which have been widely used as drug, nutrient beverage, cosmetics, and other uses related to human health throughout the world due to no side effects and cost-effectiveness [41].

Rhodiola imbricata is a distinctive medicinal plant, which grow in harsh environmental condition of high altitude Trans-Himalaya region. These stressful conditions are responsible for synthesis of secondary metabolites which are accumulated in plant root system [142]. Major phytomolecules in the root extract of this plant that exhibited pharmacological activities are salidrosides, ρ-tyrosol, rosavins, and flavonoids [48]. *Hippophae rhamnoides* is a temperate shrub growing from 3000 to 4500 meters above MSL in high altitude Himalayas [58]. Fresh *H. rhamnoides* fruits are an abundant source of vitamins, polyphenols and flavonoids [143]. A number of medicinal effects of *H. rhamnoides* have been reported which includes antioxidant [144], immunomodulatory [64], anti-stress [145], anti-tumor [146], hepatoprotective [144], and radioprotective [69]. Among the medicinal plants of Himalayas, willow (*Salix* spp.) belongs to genus *Salix* and there are more than 500 species recognized worldwide [73]. The major constituent of *Salix* leaves extract is alcoholic glycosides i.e. salicin, which showed marked anti-inflammatory effects due to its ability to inhibit prostaglandin synthesis [76]. In addition to salicin, a number of polyphenolics compounds, flavonoids and condensed tannins are also present in *S. alba* extract which also contributes to its anti-inflammatory and antioxidant activities [77]. *Prunus armeniaca* is an eatable fruit and is habituated to grow in climatic surroundings with chilly winters and hot summers [82]. A large number of diverse bioactive phytomolecules such as polyphenols, flavonoids, and carotenoids have been found in *P. armeniaca* fruits and due to by which this plant exhibited its pharmacological antioxidant properties [84, 85].

The biological activity and yield of plant extracts are deeply affected with different solvents that are used for extraction of phytomolecules [147]. Moreover, the polarity of solvent also effects the extraction of phenolic compounds from plant extracts [148]. As antioxidant capacity of plant extract is correlated with phenolic content and therefore, the present study was carried out to determine the effect of different extraction solvent on extract yield in *Rhodiola imbricata* roots, *Hippophae rhamnoides* fruits, *Salix alba* leaves and *Prunus armeniaca* seeds followed by their phytochemical analysis and biological efficacy in chicken PBL prior to the *in vivo* study.

4.2 Materials and Methods

4.2.1 Selection of herbal plant

For this study, we screened the literature reported by previous co-workers [46, 149, 150] about the high altitude trans-Himalayan flora and selected four native plants viz. *Rhodiola imbricata* (Rhodiola), *Hippophae rhamnoides* (seabuckthorn), *Salix alba* (willow), and *Prunus armeniaca* (apricot) based on their medicinal properties and there potential as feeding material in livestock rearing for the preparation of phytogenic feed additive.

4.2.2 Collection of plant material

R. imbricata roots were collected from Chang-La Top, located at the altitude of 5330 meters above MSL in the trans-Himalayan region of Ladakh sector. *Hippophae rhamnoides* fruits and *Prunus armeniaca* seeds were collected commercially from Leh market through local vendors. *Salix alba* leaves were collected from the field of DIHAR, Leh.

4.2.3 Soxhlet extraction

After collection, *R. imbricata* roots, *H. rhamnoides* fruits and *S. alba* leaves were washed, shade dried at room temperature and powder. Seeds of *P. armeniaca* were powdered without washing. Thereafter, powdered samples of *R. imbricata* roots, *H. rhamnoides* fruit, *S. alba* leaves, and *P. armeniaca* seeds were extracted with four extraction solvents (n-hexane, methanol, hydro-ethanolic (40:60), and aqueous) using a Soxhlet apparatus (Borosil Glass Works Limited, Worli, Mumbai, India) for standardization of extraction method. 30 gram of each plant sample was loaded in extraction thimble and the thimble was placed inside the chamber of Soxhlet apparatus that was filled with different extraction solvents. The extracted fractions were filtered and the solvent was removed under reduced pressure using Rota evaporator (Buchi, Switzerland). Thereafter, remaining extract material was lyophilized and stored at –80^{o}C until use.

Extract yield was calculated on the basis of the following equation:

Extract Yield (%) = Total amount of extract/Total amount of powder sample * 100

4.2.4 Characterization of the extract

After extraction of herbal plants in different extraction solvents, extracts with maximum yield were phytochemically characterized for the presence of different bioactive phytomolecules.

4.2.4.1 DPPH radical scavenging capacity

The potential of plant extract samples to scavenge DPPH radical was determined by the method described by previous investigators [151]. In brief, 30 µL of plant extract sample was treated with 600 µL of 100 µM DPPH solution prepared in methanol. Instead of extract sample, control was treated with 30 µL of solvent. After 30 m of incubation, the solution was centrifuged (Heal Force ®, Neofuge 23R, Shanghai, China) at 3000 RPM and the supernatant was transferred into 96 well microtiter plate and the decrease in absorbance at 517 nm was recorded using of ELISA microplate reader (Spectromax M2e, USA). Ascorbic acid was used as reference standard. DPPH radical scavenging capacity was determined as:

DPPH radical scavenging capacity (%) = $[(abs_{control} - abs_{sample})]/ (abs_{control})] \times 100$

Where $abs_{control}$ is the absorbance of control, and abs_{sample} is the absorbance of sample or standard.

4.2.4.2 ABTS radical scavenging capacity

The potential of plant extract samples to scavenge ABTS radical was determined by the method described by previous investigators [152]. The stock solution for this assay comprised of 7 mM ABTS solution and 2.4 mM potassium persulphate solution. For the preparation of working solution, two stock solutions were mixed in similar proportion followed by incubation in dark for 12 h. Thereafter, 1 mL of working solution was mixed with 60 mL of 96% ethanol to obtain an initial absorbance of 0.700 ± 0.02 at 734 nm. Plant extract (33.30 µL) was allowed to react with an $ABTS^{+}$ solution (266.70 µL) and the decrease in the absorbance was estimated after 7 m at 734 nm. As reference standard ascorbic acid was used. ABTS scavenging capacity was determined as:

ABTS radical scavenging capacity (%) = $[(abs_{control} - abs_{sample})]/ (abs_{control})] \times 100$

Where $abs_{control}$ is the absorbance of control, and abs_{sample} is the absorbance of sample or standard.

4.2.4.3 Total antioxidant capacity (TAC)

TAC in plant extract samples was determined by FRAP assay as suggested by previous investigators [153]. For preparing working FRAP reagent, 10 volumes of 300 mmol/L acetate buffer, pH 3.6 was mixed with 1 volume of 10 mmol/L TPTZ in 40 mmol/L HCL and 1 volume of 20 mmol/L $FeCL_3$. 15 µL of sample was allowed to react with 285 µL of FRAP reagent for 30 m in dark. Absorbance was measured at 593 nm. An aqueous solution of $FeSO_4.7H_2O$ was used for calibration and the result was expressed as µM Fe (II)/g of extract.

4.2.4.4 Total phenolic content

Total phenolic content in plant extract samples was evaluated by Folin-Ciocalteu method as suggested by Gao et al. [154]. 100 µL of extract sample was mixed with 200 µL of Folin-Ciocalteu reagent, 2 mL of H_2O, and 1 mL of 20% Na_2CO_3. After 1 h of incubation, the absorbance was measured at 765 nm. As a reference standard gallic acid was used and phenolic content was indicated as mg of gallic acid equivalent (mg GAE/g of extract).

4.2.4.5 Total flavonoid content

Total flavonoid content in plant extract samples was estimated by the method as suggested by previous investigators [155]. 0.5 mL of 2% $AlCl_3$ ethanolic solution was mixed with 0.5 mL of extract sample and after 1 h of incubation, absorbance was measured at 420 nm. Total flavonoid content was indicated as mg of quercetin equivalent (mg QE/g of extract).

4.2.4.6 Determination of carotenoids

Total carotenoid content in plant extract samples was estimated by the method as suggested by previous investigators [156]. For this assay, we mixed 0.5 mL of 5% NaCl and 2 mL hexane with plant extract. After that, we vortexed the solution for 30 seconds followed by centrifugation for 10 m. Absorbance was measured at 460 nm. As a reference standard *β*-carotene was used and total carotenoid in plant extract was indicated as mg of *β*-carotene equivalent (mg *β*-carotene/100 g of extract).

4.2.4.7 Phytochemical characterization of phytoextracts using RP-HPLC analysis

Quantification of plant extract samples for phytomolecules were carried by reverse phase HPLC (Shimadzu LC10ATvp) equipped with the SCL-10AVP system controller, CTO-10 AS VP column oven, LC10ATvp binary pump, SPD-10 Avp UV-Visible detector and CLASS-VP version 5.33 software. The phytomolecules were separated using RP-C18 column. Quantification of *R. imbricata* extract was done using mobile phase consisted of water (A), methanol (B), and acetonitrile (C). The gradient program for mobile phase was as follows: 12% B, 12% C, 76% A at 0 m to 12% B, 12% C, 76% A at 15 m, increasing linearly to 25% B, 25% C, 50% A at 25 m, and to 45% B, 45% C, 10% A at 40 m then held for 5 m [157]. The mobile phases were pumped at a flow rate of 1 ml/min and detection wavelength for acquiring chromatograph was set at 248 nm. Similarly, for quantification of *H. rhamnoides* extract mobile phases used were acetonitrile (A) and 0.4% aqueous phosphoric acid (B). The mobile phases were pumped at a flow rate of 1 ml/min, according to gradient program 12-15% A in 0-10 m, 15-20% A in 10-30 m, 20-40% A in 30-50 m, 40-12% A in 50-60 m and the detection was accomplished at UV absorbance of 360 nm [158].

Mobile phase for quantification of *S. alba* extract comprised of 0.1% ortho-phosphoric acid in water (A) and acetonitrile (B). The mobile phases were pumped at a flow rate of 1 ml/min, according to gradient program 0-25 m, 95% A: 5% B, 25-26 m, 80% A: 20% B, 26-31 m, 5% A: 95% B, 31-45 m, 90% A: 10% B and the detection was accomplished at UV absorbance of 267 nm [159]. Furthermore, quantification of *P. armeniaca* extract was done using mobile phase consisted of 3% acetic acid in water (A) and a mixture of 3% acetic acid, 25% acetonitrile and 72% water (B). The mobile phases were pumped according to gradient program 0-40 m, 30% A: 70% B, 40-45 m, 20% A: 80% B, 45-55 m, 15% A: 85% B, 55-60 m, 10% A: 90% B, with flow rate 1.2 mL/m and the detection was accomplished at UV absorbance of 280 nm [160]. The chromatographic peaks of phytomolecules were identified by matching their retention time with reference standards. For the preparation of calibration curve, standard stock solutions of referenced phytomolecules (1 mg/mL) were prepared in methanol and diluted to achieve the preferred concentration in quantification range.

4.2.5 *In vitro* evaluation for dose efficacy of plant extracts

To determine the efficacy of plant extracts, their antioxidative and cytoprotective activities were initially assessed in chicken PBL prior to *in vivo* studies.

4.2.5.1 Blood sampling and separation of peripheral blood mononuclear cells (PBMC)

We took 3 ml of blood samples from wing vein of chickens and collected those samples into sterile plastic tubes containing EDTA as an anticoagulant. For separation of PBMC, whole blood was first diluted with PBS (Ca^{2+} and Mg^{2+} free, Himedia) in 1:1 ratio and thereafter smoothly over layered on Histopaque-1077 in falcon tube (Sigma-Aldrich, St. Louis, MO) and centrifuged (Allegra^{R} X-15 R centrifuge, Beckman Coulter) for 30 m at 400 × g. We recovered the PMBC from gradient interface, washed them twofold with Ca^{2+} and Mg^{2+} free PBS, and centrifuged at 200 × g for 10 m. The last washing was performed with RPMI-1640 medium (R1145, Sigma-Aldrich). The pellets were then resuspended in 10% fetal bovine serum (FBS) rich RPMI-1640 medium. Adherent (monocytes) and non-adherent (lymphocytes) cells were separated through plastic adherence technique by incubating PBMC at 41^{0}C for 45 m [161].

4.2.5.2 Cell Culture

Trypan blue exclusion method was used to determine the cell viability and PBL were adjusted to a concentration of 1×10^{4} cells/mL in RPMI-1640 medium supplemented with 10% FBS, and antibiotics. PBL suspension (100 µL/well) was cultured in microtiter plate with 100

μL/well of different dose concentrations of *R. imbricata* extract, *H. rhamnoides* extract, *S. alba* extract, and *P. armeniaca* extract (100, 200, 400, 800 ng/mL, and 1, 2, 4, 8, 50, 100, 200, 400 μg/mL), 1 μg/mL of concanavalin A as positive control, and medium as negative control, at 41^{0}C in a 5% CO_2 incubator (CO-150, New Brunswick Scientific, USA) for 24 h.

4.2.5.3 MTT proliferation assay

The calorimetric method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay described by previous investigators [162] was used to measure the proliferative activity of plant extracts in chicken PBL. Following incubation of chicken PBL with different plant extracts for 24 h, 50 μL of MTT solution was added to each well and plate was further incubated at 41^{0}C for 4 h. Thereafter, 100 μL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the purple formazan product. By using microplate reader (Bio-Rad Laboratories, 680 microplate reader) absorbance was taken at 570 nm. Percentage cell viability was determined by employing following formula

% Cell viability = Absorbance of Test/Absorbance of Control ×100

4.2.5.4 Cytoprotective assay against H_2O_2-induced toxicity

To analyse the cytoprotective activity of plant extract against the toxic effect of H_2O_2, PBL cells were first cultured into 96 well plates and incubated for 24 h at 41^{0}C. Thereafter, cells were simultaneously treated with different concentrations of plant extracts and 100 μm H_2O_2 (Merck, India) for 2 h. MTT assay was used to determine the cell viability.

4.3. Results

4.3.1 Effect of different solvents on extraction yield

We observed that the extract yield was recorded maximum in a hydro-ethanolic solvent (30.8 %) in *R. imbricata* root extract (Figure 4.1) followed by aqueous (23.87%), methanol (18.3%) and n-hexane (2.75%). For *H. rhamnoides*, the maximum yield of extract was noticed in an aqueous solvent (32.7%) followed by hydro-ethanol (28.42%), methanol (25.08%) and n-hexane (16.47%). Similarly, *S. alba* leaves exhibit maximum extraction yield in an aqueous solvent (37.02%), followed by hydro-ethanol (31.07%), methanol (30.68%), and n-hexane (3.38%). In contrast, *P. armeniaca* seeds exhibit maximum extraction in an aqueous solvent (37.41%), followed by n-hexane (36.09%), methanol (33.03%), and hydro-ethanol (31.8%).

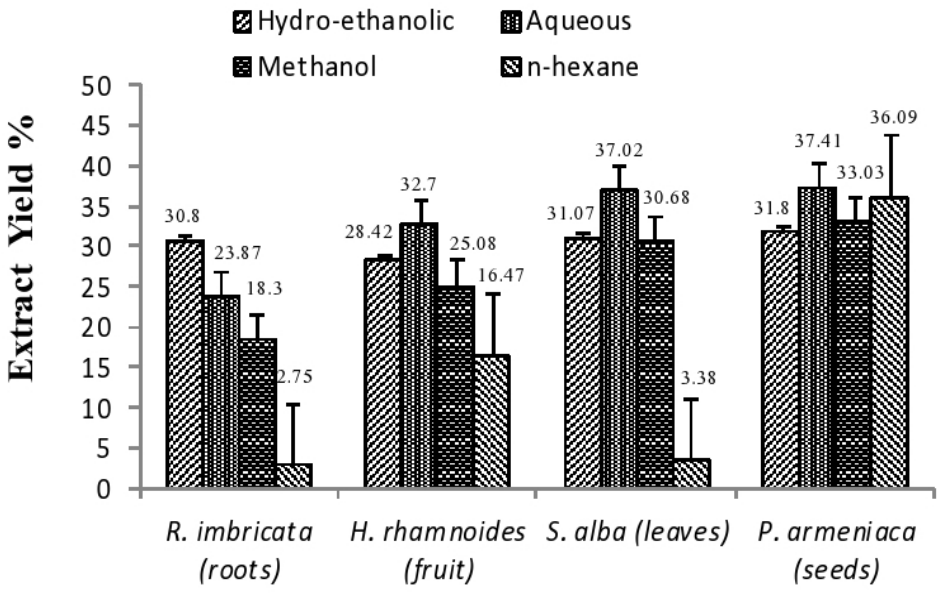

Figure 4.1: Extract yield (%) of *R. imbricata* roots, *H. rhamnoides* fruits, *S. alba* leaves, and *P. armeniaca* seeds in different extraction solvents

4.3.2. Phytochemical characterization of the extract

After extraction of herbal plants in different extraction solvents, the extracts with maximum yield in particular solvent (hydro-ethanolic extract of *R.imbricata*, aqueous extract of *H. rhamnoides*, *S. alba* and *P. armeniaca* respectively) were phytochemically characterized for the presence of different bioactive phytomolecules.

4.3.2.1 DPPH and ABTS radical scavenging capacity

Free radical scavenging capacity of different extracts was determined by DPPH and ABTS assay and all the extracts were found to scavenge the DPPH and ABTS radical in a dose-dependent manner at a concentration of 20 to 100 µg/mL. Positive control ascorbic acid was also found to produce dose-dependent inhibition of DPPH and ABTS radical at a similar concentration (Table 4.1-4.2).

Table 4.1: DPPH scavenging activity of different phytoextracts in comparison to ascorbic acid

Inhibition (%)					
DPPH radical scavenging capacity					
Concentration (µg/mL)	*R.imbricata* (hydro-ethanol)	*H. rhamnoides* (aqueous)	*S. alba* (aqueous)	*P. armeniaca* (aqueous)	Ascorbic acid
20	32.43 ± 0.71	31.85 ± 0.65	30.62 ± 0.64	29.10 ± 0.62	39.57 ± 0.76
40	36.84 ± 0.85	32.01 ± 0.78	31.01 ± 0.66	30.16 ± 0.66	45.40 ± 0.89
60	39.97 ± 0.59	35.89 ± 0.81	33.83 ± 0.59	32.51 ± 0.79	49.80 ± 0.63
80	40.96 ± 0.74	38.25 ± 0.67	37.16 ± 0.72	35.19 ± 0.73	53.98 ± 0.57
100	44.09 ± 0.82	39.47 ± 0.90	41.10 ± 0.78	40.22 ± 0.85	60.59 ± 1.08

Value are given as mean ± S.E of four replicates

Table 4.2: ABTS scavenging activity of different phytoextracts in comparison to ascorbic acid

Inhibition (%)					
ABTS radical scavenging capacity					
Concentration (µg/mL)	*R. imbricata* (hydro-ethanol)	*H. rhamnoides* (aqueous)	*S. alba* (aqueous)	*P. armeniaca* (aqueous)	Ascorbic acid
20	12.91 ± 0.37	15.63 ± 0.40	16.79 ± 0.37	15.32 ± 0.28	21.36 ± 1.12
40	25.46 ± 0.29	19.27 ± 0.25	21.13 ± 0.29	19.80 ± 0.31	29.37 ± 0.45
60	31.14 ± 0.45	24.41 ± 0.39	26.06 ± 0.40	25.54 ± 0.41	35.86 ± 0.54
80	33.43 ± 0.58	30.40 ± 0.63	31.64 ± 0.66	31.10 ± 0.57	41.18 ± 0.71
100	41.91 ± 0.72	35.25 ± 0.84	36.40 ± 0.75	37.04 ± 0.69	55.94 ± 0.96

Value are given as mean ± S.E of four replicates

4.3.2.2 TAC

TAC of different phytoextracts was estimated by FRAP assay and it was recorded to be 473.41 ± 18.47 µM Fe (II)/g of extract in hydro-ethanolic extract of *R. imbricata,* 425.54 ± 16.14 µM in aqueous extract of *Hippophae rhamnoides*, 451.68 ± 19.31 µM in aqueous extract of *S. alba* and 409.78 ± 16.61 µM in aqueous extract of *P. armeniaca,* respectively (Table 4.3).

4.3.2.3 Total phenolic content

The concentration of total phenols in phytoextracts are present in Table 4.3 and was recorded to be 219.72 ± 1.71 mg GAE/g of extract in *R. imbricata,* 76.28 ± 3.25 mg in *H. rhamnoides*, 234.14 ± 2.47 mg in *S. alba*, and 0.63 ± 0.22 mg in *P. armeniaca*, respectively.

4.3.2.4 Total flavonoids content

The concentration of total flavonoids in phytoextracts are shown in Table 4.3 and was recorded to be 184.05 ± 2.14 mg QE/g of extract in *R. imbricata*, 35.14 ± 2.18 mg in *H. rhamnoides*, 86.32 ± 1.81 mg in *S. alba*, and 0.40 ± 0.14 mg in *P. armeniaca*, respectively.

4.3.2.5 Total carotenoids content

The carotenoids content in different phytoextract are shown in Table 4.3 and was recorded to be 0.17 ± 0.04 mg/100 g extract in *R. imbricata*, 4.19 ± 0.70 mg/100 g extract in *H. rhamnoides*, 2.87 ± 0.26 mg/100 g extract in *S. alba*, and 0.68 ± 0.31 mg in *P. armeniaca*, respectively.

Table 4.3: Characterization of extracts for TAC, total phenolics, flavonoids, and carotenoids contents

Extract	TAC (µM Fe (II)/g of extract)	Total phenolic (mg GAE/g of extract	Flavonoids (mg QE/g of extract)	Carotenoids (mg/100 g extract)
R. imbricata	473.41 ± 18.47	219.72 ± 1.71	184.05 ± 2.14	0.17 ± 0.04
H. rhamnoides	425.54 ± 16.14	76.28 ± 3.25	35.14 ± 2.18	4.19 ± 0.70
S. alba	451.68 ± 19.31	234.14 ± 2.47	86.32 ± 1.81	2.87 ± 0.26
P. armeniaca	409.78 ± 16.61	0.63 ± 0.22	0.40 ± 0.14	0.68 ± 0.31

Value are given as mean ± S.E of four replicates

4.3.2.6 HPLC analysis of phytoextract

Quantification of selected plant extracts for the presence of different bioactive phytomolecules was done through RP-HPLC. HPLC analysis of a hydro-alcoholic extract of *R.imbricata* at 248 nm revealed the presence of p-tyrosol and salidroside (Figure 4.2) at a concentration of 232.40 and 191.01 µg in 1 mg of extract, respectively. Retention time for p-tyrosol and salidroside was recorded at 4.32 m and 8.15 m, respectively. Similarly, HPLC analysis of an aqueous extract of *H. rhamnoides* at 360 nm revealed the presence of gallic acid and kaempferol at a concentration of 210.24 and 26.52 µg in 1 mg of extract, respectively (Figure 4.3). Retention time for gallic acid and kaempferol was recorded at 2.32 m and 10.05 m, respectively. Furthermore, HPLC analysis of an aqueous extract of *Salix alba* leaves at 267 nm confirmed the presence of salicin and caffeic acid at a concentration of 790.18 and 18.11 µg in 1 mg of extract, respectively (Figure 4.4). Retention time for caffeic acid was recorded at 17.03 m and for salicin, retention time was 31.20 m. Moreover, HPLC analysis of an aqueous extract of *P. armeniaca* at 280 nm revealed the presence of (+)-catechins at a concentration of 320.40 µg in 1 mg of aqueous extract (Figure 4.5). Retention time for (+)-catechins was recorded at 4.87 m.

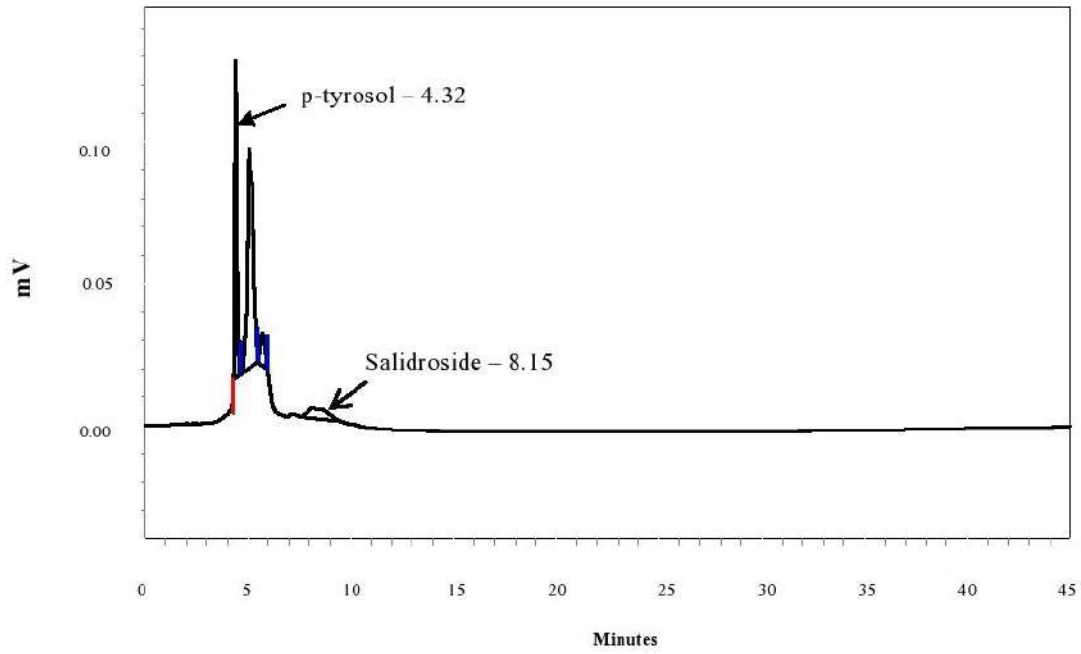

Figure 4.2: HPLC chromatogram indicating presence of p-tyrosol and salidroside with other compounds in hydro-alcoholic extract of *R. imbricata* roots

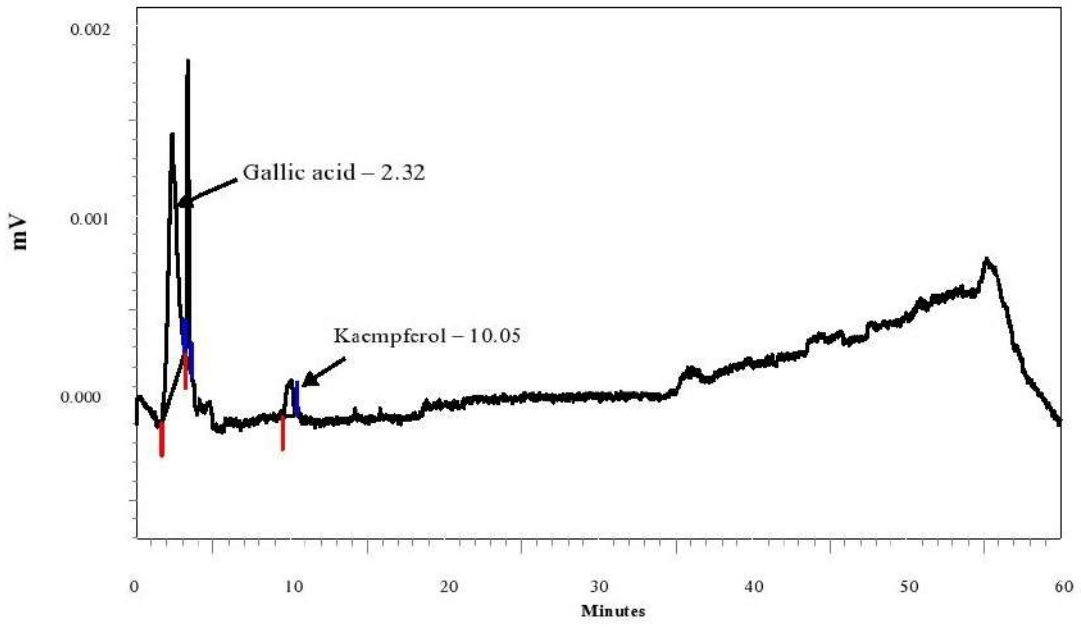

Figure 4.3: HPLC chromatogram indicating presence of gallic acid and Kaempferol with other compounds in aqueous extract of *H. rhamnoides* fruits

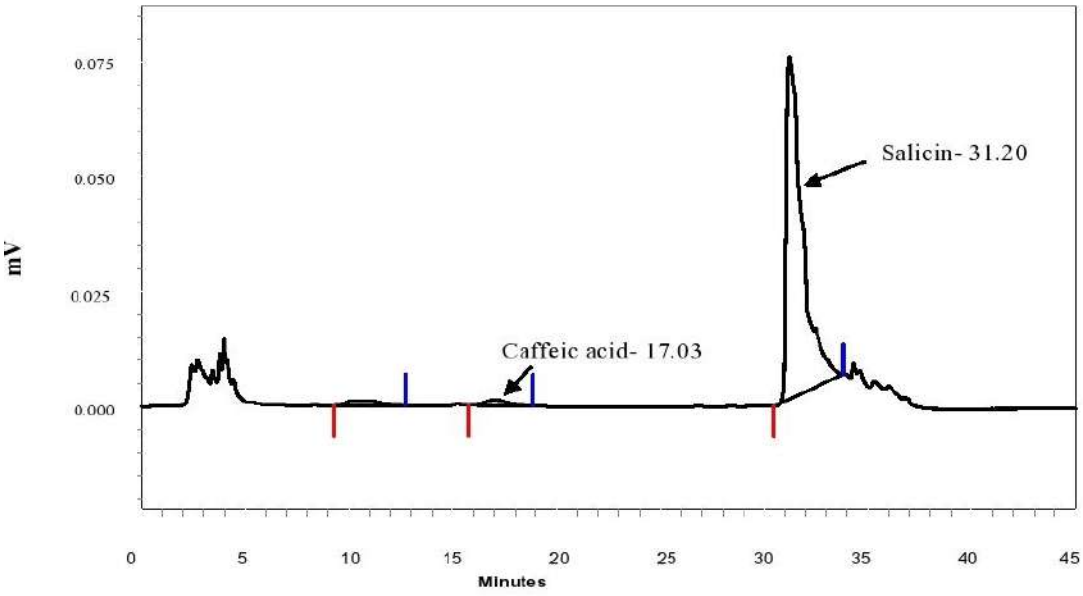

Figure 4.4: HPLC chromatogram indicating presence of caffeic acid and salicin with other compounds in aqueous extract of *Salix alba* leaves

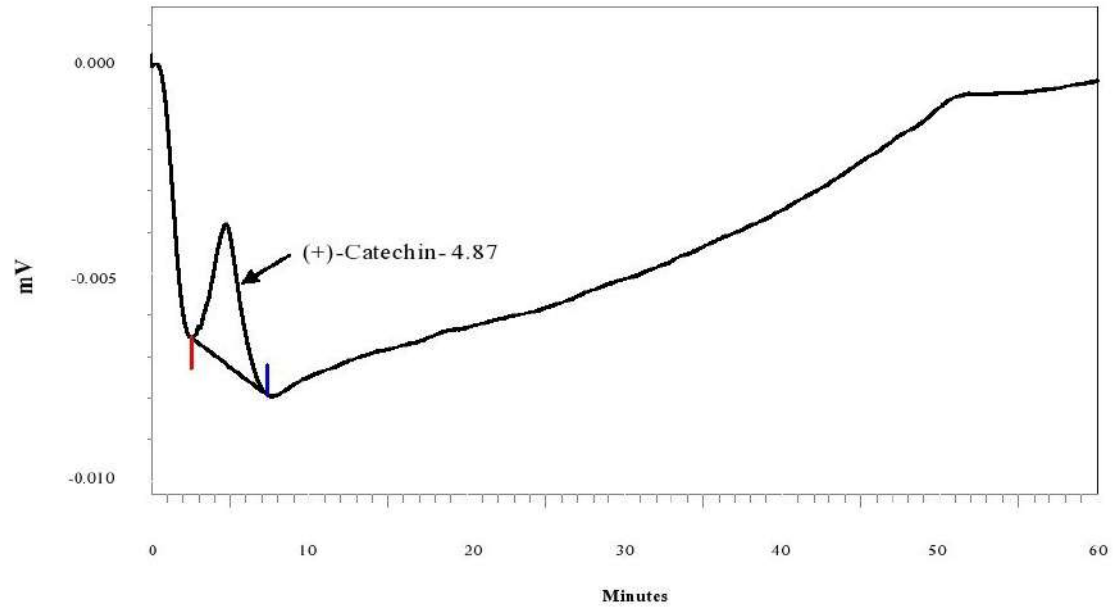

Figure 4.5: HPLC chromatogram indicating presence of (+)-catechins in aqueous extract of *P. armeniaca* seeds

4.3.3 Proliferative and cytoprotective activity of plant extracts in chicken peripheral lymphocytes

To evaluate the efficacy of plant extracts on the proliferation of chicken lymphocytes, all four extracts were cultured with lymphocytes at dissimilar dose concentrations and proliferative activity was evaluated with MTT assay. Cytoprotective activity of plant extracts against H_2O_2

induced oxidative stress in lymphocytes was also evaluated with MTT assay. Hydro-ethanolic extract of *R. imbricata* significantly increased the lymphocyte proliferation at all dose concentrations in between 800 ng/mL to 400 µg/mL in a dose-dependent manner as compared to the untreated control cells (Figure 4.6). Whereas, no change in the cell viability occurred at a dose concentration of 100 ng/mL-400 ng/mL as compared to control cells. Among the different dose concentrations of extract, the highest proliferation was recorded at 400 µg/ml. Lymphocytes that were treated with mitogen Con A represents the highest proliferation. Treatment of PBL with *R. imbricata* extract also reduced the toxicity induced by H_2O_2 at all dose concentrations in between 800 ng/mL to 400 µg/mL as compared to H_2O_2 stimulated cells (Figure 4.7). The maximum cytoprotective activity of *R. imbricata* extract was recorded at 4 µg/ml dose concentration.

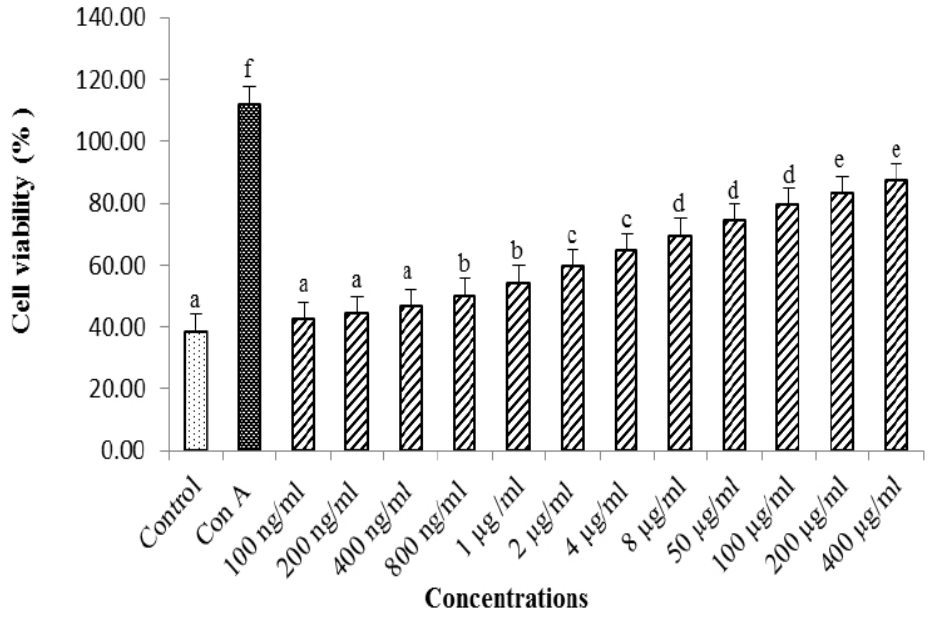

Figure 4.6: Effect of *R. imbricata* extract on chicken peripheral blood lymphocyte proliferation. Each value was compared with untreated control cells as well as within different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d, e, f}$) differ significantly ($P<0.05$).

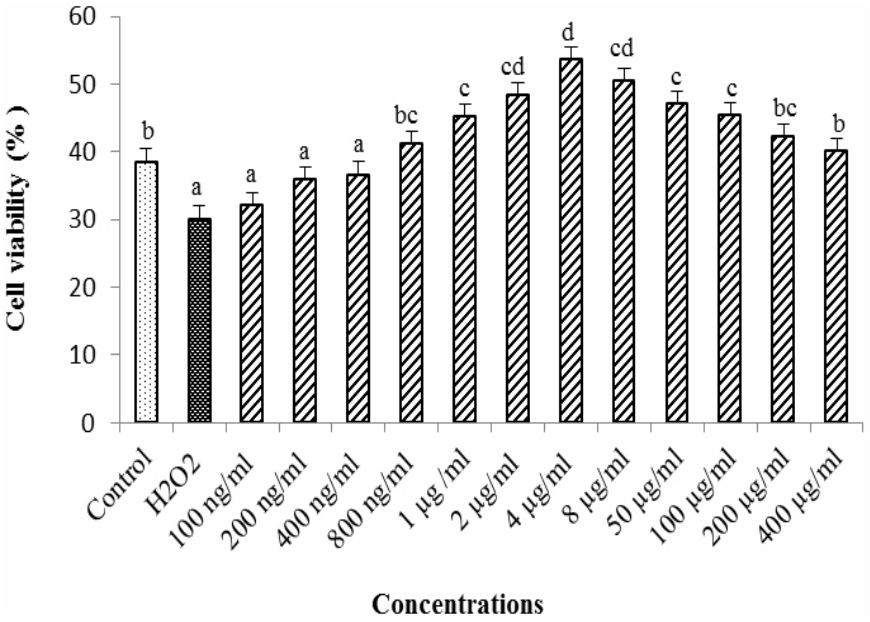

Figure 4.7: Cytoprotective activity of *R. imbricata* extract against H_2O_2 induced oxidative stress in chicken peripheral blood lymphocyte. Each value was compared with H_2O_2 stimulated cells as well as within different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d}$) differ significantly ($P<0.05$).

Aqueous extract of *H. rhamnoides* fruit also stimulated the lymphocytes proliferation at all dose concentrations (100 ng/mL-400 µg/mL) as compared to untreated control cells. Among different dose concentrations, the maximum proliferation activity was noticed at 400 µg/ml followed by 200 and 100 µg/ml (Figure 4.8). Further, *H. rhamnoides* extract also protects the PBL from the toxic effects of H_2O_2 at all of the tested concentrations as compared to H_2O_2 stimulated cells (Figure 4.9). The maximum cytoprotective activity of *H. rhamnoides* extract was recorded at 2 µg/ml dose concentration. With the treatment of aqueous extract of *S. alba* leaves a dose-dependent increase in the proliferation of chicken lymphocyte was recorded between the concentration of 400 ng/mL- 400 µg/mL compared with untreated control cells, however, no change in the cell viability was recorded at dose concentration of 100 ng/mL-200 ng/mL in comparison to control cells (Figure 4.10). Moreover, treatment of *S. alba* extract with lymphocytes also reduced the H_2O_2 induced cytotoxicity at a dose concentration of 400 ng/mL-400 µg/mL compared with H_2O_2 stimulated cells (Figure 4.11). The maximum cytoprotective activity of *S. alba* extract was recorded at 4 µg/ml dose concentration.

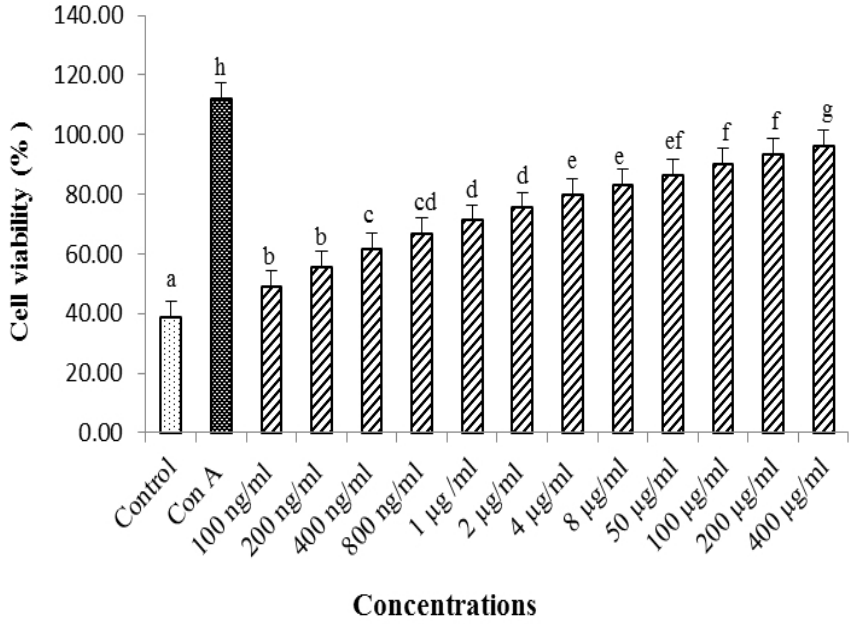

Figure 4.8: Effect of *H. rhamnoides* extract on chicken peripheral blood lymphocyte proliferation. Each value was compared with untreated control cells as well as with in different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d, e, f, g, h}$) differ significantly ($P<0.05$).

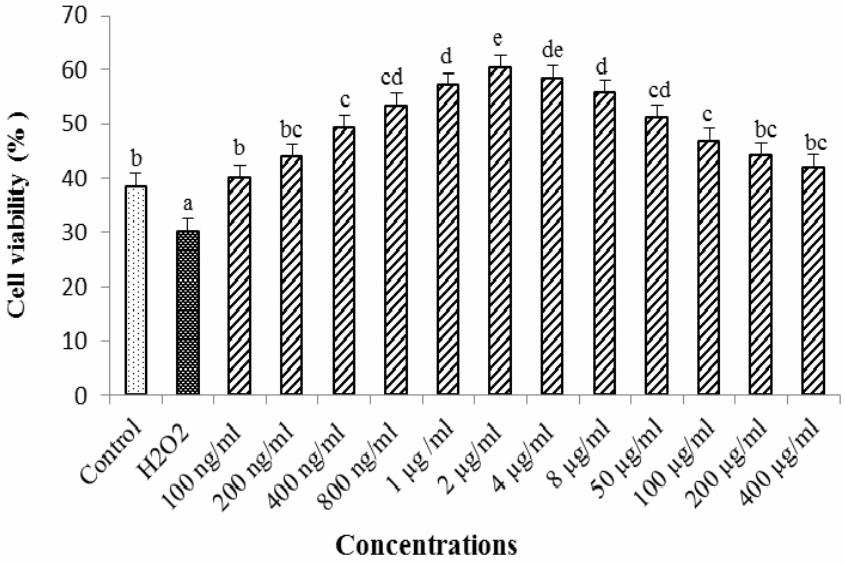

Figure 4.9: Cytoprotective activity of *H. rhamnoides* extract against H_2O_2 induced oxidative stress in chicken peripheral blood lymphocyte. Each value was compared with H_2O_2 stimulated cells as well as with in different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d, e}$) differ significantly ($P<0.05$).

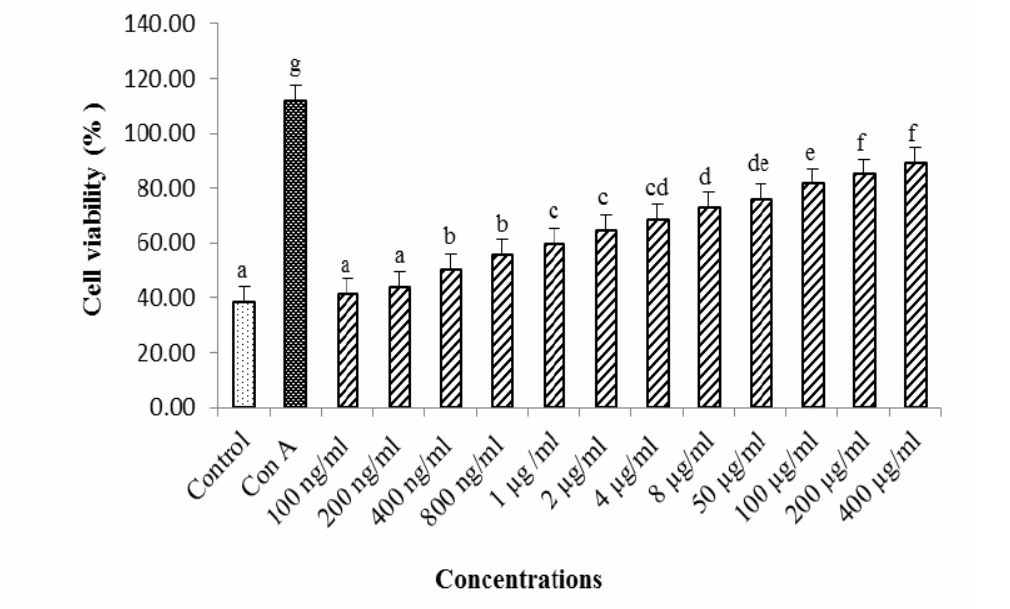

Figure 4.10: Effect of *S. alba* extract on chicken peripheral blood lymphocyte proliferation. Each value was compared with untreated control cells as well as with in different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d, e, f, g}$) differ significantly ($P<0.05$).

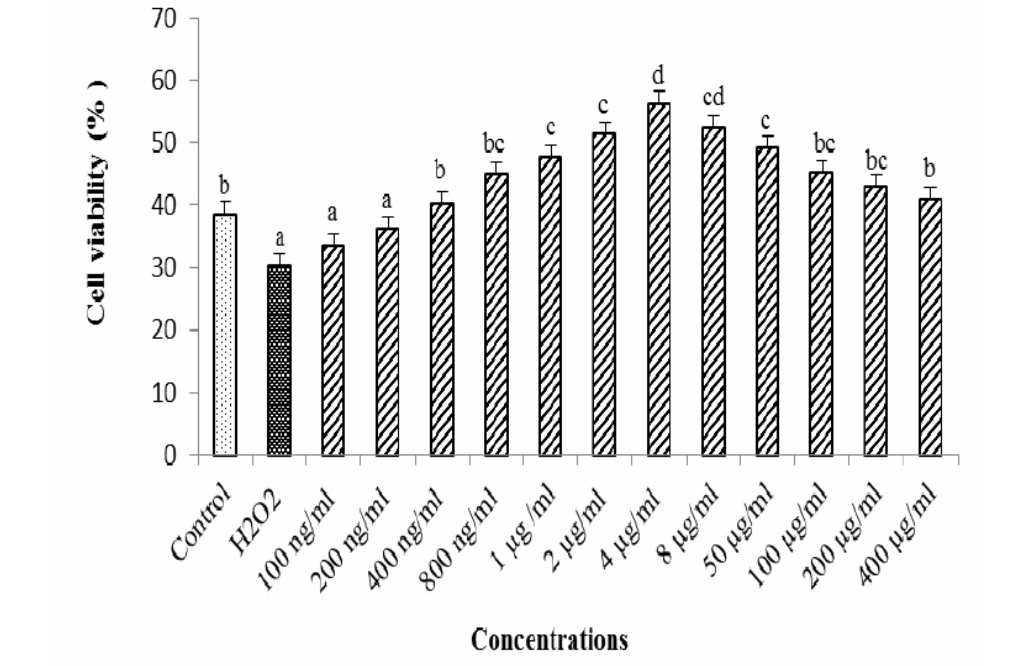

Figure 4.11: Cytoprotective activity of *S. alba* extract against H_2O_2 induced oxidative stress in chicken peripheral blood lymphocyte. Each value was compared with H_2O_2 stimulated cells as well as with in different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d}$) differ significantly ($P<0.05$).

Furthermore, aqueous extract of *P. armeniaca* seeds was also found to increase the proliferation of PBL at all of the tested concentration compared with the untreated control cells (Figure 4.12). Among the different dose concentrations, the maximum proliferation was noticed at 400 µg/ml. Lymphocytes that were treated with mitogen Con A represents the highest proliferation. Moreover, treatment of lymphocytes with *P. armeniaca* extract reduced the H_2O_2 induced cytotoxicity at a dose concentration of 100 ng/mL-50 µg/mL compared with H_2O_2 stimulated cells. The maximum cytoprotective activity of *P. armeniaca* extract was recorded at 2 µg/ml dose concentration (Figure 4.13).

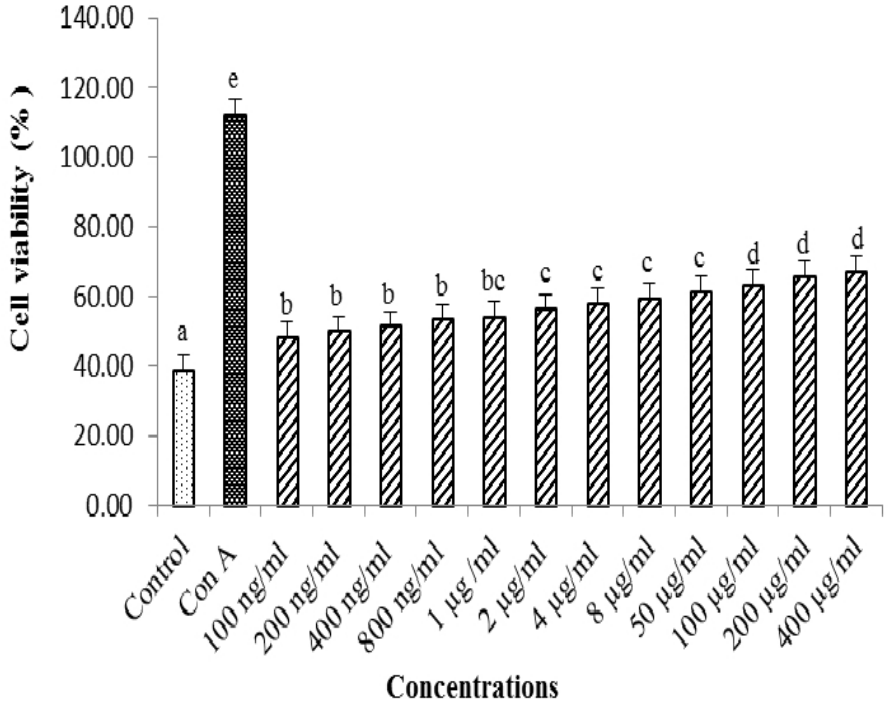

Figure 4.12: Effect of *P. armeniaca* extract on chicken peripheral blood lymphocyte proliferation. Each value was compared with untreated control cells as well as with in different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d, e}$) differ significantly ($P<0.05$).

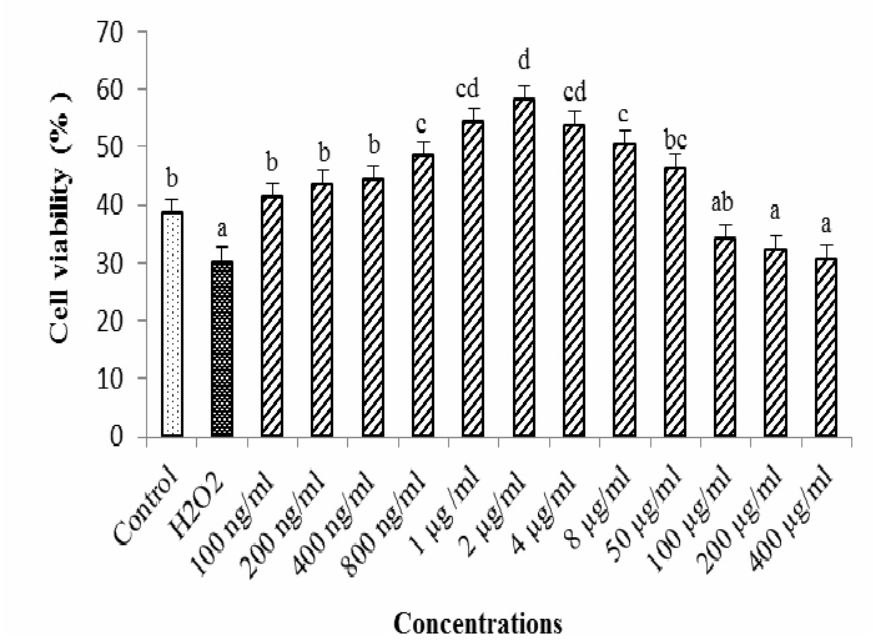

Figure 4.13: Cytoprotective activity of *P. armeniaca* extract against H_2O_2 induced oxidative stress in chicken peripheral blood lymphocyte. Each value was compared with H_2O_2 stimulated cells as well as with in different dose concentrations with Duncan's multiple range test. Each bar represents the mean ± SE value obtained from four culture wells. Bars having different superscript ($^{a, b, c, d}$) differ significantly ($P<0.05$).

4.4. Discussion

It has been highly described that polyphenolic content in plant herbs are associated with their antioxidant capacity [163] and soxhlet extraction is a very good technique in extracting phenolics compounds from plant herbs in a short period of time with the help of different extraction solvents having different polarities [148]. Here we studied the effect of different extraction solvent in terms of extraction yield. The result indicated that extraction of *R. imbricata* roots exhibits higher extract yield in hydro-ethanolic (60%) solvent. Whereas, extraction of *H. rhamnoides* fruits, *S. alba* leaves, and *P. armeniaca* exhibits higher yield of extract in an aqueous solvent. As the solvent polarity played a significant role in extracting phytomolecules from plant herbs and therefore it might be possible that the main bioactive phytomolecules exist in these plants were more polar in nature.

DPPH and $ABTS^+$ are stable free radical and are widely used to estimate the antioxidant activity of plant extracts [164]. In the present study, all four extract was found to have better scavenging ability in a dose-dependent manner, as compared to control. ABTS assay showed the same phenomenon where each extract scavenged the ABTS radical in a

dose-dependent manner. TAC represents the overall antioxidant capacity of plant extract in reducing oxidative stress [165] and in this study, all four extracts were found to possess better TAC. This increase in TAC and free radical scavenging activity in plant extracts could be attributed to the higher content of polyphenolic compounds present in these plants. These plants are habituated to grow in stressful surroundings of high altitude and such stressful conditions could up-regulate the pathway of synthesis of secondary metabolites and increased the production of antioxidant-rich polyphenolic compounds in these plants [77, 84, 142].

In the present study, all plant extracts were found to be rich in total phenolics, flavonoids, and carotenoid contents. Further, in this study HPLC quantification of extract revealed the presence of bioactive phytomolecules in *R. imbricata* (p-tyrosol and salidroside), *H. rhamnoides* (gallic acid and kaempferol), *S. alba* (salicin and caffeic acid) and *P. armeniaca* ((+)-catechins) at a higher concentration and the reported antioxidant properties of these plant extracts could also have been due to the synergistic effect of their phenolics, flavonoids, and carotenoids content. These results confirmed the previous reports that phenolics and flavonoids are the major phytomolecules in selected plant extracts and probably due to these constituents, these plant extracts exhibit their pharmacological antioxidant properties which are important for the scavenging of free radicals under stressful conditions of high altitude [59, 85, 166, 167]

In the present study, we assessed the efficacy of selected plant extracts in chicken PBL. The results indicated that all the four extracts induced dose-dependent proliferation of chicken PBL with greater activation of lymphocytes at higher dose concentrations. In addition, significant cytoprotective activity observed against H_2O_2 induced oxidative stress in lymphocytes. The capability of a plant extract to stimulate lymphocyte proliferation and enhance cytoprotection against free radicals is mostly attributed to its higher polyphenolic and carotenoids content [168, 169]. Since the bioactive phytomolecules salidroside and p-tyrosol were potent antioxidants found in *R. imbricata* extract. So, it is very likely that these phytomolecules may stimulate the proliferation of T lymphocytes and improved the cellular immunity in chickens.

H. rhamnoides was also found to be rich in carotenoids and polyphenolic compounds (gallic acid and kaempferol) and probably due to the synergistic effect of present polyphenols and carotenoids this plant extract stimulated the lymphocyte proliferation and reduced the H_2O_2 induced cytotoxic effects. The major bioactive phytomolecules found in this study in

aqueous extract of *S. alba* leaves was caffeic acid and salicin and might be these phytomolecules activated the lymphocyte proliferation and enhanced the cellular immune response in chickens. Aqueous extract of *P. armeniaca* was also found to possess a higher concentration of (+)-catechins, polyphenols and carotenoids and probably due to their synergistic effect *P. armeniaca* protects the chicken lymphocytes from H_2O_2 induced toxicity. Moreover, *in vitro* study also indicated the mitogenic activity of plant extracts in chicken lymphocytic cells in the absence of lymphocyte mitogen concanavalin A.

4.5 Conclusion

Hydro-ethanolic extract of *Rhodiola imbricata* roots and aqueous extract of *Hippophae rhamnoides* fruits, S*alix alba* leaves and *Prunus armeniaca* seeds were phytochemically characterized which revealed rich in polyphenols, flavonoids, and carotenoids content. Moreover, *in-vitro* studies indicated enhanced antioxidative and cytoprotective activities of these phytoextracts on chicken peripheral blood lymphocytes. Therefore, considering the pharmacological antioxidant properties of these four extracts their *in-vivo* efficacy might be evaluated on different physio-biochemical indices and growth performance of broiler chickens.

CHAPTER 5

TO EVALUATE THE EFFICACY OF PHYTOEXTRACTS ON PHYSIO-BIOCHEMICAL INDICES, GROWTH PERFORMANCE, SURVIVABILITY OF BROILER CHICKEN AND COST ECONOMICS OF THEIR REARING AT HIGH ALTITUDE

Abstract

The current study was carried out to know the effect of *R. imbricata, H. rhamnoides, S. alba,* and *P. armeniaca* extracts on physio-biochemical indices, growth performance, survivability of broiler chicken and evaluate cost economic of its rearing at high altitude. The study was conducted in four different experimental field trials, and during each experimental trial, 105 one day old RIR cross breed broiler chicks were randomly assigned to seven experimental groups. There were 3 replications per experimental group with 5 chickens per replicate pen. Experimental groups included control (fed the basal diet), and treatments T1, T2, T3, T4, T5, and T6 which received an hydro-ethanolic extract of *Rhodiola imbricata* in experiment 1, aqueous extract of *H. rhamnoides* fruits in experiment 2, aqueous extract of *S. alba* leaves in experiment 3, and aqueous extract of *P. armeniaca* seeds in experiment 4 in drinking water at concentration of 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively, along with basal diet. Blood samples were collected at 0, 21st, and 42nd day for analysis of physio-biochemical indices. Mortality was recorded daily and cost economics was calculated. During the each experimental trial, birds that were supplemented with phytoextracts at different dose concentration has shown better (P<0.05) physio-biochemical indices, higher body weight and improved FCR as compared to control birds. Interestingly, lower mortality rate due to ascites and coccidiosis was recorded in treated birds and therefore higher net return was observed. Hence, present investigation demonstrated the beneficial effect of these extracts in broiler chickens at high altitude. Therefore, may be used in formulation of feed additive for poultry ration.

5.1 Introduction

Increased production of free radicals at high altitude due to hypobaric hypoxia leads to the generation of oxidative stress [170]. A major outcome of oxidative stress is the marked increase in cellular dysfunction, decrease in the productiveness of antioxidant defense system,

alteration in immune responses and adverse biochemical changes in the animal body [4, 171]. Oxidative stress at high altitude hampers the growth rate of poultry birds by creating a catabolic breakdown and more tissue damage [172]. Dietary supplementation of broiler feed with antioxidants rich herbal preparations having free radical scavenging activity is an effective way of solving problems associated with oxidative stress [5, 58]. *R. imbricata, H. rhamnoides, S. alba,* and *P. armeniaca* are the important herbal plants of high altitude Himalayas and they are shown to possess antioxidative, anti-inflammatory, and immunomodulatory properties in a number of previous studies in animal models [54, 64, 77, 91]. However, the efficacy of these plants extracts on broilers physio-biochemical indices, growth performance, survivability rate, and cost economics of their rearing has not been studied so far at high altitude. Therefore, the current study was performed to examine the dietary supplemental effect of *R. imbricata, H. rhamnoides, S. alba,* and *P. armeniaca* extract on broiler physio-biochemical indices, growth performance, survivability rate and cost economics of their rearing at high altitude.

5.2 Material and Methods

5.2.1 Experimental design

The *in-vivo* experiment was approved by Institutional Animal Ethics Committee of DIHAR and all the methods were performed as per the guidelines of animal experimentation. The experiment was carried out under deep litter system in the solar poultry house of DIHAR having a stacking density of 0.80 square feet per bird in 2x2 feet of pen size (5 birds/pen). During each experimental trial, 105 one day old RIR cross-bred broiler chicks were distributed into 7 experimental groups as per complete randomized design. There were 3 replications per experimental group with 5 chickens per replicate pen. Experimental groups included control (fed the basal diet), and treatments T1, T2, T3, T4, T5, and T6 which received an hydro-ethanolic extract of *Rhodiola imbricata* in experiment 1, aqueous extract of *H. rhamnoides* fruits in experiment 2, aqueous extract of *S. alba* leaves in experiment 3, and aqueous extract of *P. armeniaca* seeds in experiment 4 in drinking water at concentration of 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively, along with the basal diet. The ingredients and analysed composition of basal diet are presented in Table 3.1. The experimental period for each study was 0 to 42 days of broiler chick age. Antibiotics, anticoccidials or any other commercially available vitamin/mineral supplement were not added to drinking water in order to avoid interference. Each chicken was individually

weighed at every week interval during each experimental trial. Throughout the experiment, water and feed intake was measured. FCR was calculated from the ratio between feed intake and weight gain by a chick. During each experimental trial post-mortem examination of dead birds was done to find out the cause of death. Economics of each experiment trial was also estimated based on the rearing cost of birds.

5.2.2 Blood collection

During each experimental trial, blood samples were taken at 0, 21, and 42 days for examination of physio-biochemical indices. For a collection of a blood sample, we randomly picked the chickens from replicate pens of each experimental group and took 3 ml of a blood sample from wing vein of chickens and collected those samples into sterile plastic tubes containing EDTA as an anticoagulant (Figure 5.1). EDTA tubes containing blood were centrifuged at 3500 RPM for 10 m at 4^0C to acquire clear plasma and stored at -80^0C until use. Isolated plasma samples were then analysed for different physio-biochemical indices.

Figure 5.1: Collection of venous blood samples from chickens wings

5.2.3 Physio-biochemical indices

5.2.3.1 Determination of blood biochemical parameters

Plasma glucose content was estimated by GOD-POD method using biochemical kit supplied by Span Diagnostics, India (Cat. No. 93DP100-80). Plasma cholesterol and triglyceride were estimated by CHOD-PAP and GPO-PAP methods respectively using Span Diagnostics kits (Cat. No. 71LS200-60, 72LS200-60). High density lipoprotein (HDL) and low density lipoprotein (LDL) were assayed with PVS-PEGME coupled precipitation method using biochemical kits supplied by ERBA Mannheim Diagnostics, Germany (Cat. No. BLT00028,

BLT00041 respectively). Plasma total protein and albumin were estimated by Biuret and BCG methods respectively using ERBA Mannheim Diagnostics kits (Cat. No. BLT00054, BLT0002). Creatinine concentration was estimated by Jaffe method using Span Diagnostic kit (Cat. No. 85LS201-62). Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by IFCC method using ERBA Mannheim Diagnostics kits (Cat. No. BLT00051, BLT00053 respectively). All the analysis was done in biochemical semi-auto analyser (BIOTRON BTR-830). Plasma albumin concentration was estimated by subtracting the value of albumin from total protein.

5.2.3.2 Determination of plasma antioxidant parameters

5.2.3.2.1 DPPH radical scavenging capacity

Plasma free radical scavenging capacity was determined as described earlier in the section 4.2.4.1.

5.2.3.2.2 TAC

Plasma TAC was determined as described earlier in the section 4.2.4.3. Results were expressed as FRAP value (μM Fe (II)/L of plasma).

5.2.3.2.3 Lipid peroxidation (LPO)

The LPO assay was performed by measuring malondialdehyde (MDA) concentration in plasma samples. The level of MDA in plasma samples was analysed by the method suggested by previous investigators [173]. For this assay, we first dissolved 375 mg of thiobarbituric acid (TBA) in to 2 mL of 0.25 N hydrochloric acid and after that we added 15 g of trichloroacetic acid (TCA) and made a final volume of 100 mL. Thereafter, to properly dissolve the TBA the solution was heated at 55^0C in a water bath (GFL water bath, Burgwedal, Germany) for 15 m. After that, 250 μL of plasma sample was merged with 500 μL of TCA-TBA-HCL solution and mixed properly. The solution was further heated for 15 m and upon cooling, the solution was centrifuged at 3000 RPM for 5 m in a centrifuge to remove the flocculent precipitate and the stable pink colour was separated out for the determination of absorption at 535 nm against a blank that contained all reagents except the plasma samples. Plasma MDA concentration was indicated as nmol/mL.

5.2.3.3 Determination of inflammatory cytokines

Plasma IL-1 concentration was estimated by LEGEND MAX IL-1 ELISA kit (Biolegend, San Diego, CA, Cat. No. 433401). Plasma IL-2 concentration was estimated with LEGEND MAX

IL-2 ELISA kit (Biolegend, San Diego, CA, Cat. No. 431007). Plasma IL-6 concentration was estimated by LEGEND MAX IL-6 ELISA kit (Biolegend, San Diego, CA, Cat. No. 431307) following the instructions in respective kit manuals.

5.2.4 Statistical analysis

As described earlier in the section 3.2.4. For growth performance, 3 replicates pen per group (5 broiler chickens per replicate pen) served as experimental unit.

5.3 Results

5.3.1 Physio-biochemical indices

5.3.1.1 Blood biochemical profile in experimental trial 1

In experiment 1, with supplementation of hydro-ethanolic extract of *R. imbricata* a significant increase in plasma total protein was recorded in the birds in T5 group at 42 day. Total protein concentration was also increased in T3, T4, and T6 groups as compared to control group at 21 and 42 day (Table 5.1). No differences were observed in albumin concentration among the experimental groups. Plasma globulin concentration was increased in T3, T4, T5, and T6 groups as compared to control group at 21 day. Moreover, at 42 day there was a significant increase in globulin level was observed in all treatment groups as compared to control group and highest concentration was observed in T5 group. A significant decrease in A/G ratio was noticed in treatment groups as compared with control group. Numerically lowest A/G ratio was recorded in T5 group. Mean concentrations of cholesterol and triglyceride were recorded significantly lower in T2 and T5 groups in comparison to control group at 21 and 42 days, but the mean values in remaining treatment groups did not differ with control group (Table 5.1)

Plasma HDL concentration was increased significantly in treatment group birds as compared to the control group birds at 21 and 42 days. Within the treatment groups, birds in T5 group represent highest HDL concentration. A significant decrease in LDL concentration was recorded in treatment group birds as compared with control group at 21 and 42 days. Within the treatment groups, birds in T5 group had lowest LDL concentration. A significant increase in plasma glucose was noticed in control group birds. Within the treatment groups, the lowest glucose concentration was observed in T1 and T4 groups at 21 day. Moreover, at 42 day the lowest glucose concentration was noticed in T5 group. AST and ALT levels were reduced in treatment groups as compared with control group. Within the treatment groups, birds in T5 group represent lowest ALT and AST concentration. However, no difference in creatinine concentration was observed among the experimental groups (Table 5.1).

5.3.1.2 Blood biochemical profile in experimental trial 2

In experiment 2, supplementation of *Hippophae rhamnoides* aqueous extract significantly increased the concentration of plasma total protein, albumin, and globulin in all the treatment group birds as compared with control group (Table 5.2). Within the treatment groups, the highest concentration of total protein and globulin was observed in T3 group birds at 42 day. A significant decrease in A/G ratio was noticed in treatment group birds as compared to control group. Within the treatment groups, the lowest A/G ratio was observed in T3 group birds at 42 day. Mean concentration of plasma cholesterol and LDL was reduced significantly in treatment group as compared to control group at 21 and 42 days. Within the treatment groups, lowest concentration of cholesterol and LDL was observed in T3 and T4 group birds.

HDL level was found to be significantly higher in treatment group birds as compared to control group. Within the treatment groups, birds in the T3 group represent highest HDL concentration. We observed a significant increase in plasma glucose level in the control group birds as compared to treatment group birds and the lowest glucose concentration was noticed in the T3 treatment group at 21 and 42 days. The concentration of AST was reduced in T3 and T4 group birds as compared to control group at 21 day. Similarly, at 42 day, AST concentration was reduced in T3, T4, and T5 groups as compared to control group birds. However, *H. rhamnoides* supplementation did not show any significant difference in triglyceride, creatinine and ALT concentrations among the experimental groups (Table 5.2).

Table 5.1: Effect of *R. imbricata* on blood biochemical parameters of broiler chickens at high altitude (experiment 1)

Groups	0 day	21st day	42nd day	Groups	0 day	21st day	42nd day
Total protein (g/dL)				**HDL (mg/dL)**			
Control	3.95 ± 0.10	4.62^{a} ± 0.12	4.55^{a} ± 0.17	Control	16.54 ± 0.36	24.71^{a} ± 0.34	23.24^{a} ± 0.44
T1	3.91 ± 0.06	4.66^{a} ± 0.17	5.28^{b} ± 0.21	T1	17.16 ± 0.36	25.44^{b} ± 0.24	39.04^{b} ± 0.40
T2	3.96 ± 0.11	4.36^{a} ± 0.18	5.17^{b} ± 0.27	T2	16.32 ± 0.35	29.58^{d} ± 0.25	48.26^{d} ± 0.48
T3	3.95 ± 0.07	5.20^{c} ± 0.26	6.06^{c} ± 0.25	T3	16.29 ± 0.24	27.52^{c} ± 0.28	42.34^{b} ± 0.51
T4	3.94 ± 0.10	5.17^{c} ± 0.21	5.92^{c} ± 0.19	T4	17.04 ± 0.21	27.12^{c} ± 0.32	44.19^{c} ± 0.61
T5	3.96 ± 0.07	5.59^{d} ± 0.24	6.31^{d} ± 0.30	T5	16.50 ± 0.22	32.16^{e} ± 0.32	54.42^{e} ± 0.52
T6	3.90 ± 0.09	4.87^{b} ± 0.19	5.67^{c} ± 0.21	T6	16.81 ± 0.21	24.38^{a} ± 0.23	39.38^{b} ± 0.41
Albumin (g/dL)				**LDL (mg/dL)**			
Control	2.44 ± 0.07	2.71 ± 0.14	2.90 ± 0.13	Control	48.16 ± 1.68	44.31^{c} ± 1.62	43.59^{d} ± 1.68
T1	2.44 ± 0.05	2.67 ± 0.09	3.11 ± 0.15	T1	48.55 ± 1.40	42.90^{b} ± 1.74	35.24^{c} ± 1.63
T2	2.42 ± 0.05	2.58 ± 0.13	3.10 ± 0.10	T2	49.04 ± 1.76	40.63^{b} ± 1.93	31.16^{b} ± 1.92
T3	2.43 ± 0.09	2.59 ± 0.17	2.97 ± 0.18	T3	48.62 ± 1.66	41.86^{b} ± 1.79	33.46^{b} ± 1.58
T4	2.42 ± 0.06	2.63 ± 0.11	3.01 ± 0.15	T4	48.10 ± 1.55	41.46^{b} ± 1.75	32.77^{b} ± 1.98
T5	2.41 ± 0.10	2.60 ± 0.14	2.94 ± 0.15	T5	49.12 ± 1.76	38.35^{a} ± 1.85	29.18^{a} ± 1.88
T6	2.40 ± 0.08	2.69 ± 0.16	3.12 ± 0.21	T6	48.21 ± 1.77	44.29^{c} ± 1.82	35.18^{c} ± 1.75
Globulin (g/dL)				**Glucose (mg/dL)**			
Control	1.51 ± 0.08	1.91^{a} ± 0.06	1.65^{a} ± 0.12	Control	288.75 ± 6.71	287.25^{c} ± 8.98	278.00^{d} ± 6.64
T1	1.47 ± 0.06	1.99^{ab} ± 0.08	2.17^{b} ± 0.11	T1	287.25 ± 5.57	240.75^{a} ± 8.17	267.00^{cd} ± 5.52
T2	1.54 ± 0.06	1.78^{a} ± 0.09	2.07^{b} ± 0.09	T2	292.50 ± 5.86	262.75^{b} ± 8.56	269.25^{d} ± 5.12
T3	1.50 ± 0.09	2.61^{c} ± 0.08	3.09^{c} ± 0.17	T3	287.00 ± 6.98	263.75^{b} ± 7.58	226.00^{b} ± 9.06
T4	1.52 ± 0.07	2.54^{c} ± 0.13	2.91^{c} ± 0.20	T4	287.50 ± 7.50	240.00^{a} ± 9.54	266.00^{cd} ± 7.56
T5	1.55 ± 0.11	2.99^{d} ± 0.11	3.37^{d} ± 0.21	T5	290.50 ± 5.36	265.00^{b} ± 6.55	197.75^{a} ± 6.60
T6	1.50 ± 0.09	2.18^{b} ± 0.08	2.55^{b} ± 0.17	T6	287.50 ± 7.41	249.70^{ab} ± 7.32	246.00^{bc} ± 6.27
A/G ratio (g/dL)				**Creatinine (mg/dL)**			
Control	1.61 ± 0.07	1.42^{c} ± 0.04	1.76^{d} ± 0.12	Control	0.18 ± 0.04	0.14 ± 0.02	0.20 ± 0.04
T1	1.66 ± 0.08	1.34^{b} ± 0.06	1.43^{c} ± 0.10	T1	0.20 ± 0.04	0.13 ± 0.05	0.13 ± 0.03
T2	1.57 ± 0.08	1.45^{c} ± 0.09	1.50^{c} ± 0.10	T2	0.22 ± 0.06	0.16 ± 0.03	0.16 ± 0.02

T3	1.62 ± 0.10	0.99^{a} ± 0.07	0.96^{a} ± 0.08		T3	0.21 ± 0.03	0.17 ± 0.03	0.16 ± 0.02
T4	1.59 ± 0.10	1.04^{a} ± 0.05	1.03^{ab} ± 0.14		T4	0.18 ± 0.05	0.16 ± 0.04	0.17 ± 0.04
T5	1.55 ± 0.11	0.87^{a} ± 0.06	0.87^{a} ± 0.09		T5	0.22 ± 0.06	0.13 ± 0.02	0.19 ± 0.04
T6	1.61 ± 0.10	1.23^{b} ± 0.05	1.22^{b} ± 0.13		T6	0.17 ± 0.04	0.13 ± 0.02	0.13 ± 0.02
Cholesterol (mg/dL)					**AST (IU/L)**			
Control	215.75 ± 2.98	212.00^{b} ± 3.62	208.75^{b} ± 3.96		Control	88.27 ± 2.43	85.00^{c} ± 2.97	82.00^{d} ± 2.73
T1	218.50 ± 3.66	207.25^{b} ± 4.32	206.25^{b} ± 3.70		T1	88.50 ± 1.95	61.50^{a} ± 2.02	56.25^{c} ± 2.01
T2	214.25 ± 2.95	190.00^{a} ± 4.60	181.25^{a} ± 5.17		T2	86.00 ± 2.35	59.00^{a} ± 1.47	53.25^{c} ± 2.75
T3	212.75 ± 3.68	207.75^{b} ± 4.80	205.00^{b} ± 5.30		T3	85.75 ± 2.25	57.25^{a} ± 2.05	46.77^{a} ± 2.04
T4	216.50 ± 4.92	209.25^{b} ± 6.28	201.00^{b} ± 3.67		T4	87.75 ± 2.50	65.00^{b} ± 2.61	54.50^{c} ± 2.75
T5	212.50 ± 3.37	184.00^{a} ± 4.14	176.25^{a} ± 3.30		T5	88.00 ± 2.00	60.25^{a} ± 2.01	45.00^{a} ± 2.12
T6	216.00 ± 3.65	212.25^{b} ± 5.10	207.50^{b} ± 5.05		T6	88.25 ± 2.54	61.00^{a} ± 2.04	48.45^{b} ± 1.89
Triglyceride (mg/dL)					**ALT (IU/L)**			
Control	108.82 ± 1.91	101.24^{c} ± 1.06	99.54^{c} ± 0.67		Control	16.00 ± 1.75	15.00^{c} ± 0.81	14.00^{c} ± 0.48
T1	109.44 ± 1.76	100.37^{c} ± 1.63	98.71^{c} ± 0.64		T1	15.25 ± 1.43	10.00^{b} ± 0.40	11.32^{b} ± 0.77
T2	107.48 ± 1.71	092.64^{b} ± 1.30	85.61^{b} ± 1.24		T2	16.00 ± 0.91	12.25^{b} ± 0.62	10.00^{b} ± 0.40
T3	108.75 ± 1.79	099.70^{c} ± 1.16	95.36^{c} ± 0.72		T3	16.25 ± 1.43	10.25^{b} ± 0.85	11.00^{b} ± 0.81
T4	109.35 ± 1.31	098.44^{c} ± 1.00	93.69^{c} ± 0.89		T4	16.25 ± 0.62	11.75^{b} ± 1.03	10.00^{b} ± 0.81
T5	107.65 ± 1.33	088.52^{a} ± 1.26	78.24^{a} ± 0.98		T5	15.50 ± 1.65	08.75^{a} ± 0.62	08.32^{a} ± 0.63
T6	108.17 ± 1.24	100.55^{c} ± 1.01	97.47^{c} ± 0.88		T6	16.25 ± 1.54	11.00^{b} ± 0.70	11.75^{b} ± 0.75

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *R. imbricata* extract at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c, d, e}$) in a columns differ significantly ($P<0.05$).

Table 5.2: Effect of *H. rhamnoides* on blood biochemical parameters of broiler chickens at high altitude (experiment 2)

Groups	0 day	21st day	42nd day
Total protein (g/dL)			
Control	3.36 ± 0.18	3.51^{a} ± 0.07	3.57^{a} ± 0.13
T1	3.31 ± 0.15	4.72^{b} ± 0.09	5.64^{c} ± 0.08
T2	3.35 ± 0.13	4.76^{b} ± 0.14	5.23^{b} ± 0.09
T3	3.33 ± 0.13	4.89^{b} ± 0.12	5.77^{d} ± 0.11
T4	3.40 ± 0.14	4.65^{b} ± 0.13	5.30^{b} ± 0.27
T5	3.37 ± 0.17	4.74^{b} ± 0.10	5.12^{b} ± 0.21
T6	3.34 ± 0.14	4.60^{b} ± 0.14	5.51^{c} ± 0.13
Albumin (g/dL)			
Control	2.08 ± 0.12	2.18^{a} ± 0.08	2.23^{a} ± 0.15
T1	2.05 ± 0.12	2.88^{b} ± 0.10	3.10^{b} ± 0.08
T2	2.04 ± 0.11	2.87^{b} ± 0.11	2.98^{b} ± 0.06
T3	2.03 ± 0.16	2.91^{b} ± 0.21	3.05^{b} ± 0.19
T4	2.06 ± 0.15	2.78^{b} ± 0.17	3.02^{b} ± 0.06
T5	2.07 ± 0.12	2.80^{b} ± 0.11	2.89^{b} ± 0.08
T6	2.06 ± 0.13	2.79^{b} ± 0.15	3.00^{b} ± 0.14
Globulin (g/dL)			
Control	1.28 ± 0.08	1.33^{a} ± 0.15	1.34^{a} ± 0.20
T1	1.26 ± 0.10	1.84^{b} ± 0.18	2.54^{b} ± 0.19
T2	1.31 ± 0.14	1.89^{b} ± 0.16	2.25^{b} ± 0.13
T3	1.30 ± 0.19	1.98^{b} ± 0.23	2.72^{c} ± 0.28
T4	1.34 ± 0.11	1.87^{b} ± 0.21	2.28^{b} ± 0.23
T5	1.30 ± 0.09	1.94^{b} ± 0.15	2.23^{b} ± 0.23
T6	1.28 ± 0.09	1.81^{b} ± 0.18	2.51^{b} ± 0.16
A/G ratio (g/dL)			
Control	1.63 ± 0.10	1.64^{c} ± 0.12	1.66^{d} ± 0.14

Groups	0 day	21st day	42nd day
HDL (mg/dL)			
Control	19.81 ± 0.80	20.06^{a} ± 0.77	20.40^{a} ± 0.84
T1	19.70 ± 0.73	27.16^{b} ± 0.80	31.64^{b} ± 1.04
T2	20.04 ± 0.69	26.19^{b} ± 0.75	31.56^{b} ± 0.82
T3	19.50 ± 0.86	30.56^{c} ± 0.85	44.17^{d} ± 1.02
T4	20.16 ± 0.71	27.49^{b} ± 0.81	38.12^{c} ± 0.90
T5	19.71 ± 0.81	26.83^{b} ± 0.85	37.89^{c} ± 0.91
T6	19.39 ± 0.71	27.07^{b} ± 0.80	32.23^{b} ± 0.85
LDL (mg/dL)			
Control	53.35 ± 1.07	51.86^{c} ± 0.96	50.43^{c} ± 0.88
T1	53.91 ± 0.90	44.80^{b} ± 1.01	41.05^{b} ± 0.92
T2	54.12 ± 1.15	46.04^{b} ± 1.21	40.59^{b} ± 1.10
T3	53.80 ± 1.01	42.70^{a} ± 0.89	36.24^{a} ± 0.95
T4	53.26 ± 0.91	42.91^{a} ± 1.03	37.12^{a} ± 1.07
T5	54.07 ± 1.10	45.77^{b} ± 1.04	40.25^{b} ± 0.90
T6	53.90 ± 0.97	45.90^{b} ± 0.90	41.18^{b} ± 0.83
Glucose (mg/dL)			
Control	322.75 ± 12.45	311.75^{d} ± 9.85	309.50^{d} ± 6.11
T1	324.00 ± 13.63	291.25^{c} ± 6.30	280.25^{c} ± 4.28
T2	321.75 ± 13.02	274.00^{b} ± 9.05	260.00^{bc} ± 9.46
T3	324.25 ± 14.41	249.25^{a} ± 6.79	213.00^{a} ± 3.87
T4	326.50 ± 12.41	266.25^{b} ± 6.32	253.00^{b} ± 4.43
T5	324.25 ± 13.77	294.25^{c} ± 7.16	300.75^{d} ± 6.23
T6	327.00 ± 12.94	297.25^{c} ± 7.25	302.25^{d} ± 9.91
Creatinine (mg/dL)			
Control	0.20 ± 0.05	0.30 ± 0.04	0.26 ± 0.03

T1	1.63 ± 0.08	1.57^{b} ± 0.09	1.22^{b} ± 0.10	T1	0.20 ± 0.04	0.27 ± 0.03	0.25 ± 0.02
T2	1.56 ± 0.07	1.52^{b} ± 0.11	1.32^{c} ± 0.08	T2	0.20 ± 0.06	0.30 ± 0.04	0.27 ± 0.02
T3	1.56 ± 0.09	1.47^{a} ± 0.10	1.12^{a} ± 0.11	T3	0.22 ± 0.05	0.28 ± 0.04	0.25 ± 0.04
T4	1.54 ± 0.08	1.49^{a} ± 0.09	1.32^{c} ± 0.09	T4	0.23 ± 0.03	0.31 ± 0.03	0.27 ± 0.03
T5	1.59 ± 0.11	1.44^{a} ± 0.11	1.30^{c} ± 0.10	T5	0.22 ± 0.02	0.27 ± 0.04	0.25 ± 0.05
T6	1.61 ± 0.13	1.54^{b} ± 0.12	1.20^{b} ± 0.08	T6	0.21 ± 0.02	0.31 ± 0.07	0.28 ± 0.05
Cholesterol (mg/dL)				**AST (IU/L)**			
Control	178.25 ± 05.23	169.67^{c} ± 04.92	165.32^{d} ± 03.88	Control	80.75 ± 7.58	60.00^{b} ± 2.79	75.00^{b} ± 4.67
T1	177.50 ± 08.42	158.00^{b} ± 04.30	138.50^{b} ± 02.59	T1	81.50 ± 6.39	62.25^{b} ± 3.70	74.00^{b} ± 6.48
T2	179.50 ± 10.96	155.17^{b} ± 04.78	139.67^{b} ± 02.19	T2	79.25 ± 4.64	53.25^{ab} ± 7.71	67.00^{ab} ± 5.47
T3	177.50 ± 13.24	142.50^{a} ± 04.98	128.50^{a} ± 03.30	T3	79.50 ± 7.96	45.25^{a} ± 4.19	54.75^{a} ± 4.05
T4	180.00 ± 07.56	147.57^{a} ± 06.16	131.67^{a} ± 02.24	T4	80.00 ± 7.49	45.25^{a} ± 4.62	56.75^{a} ± 5.32
T5	175.25 ± 07.92	158.67^{b} ± 06.47	137.00^{b} ± 02.64	T5	79.00 ± 6.27	58.00^{b} ± 3.81	61.25^{a} ± 4.11
T6	178.00 ± 07.39	158.00^{b} ± 05.81	143.67^{c} ± 02.71	T6	79.75 ± 6.81	57.25^{ab} ± 3.25	63.75^{ab} ± 4.93
Triglyceride (mg/dL)				**ALT (IU/L)**			
Control	138.87 ± 05.49	130.25 ± 09.15	123.25 ± 06.96	Control	18.25 ± 1.25	12.00 ± 0.40	12.25 ± 0.62
T1	136.63 ± 08.16	127.38 ± 08.68	120.50 ± 05.48	T1	18.75 ± 1.65	11.75 ± 1.03	11.00 ± 1.08
T2	131.93 ± 09.94	123.97 ± 08.05	120.00 ± 05.96	T2	18.50 ± 0.64	13.25 ± 0.62	10.75 ± 0.62
T3	135.21 ± 12.83	128.17 ± 09.83	121.00 ± 08.22	T3	17.75 ± 2.65	11.25 ± 0.62	09.75 ± 0.85
T4	132.48 ± 07.81	121.33 ± 10.96	118.75 ± 09.11	T4	18.00 ± 1.35	12.75 ± 1.65	11.00 ± 0.81
T5	134.87 ± 07.19	123.94 ± 09.11	119.25 ± 06.04	T5	17.75 ± 0.75	11.75 ± 0.85	10.25 ± 1.03
T6	135.92 ± 03.94	127.16 ± 08.37	123.00 ± 04.91	T6	18.25 ± 0.85	12.75 ± 1.03	10.00 ± 1.08

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *H. rhamnoides* extract at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a column differ significantly ($P<0.05$).

5.3.1.3 Blood biochemical profile in experimental trial 3

In experiment 3, the addition of *S. alba* extract in broiler drinking water significantly increased the concentration of plasma total protein, albumin, and globulin in treatment group birds as compared with control group (Table 5.3). Within the treatment groups, birds in T4 group represented highest total protein and albumin concentration whereas, we did not observe any difference in the level of A/G ratio among the control and treatment groups. The concentration of plasma cholesterol was decreased in treatment group birds as compared to control group and within the treatment groups, lowest cholesterol level was observed in T4 group. The concentration of triglyceride was significantly decreased in T4 group birds as compared to control group. However, triglyceride concentration did not differ among the control and remaining treatment groups (Table 5.3).

At 21 day, we observed a significant increase in the concentration of HDL in T4 group birds as compared to control, T1 and T2 group whereas, at 42 day, the concentration of HDL was recorded significantly higher in T4 group birds among all the experimental groups. Moreover, the concentration of LDL was reduced in T3, T4, T5, and T6 groups as compared to control group at 21 and 42 day. In between the treatment groups, the numerically lowest LDL concentration was recorded in T4 group birds. In addition to this, we observed a significant increase in glucose concentration in control group birds as compared to treatment groups at 21 and 42 day. No differences were recorded in the concentration of A/G ratio, creatinine, and ALT among the experimental groups whereas, AST level was reduced in T1 and T4 group birds as compared to control group at 42 day (Table 5.3).

Table 5.3: Effect of *S. alba leaves* extract on blood biochemical parameters of broiler chickens at high altitude (experiment 3)

Groups	0 day	21st day	42nd day	Groups	0 day	21st day	42nd day
Total protein (g/dL)				**HDL (mg/dL)**			
Control	4.12 ± 0.23	4.16^{a} ± 0.24	4.28^{a} ± 0.26	Control	22.36 ± 0.55	24.12^{a} ± 0.48	26.08^{a} ± 0.80
T1	4.09 ± 0.22	5.19^{b} ± 0.27	6.13^{b} ± 0.32	T1	22.84 ± 0.61	24.80^{a} ± 0.39	27.22^{a} ± 0.66
T2	4.13 ± 0.17	5.24^{b} ± 0.30	6.28^{bc}± 0.38	T2	23.07 ± 0.49	25.34^{a} ± 0.55	29.70^{ab}± 0.70
T3	4.07 ± 0.25	5.26^{b} ± 0.27	6.33^{bc}± 0.34	T3	22.61 ± 0.65	29.27^{ab}± 0.60	35.44^{b} ± 0.79
T4	4.10 ± 0.20	5.34^{c} ± 0.31	6.41^{c} ± 0.36	T4	22.16 ± 0.52	33.81^{b} ± 0.71	41.25^{c} ± 0.85
T5	4.14 ± 0.21	5.16^{b} ± 0.30	6.09^{b} ± 0.33	T5	23.00 ± 0.55	27.92^{ab}± 0.50	34.16^{b} ± 0.70
T6	4.12 ± 0.18	5.21^{b} ± 0.28	6.11^{b} ± 0.32	T6	22.57 ± 0.51	27.80^{ab}± 0.68	35.07^{b} ± 0.80
Albumin (g/dL)				**LDL (mg/dL)**			
Control	2.56 ± 0.09	2.62^{a} ± 0.12	2.80^{a} ± 0.11	Control	50.87 ± 0.78	46.72^{c} ± 0.64	45.90^{c} ± 0.89
T1	2.57 ± 0.11	3.15^{b} ± 0.16	3.91^{b} ± 0.18	T1	51.19 ± 0.70	44.11^{bc}± 0.69	43.72^{c} ± 0.76
T2	2.56 ± 0.12	3.19^{b} ± 0.14	4.01^{c} ± 0.22	T2	50.76 ± 0.84	46.68^{c} ± 0.75	44.37^{c} ± 0.80
T3	2.52 ± 0.08	3.22^{b} ± 0.16	4.04^{c} ± 0.19	T3	50.93 ± 0.95	42.35^{b} ± 0.81	40.59^{b} ± 0.92
T4	2.53 ± 0.08	3.26^{b} ± 0.19	4.12^{d} ± 0.18	T4	51.35 ± 0.68	38.09^{a} ± 0.90	34.26^{a} ± 0.95
T5	2.56 ± 0.10	3.15^{b} ± 0.16	3.90^{b} ± 0.16	T5	51.28 ± 0.80	42.70^{b} ± 0.66	37.69^{ab}± 0.75
T6	2.54 ± 0.09	3.18^{b} ± 0.17	3.93^{b} ± 0.21	T6	50.81 ± 0.77	41.90^{ab}± 0.73	37.80^{ab}± 0.88
Globulin (g/dL)				**Glucose (mg/dL)**			
Control	1.56 ± 0.08	1.54^{a} ± 0.07	1.48^{a} ± 0.10	Control	212.75 ± 12.51	227.00^{d} ± 8.54	247.00^{d} ± 9.49
T1	1.52 ± 0.11	2.04^{b} ± 0.16	2.22^{b} ± 0.15	T1	214.50 ± 10.04	196.75^{bc}± 4.78	218.25^{ab}± 4.64
T2	1.57 ± 0.08	2.05^{b} ± 0.14	2.27^{b} ± 0.18	T2	212.50 ± 11.39	204.00^{c} ± 6.28	211.75^{a} ± 7.33
T3	1.55 ± 0.09	2.04^{b} ± 0.15	2.29^{b} ± 0.16	T3	212.75 ± 14.41	194.75^{bc}± 5.48	232.75^{c} ± 4.09
T4	1.57 ± 0.06	2.08^{b} ± 0.11	2.29^{b} ± 0.13	T4	212.25 ± 15.29	182.00^{b} ± 3.55	210.75^{a} ± 7.84
T5	1.58 ± 0.10	2.01^{b} ± 0.09	2.19^{b} ± 0.15	T5	212.00 ± 11.69	173.75^{a} ± 4.19	235.00^{c} ± 4.08
T6	1.58 ± 0.12	2.03^{b} ± 0.12	2.18^{b} ± 0.14	T6	211.75 ± 09.20	216.25^{cd}± 5.79	231.25^{c} ± 5.10
A/G ratio (g/dL)				**Creatinine (mg/dL)**			
Control	1.64 ± 0.06	1.70 ± 0.06	1.89 ± 0.10	Control	0.30 ± 0.04	0.25 ± 0.06	0.22 ± 0.04

T1	1.69 ± 0.08	1.54 ± 0.06	1.76 ± 0.12	T1	0.30 ± 0.03	0.23 ± 0.02	0.20 ± 0.02
T2	1.63 ± 0.10	1.56 ± 0.07	1.77 ± 0.09	T2	0.29 ± 0.02	0.23 ± 0.02	0.20 ± 0.04
T3	1.63 ± 0.05	1.58 ± 0.04	1.76 ± 0.11	T3	0.29 ± 0.05	0.21 ± 0.03	0.19 ± 0.02
T4	1.61 ± 0.07	1.57 ± 0.08	1.80 ± 0.08	T4	0.29 ± 0.05	0.22 ± 0.02	0.20 ± 0.02
T5	1.62 ± 0.06	1.57 ± 0.06	1.78 ± 0.09	T5	0.27 ± 0.04	0.23 ± 0.02	0.22 ± 0.03
T6	1.61 ± 0.06	1.57 ± 0.07	1.80 ± 0.10	T6	0.30 ± 0.05	0.24 ± 0.04	0.19 ± 0.02
Cholesterol (mg/dL)				**AST (IU/L)**			
Control	149.25 ± 11.47	149.00^{c} ± 6.36	146.75^{d} ± 6.49	Cont	46.00 ± 7.08	49.00 ± 2.44	60.75^{b} ± 3.47
T1	148.50 ± 10.16	134.00^{b} ± 5.78	129.00^{c} ± 3.89	T1	46.50 ± 5.85	47.00 ± 2.91	49.25^{a} ± 3.01
T2	148.25 ± 09.27	127.75^{ab} ± 3.49	119.25^{bc} ± 2.09	T2	45.50 ± 7.14	45.75 ± 5.06	55.00^{ab} ± 3.26
T3	150.00 ± 11.86	130.25^{ab} ± 4.73	107.25^{b} ± 3.52	T3	45.70 ± 7.26	46.25 ± 3.47	53.00^{ab} ± 3.26
T4	150.50 ± 11.47	122.75^{a} ± 2.65	095.00^{a} ± 2.85	T4	46.00 ± 7.52	42.75 ± 3.30	45.25^{a} ± 1.83
T5	148.50 ± 11.66	126.75^{ab} ± 3.01	110.00^{b} ± 5.93	T5	45.25 ± 7.04	47.75 ± 2.05	53.75^{ab} ± 2.05
T6	149.00 ± 13.47	139.00^{bc} ± 3.44	102.00^{ab} ± 3.08	T6	46.00 ± 6.16	50.00 ± 2.94	55.00^{ab} ± 5.09
Triglyceride (mg/dL)				**ALT (IU/L)**			
Control	124.42 ± 3.22	119.08^{b} ± 2.59	112.50^{b} ± 2.32	Control	16.20 ± 0.95	15.10 ± 1.12	17.35 ± 1.30
T1	123.76 ± 2.91	117.14^{ab} ± 1.79	111.62^{b} ± 1.53	T1	16.00 ± 1.15	14.90 ± 1.04	16.70 ± 0.96
T2	122.08 ± 2.35	115.42^{ab} ± 1.88	109.56^{ab} ± 2.04	T2	17.25 ± 1.40	15.40 ± 0.98	17.00 ± 1.14
T3	122.39 ± 2.89	112.12^{ab} ± 2.45	107.38^{ab} ± 1.47	T3	17.10 ± 1.70	15.20 ± 1.22	17.10 ± 0.91
T4	124.01 ± 1.91	110.31^{a} ± 2.73	103.59^{a} ± 2.08	T4	16.35 ± 0.85	14.70 ± 1.10	16.95 ± 1.35
T5	122.44 ± 1.88	116.68^{ab} ± 2.27	110.22^{b} ± 1.98	T5	16.85 ± 1.10	14.95 ± 1.35	16.83 ± 1.50
T6	122.06 ± 1.96	118.46^{b} ± 2.42	109.61^{ab} ± 1.95	T6	17.20 ± 0.90	15.42 ± 1.07	17.20 ± 1.19

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *S. alba* extract at a concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a column differ significantly ($P<0.05$).

Table 5.4: Effect of *P. armeniaca* extract on blood biochemical parameters of broiler chickens at high altitude (experiment 4)

Groups	0 day	21st day	42nd day	Groups	0 day	21st day	42nd day
Total protein (g/dL)				**HDL (mg/dL)**			
Control	3.86 ± 0.09	4.11^{a} ± 0.15	4.15^{a} ± 0.21	Control	18.26 ± 0.40	21.81 ± 0.44	23.45 ± 0.51
T1	3.87 ± 0.13	4.60^{bc} ± 0.17	5.33^{c} ± 0.19	T1	18.52 ± 0.51	22.27 ± 0.47	24.12 ± 0.49
T2	3.80 ± 0.10	4.54^{b} ± 0.14	5.22^{b} ± 0.17	T2	17.84 ± 0.49	22.90 ± 0.55	23.81 ± 0.60
T3	3.81 ± 0.13	4.76^{d} ± 0.19	5.51^{d} ± 0.20	T3	18.90 ± 0.46	23.04 ± 0.51	25.19 ± 0.58
T4	3.84 ± 0.08	4.64^{c} ± 0.16	5.40^{cd} ± 0.23	T4	17.89 ± 0.42	22.10 ± 0.55	24.33 ± 0.60
T5	3.80 ± 0.05	4.47^{b} ± 0.14	5.16^{b} ± 0.18	T5	18.12 ± 0.50	22.65 ± 0.57	25.00 ± 0.62
T6	3.81 ± 0.07	4.51^{b} ± 0.15	5.28^{bc} ± 0.16	T6	18.55 ± 0.40	22.37 ± 0.38	24.26 ± 0.55
Albumin (g/dL)				**LDL (mg/dL)**			
Control	2.14 ± 0.06	2.17^{a} ± 0.10	2.23^{a} ± 0.15	Control	45.19 ± 0.79	41.32 ± 0.76	40.11 ± 0.84
T1	2.15 ± 0.04	2.61^{c} ± 0.13	3.16^{b} ± 0.21	T1	46.11 ± 0.82	40.16 ± 0.90	39.85 ± 0.82
T2	2.14 ± 0.06	2.53^{bc} ± 0.09	3.14^{b} ± 0.18	T2	45.80 ± 0.85	41.03 ± 0.90	40.10 ± 0.78
T3	2.10 ± 0.08	2.68^{d} ± 0.15	3.21^{b} ± 0.20	T3	46.31 ± 0.91	39.56 ± 0.95	38.83 ± 0.90
T4	2.11 ± 0.10	2.59^{c} ± 0.14	3.20^{b} ± 0.23	T4	45.21 ± 0.90	40.76 ± 0.92	40.21 ± 0.89
T5	2.10 ± 0.08	2.41^{b} ± 0.10	3.12^{b} ± 0.18	T5	46.07 ± 0.87	41.09 ± 0.90	39.74 ± 0.92
T6	2.11 ± 0.11	2.45^{b} ± 0.12	3.17^{b} ± 0.20	T6	45.89 ± 0.91	40.70 ± 0.95	39.45 ± 0.93
Globulin (g/dL)				**Uric acid (mg/dL)**			
Control	1.72 ± 0.08	1.94 ± 0.11	1.92^{a} ± 0.09	Control	5.02 ± 0.14	5.38 ± 0.16	6.18 ± 0.22
T1	1.72 ± 0.08	1.99 ± 0.16	2.17^{c} ± 0.14	T1	5.01 ± 0.18	5.41 ± 0.24	6.18 ± 0.26
T2	1.66 ± 0.06	2.01 ± 0.14	2.08^{b} ± 0.17	T2	5.01 ± 0.17	5.37 ± 0.21	6.15 ± 0.23
T3	1.71 ± 0.09	2.08 ± 0.19	2.30^{d} ± 0.21	T3	5.06 ± 0.11	5.42 ± 0.26	6.17 ± 0.25
T4	1.73 ± 0.10	2.05 ± 0.15	2.20^{c} ± 0.16	T4	5.02 ± 0.16	5.44 ± 0.19	6.10 ± 0.23
T5	1.70 ± 0.06	2.06 ± 0.21	2.04^{b} ± 0.13	T5	5.02 ± 0.14	5.44 ± 0.21	6.12 ± 0.27
T6	1.70 ± 0.06	2.06 ± 0.20	2.11^{bc} ± 0.15	T6	5.01 ± 0.15	5.37 ± 0.22	6.15 ± 0.24
Glucose (mg/dL)				**Creatinine (mg/dL)**			
Control	316.25 ± 7.85	323.25^{c} ± 5.37	308.25^{d} ± 6.68	Control	0.87 ± 0.13	1.25 ± 0.06	1.22 ± 0.12
T1	316.00 ± 6.67	282.25^{a} ± 7.92	257.00^{a} ± 5.95	T1	0.90 ± 0.11	1.27 ± 0.04	1.22 ± 0.06
T2	316.75 ± 9.53	309.00^{b} ± 6.45	293.25^{c} ± 4.78	T2	0.92 ± 0.12	1.22 ± 0.20	1.20 ± 0.05

T3	314.50 ± 7.92	285.25^{a} ± 6.20	261.50^{a} ± 4.29	T3	0.87 ± 0.12	1.22 ± 0.07	1.18 ± 0.07
T4	314.25 ± 9.62	310.75^{b} ± 5.97	289.00^{c} ± 9.85	T4	0.95 ± 0.09	1.22 ± 0.16	1.32 ± 0.12
T5	318.75 ± 5.54	313.00^{b} ± 4.60	301.50^{cd} ± 7.59	T5	0.90 ± 0.09	1.20 ± 0.04	1.22 ± 0.04
T6	315.50 ± 7.59	289.40^{a} ± 6.70	272.75^{b} ± 4.87	T6	0.92 ± 0.13	1.21 ± 0.05	1.30 ± 0.10
Cholesterol (mg/dL)				**AST (IU/L)**			
Control	184.50 ± 10.50	193.00^{c} ± 06.09	189.75^{c} ± 10.54	Control	98.50 ± 2.10	89.75^{d} ± 1.31	84.00^{c} ± 3.24
T1	184.75 ± 09.19	175.25^{ab} ± 05.55	159.25^{b} ± 04.84	T1	97.25 ± 3.19	68.50^{b} ± 3.40	56.25^{a} ± 2.32
T2	185.25 ± 11.03	174.75^{ab} ± 13.13	153.25^{ab} ± 04.58	T2	97.00 ± 3.89	64.00^{b} ± 2.16	57.75^{a} ± 2.56
T3	186.50 ± 09.57	166.75^{a} ± 05.46	147.00^{a} ± 07.22	T3	97.25 ± 1.65	57.00^{a} ± 1.82	54.75^{a} ± 1.49
T4	184.25 ± 11.50	175.25^{ab} ± 15.63	166.25^{b} ± 05.02	T4	98.25 ± 0.85	72.75^{bc} ± 2.92	57.25^{a} ± 1.93
T5	186.25 ± 10.75	173.00^{ab} ± 15.86	168.75^{b} ± 04.05	T5	98.50 ± 2.95	79.75^{c} ± 1.49	68.75^{b} ± 1.79
T6	185.75 ± 10.15	179.75^{b} ± 10.11	169.25^{b} ± 04.78	T6	98.00 ± 1.29	68.00^{b} ± 3.58	61.00^{a} ± 1.47
Triglyceride (mg/dL)				**ALT (IU/L)**			
Control	124.50 ± 2.50	126.50^{b} ± 2.21	121.50^{b} ± 1.25	Control	23.50 ± 2.10	19.25^{b} ± 1.49	18.50^{c} ± 1.32
T1	125.75 ± 3.27	123.75^{ab} ± 2.46	116.25^{ab} ± 2.17	T1	22.50 ± 2.10	18.50^{b} ± 1.19	14.50^{b} ± 1.75
T2	124.25 ± 3.27	124.50^{ab} ± 3.12	120.75^{b} ± 1.25	T2	23.75 ± 1.65	16.25^{ab} ± 1.31	12.75^{b} ± 1.31
T3	123.50 ± 2.59	118.25^{a} ± 1.03	111.50^{a} ± 1.84	T3	23.00 ± 2.79	13.75^{a} ± 1.37	08.50^{a} ± 0.64
T4	124.75 ± 1.10	122.75^{ab} ± 1.25	115.75^{ab} ± 2.32	T4	22.50 ± 1.32	17.25^{ab} ± 1.10	14.75^{b} ± 1.31
T5	123.25 ± 1.25	122.25^{ab} ± 1.43	120.00^{b} ± 1.47	T5	22.75 ± 1.97	19.00^{b} ± 1.29	13.75^{b} ± 1.32
T6	124.50 ± 2.50	126.50^{b} ± 2.21	121.50^{b} ± 1.25	T6	23.00 ± 0.91	16.75^{ab} ± 1.25	12.50^{b} ± 0.95

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *P. armeniaca* extract at a concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Mean bearing the different superscript $^{(a, b, c, d)}$ in column differ significantly ($P<0.05$).

5.3.1.4 Blood biochemical profile in experimental trial 4

In experiment 4, we observed a significant increase in plasma total protein and albumin concentration in treatment group birds as compared to control group birds with the supplementation of *P. armeniaca* extract (Table 5.4). Within treatment groups, birds in T3 group represented the highest level of total protein. We did not observe any difference in the level of globulin among the experimental groups at 21 day whereas at 42 day globulin level was increased in all treatment groups as compared with control group. The level of plasma cholesterol was significantly reduced in treatment groups as compared to control group at 21 and 42 day. Within the treatment groups, lowest cholesterol level was observed in T3 group birds (Table 5.4).

In addition to this, we observed a significant decrease in triglyceride level in T3 group birds as compared with control and remaining treatment groups. AST and ALT level was significantly reduced in treatment group birds as compared with control group at 21 and 42 day. Within treatment groups, lowest ALT concentration was recorded in T3 group. Furthermore, we did not observe any differences in the concentration of HDL, LDL, creatinine and uric acid within the experimental groups (Table 5.4).

5.3.1.5 Antioxidant status in chicken plasma in experimental trial 1

In experiment 1, we observed a significant increase in TAC in chicken plasma samples that were supplemented with *R. imbricata* extract compared with the control group at both 21 and 42 day (Table 5.5). Among the treatment groups, birds in the T5 group represent the highest TAC throughout the experiment. Moreover, free radical scavenging activity in treatment group birds was increased as compared to the birds in the control group at both 21 and 42 day. Within the treatment groups, birds in the T5 group represent the highest free radical scavenging activity. LPO status was estimated by determining MDA concentration in plasma samples and it was significantly decreased in T5 group as compared to control group at 21 day. However, there was no difference observed in MDA level in between control and remaining treatment groups at 21 day. In addition to this, we observed a significant decrease in MDA concentration in treatment groups as compared with control group at 42 day (Table 5.5).

Table 5.5: Effect of *R. imbricata* on oxidative stress parameters like MDA, TAC, and DPPH radical-scavenging activity in broiler chickens (experiment 1)

Groups	0 day	21st day	42nd day
MDA(nmol/mL)			
Control	8.55 ± 0.33	7.98^{b} ± 0.22	7.76^{c} ± 0.12
T1	8.62 ± 0.34	7.69^{ab} ± 0.22	6.32^{b} ± 0.16
T2	8.50 ± 0.17	7.91^{b} ± 0.18	6.39^{b} ± 0.09
T3	8.44 ± 0.24	7.69^{ab} ± 0.15	6.41^{b} ± 0.07
T4	8.51 ± 0.23	7.77^{ab} ± 0.19	6.62^{b} ± 0.11
T5	8.45 ± 0.20	7.46^{a} ± 0.22	5.67^{a} ± 0.13
T6	8.60 ± 0.13	7.73^{ab} ± 0.26	6.67^{b} ± 0.12
TAC (µmol/L)			
Control	572.25 ± 23.94	676.00^{a} ± 29.53	629.50^{a} ± 33.10
T1	568.00 ± 22.58	761.50^{b} ± 44.52	781.00^{b} ± 48.88
T2	569.00 ± 20.29	691.00^{ab} ± 42.45	778.00^{b} ± 50.00
T3	569.25 ± 22.45	691.75^{ab} ± 35.96	765.00^{b} ± 43.77
T4	570.50 ± 23.48	773.00^{b} ± 32.78	791.00^{b} ± 42.68
T5	571.00 ± 20.12	808.25^{c} ± 38.60	856.00^{c} ± 46.58
T6	570.00 ± 23.46	780.50^{b} ± 37.95	784.00^{b} ± 48.38
DPPH radical-scavenging activity (%)			
Control	42.32 ± 2.55	45.06^{a} ± 1.82	43.70^{a} ± 2.21
T1	42.08 ± 3.21	51.35^{b} ± 2.48	58.64^{b} ± 2.10
T2	42.70 ± 3.45	50.61^{ab} ± 3.30	58.20^{b} ± 2.93
T3	42.77 ± 3.88	53.80^{b} ± 3.10	56.13^{b} ± 1.66
T4	42.83 ± 2.57	55.74^{b} ± 2.80	56.08^{b} ± 2.65
T5	41.80 ± 2.36	57.25^{b} ± 2.71	65.23^{c} ± 1.90
T6	41.99 ± 2.59	56.09^{b} ± 3.28	57.08^{b} ± 2.70

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *R. imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c}$) in a columns differ significantly ($P<0.05$).

5.3.1.6 Antioxidant status in chicken plasma in experimental trial 2

In experimental trial 2, we observed a significant increase in TAC in treatment groups that were supplemented with *H. rhamnoides* extract compared with the control group at both 21 and 42 day (Table 5.6). Birds in the T3 group represent the highest TAC throughout the experiment. Moreover, free radical scavenging activity in treatment group birds was increased as compared to the birds in the control group at both 21 and 42 day. Within the treatment groups, birds in the T3 group represent the highest free radical scavenging activity. Furthermore, the concentration of MDA was decreased in treatment groups as compared with control group and lowest MDA concentration was observed in T3 group birds at 42 day (Table 5.6).

Table 5.6: Effect of *H. rhamnoides* on oxidative stress parameters like MDA, TAC, and DPPH radical-scavenging activity in broiler chickens (experiment 2)

Groups	0 day	21st day	42nd day
MDA (nmol/mL)			
Control	8.61 ± 0.58	8.31^{c} ± 0.30	8.06^{c} ± 0.18
T1	8.58 ± 0.68	6.05^{a} ± 0.26	5.81^{b} ± 0.12
T2	8.59 ± 0.64	6.32^{a} ± 0.24	5.67^{b} ± 0.14
T3	8.61 ± 0.67	5.91^{a} ± 0.13	4.04^{a} ± 0.32
T4	8.63 ± 0.63	6.47^{ab} ± 0.16	5.49^{b} ± 0.18
T5	8.59 ± 0.70	6.94^{b} ± 0.14	5.47^{b} ± 0.13
T6	8.97 ± 0.48	6.93^{b} ± 0.16	5.50^{b} ± 0.08
TAC (µmol/L)			
Control	1122.43 ± 12.11	1139.66^{a} ± 17.60	1186.32^{a} ± 17.35
T1	1120.59 ± 10.15	1328.63^{d} ± 17.18	1547.37^{b} ± 18.34
T2	1121.31 ± 07.20	1339.49^{d} ± 18.08	1665.58^{c} ± 18.80
T3	1119.32 ± 07.47	1414.76^{e} ± 20.30	1698.53^{d} ± 20.99
T4	1118.33 ± 08.89	1254.24^{c} ± 15.79	1613.26^{c} ± 20.71
T5	1123.83 ± 06.35	1266.81^{c} ± 16.50	1649.61^{c} ± 20.29
T6	1119.82 ± 05.62	1213.81^{b} ± 18.58	1605.79^{b} ± 20.25
DPPH radical-scavenging activity (%)			
Control	41.24 ± 0.83	42.17^{a} ± 2.01	44.88^{a} ±1.99
T1	41.77 ± 0.80	51.59^{b} ± 1.61	62.83^{c} ± 3.25
T2	41.11 ± 0.57	52.29^{b} ± 0.96	62.17^{bc}±0.90
T3	41.60 ± 0.98	57.44^{c} ± 0.80	65.37^{c} ± 1.74
T4	42.02 ± 0.74	50.24^{b} ± 0.87	60.55^{bc}± 2.39
T5	41.29 ± 1.03	51.38^{b} ± 0.81	58.91^{b} ± 2.46
T6	41.34 ± 0.69	49.07^{b} ± 2.29	55.78^{b} ± 0.80

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *H. rhamnoides* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a columns differ significantly ($P<0.05$).

5.3.1.7 Antioxidant status in chicken plasma in experimental trial 3

During experimental trial 3, TAC in chicken plasma samples was recorded significantly higher in all the treatments that were supplemented with *S. alba* extract as compared with the control group at both 21 and 42 day. Birds in the T4 group represent the highest TAC throughout the experiment (Table 5.7). Free radical scavenging activity in treatment group birds was increased as compared to the control group birds at 21 day. In addition to this, we observed a significant increase in free radical scavenging activity in T4 group birds at 42 day. The concentration of MDA was decreased in treatment groups as compared with control group and lowest MDA level was observed in T4 group throughout the experiment (Table 5.7).

Table 5.7: Effect of *S. alba* on oxidative stress parameters like MDA, TAC, and DPPH radical-scavenging activity in broiler chickens (experiment 3)

Groups	0 day	21st day	42nd day
MDA (nmol/mL)			
Control	7.44 ± 0.32	7.36^{d} ± 0.23	7.38^{e} ± 0.26
T1	7.48 ± 0.20	5.92^{c} ± 0.19	6.33^{d} ± 0.21
T2	7.51 ± 0.21	5.74^{c} ± 0.16	6.03^{c} ± 0.19
T3	7.48 ± 0.26	4.95^{b} ± 0.17	5.89^{b} ± 0.16
T4	7.50 ± 0.29	4.36^{a} ± 0.22	5.40^{a} ± 0.17
T5	7.53 ± 0.34	5.79^{c} ± 0.18	6.14^{c} ± 0.21
T6	7.46 ± 0.30	6.05^{c} ± 0.20	6.54^{d} ± 0.22
TAC (µmol/L)			
Control	1113.76 ± 07.55	1132.81^{a} ± 08.27	1124.59^{a} ± 10.24
T1	1115.56 ± 07.72	1498.68^{c} ± 10.64	1449.08^{c} ± 17.57
T2	1113.27 ± 08.38	1522.73^{c} ± 10.23	1476.38^{c} ± 17.44
T3	1114.08 ± 09.51	1593.80^{d} ± 09.46	1531.12^{d} ± 11.45
T4	1114.26 ± 07.98	1646.77^{e} ± 11.42	1592.06^{e} ± 15.66
T5	1112.01 ± 09.74	1516.50^{c} ± 10.90	1412.72^{b} ± 11.56
T6	1115.68 ± 09.17	1440.23^{b} ± 08.43	1390.49^{b} ± 09.96
DPPH radical-scavenging activity (%)			
Control	43.08 ± 2.52	45.81^{a} ± 2.41	45.26^{a} ± 1.33
T1	42.46 ± 2.09	51.73^{b} ± 1.65	49.06^{a} ± 1.47
T2	42.97 ± 2.51	52.92^{b} ± 2.00	49.27^{a} ± 1.44
T3	42.87 ± 2.38	58.15^{c} ± 2.17	56.44^{b} ± 1.43
T4	42.83 ± 1.85	64.17^{d} ± 2.59	60.61^{c} ± 1.13
T5	42.01 ± 1.17	51.44^{b} ± 1.92	48.44^{a} ± 1.14
T6	42.92 ± 1.05	50.14^{b} ± 1.63	47.78^{a} ± 1.07

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *S. alba* extract at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c, d, e}$) in a columns differ significantly ($P<0.05$).

5.3.1.8 Antioxidant status in chicken plasma in experimental trial 4

In experimental trial 4, we observed a significant increase in TAC in treatment groups that were supplemented with *P. armeniaca* extract compared with the control group at both 21 and 42 day (Table 5.8). Birds in the T3 group represent the maximum TAC throughout the experiment. Free radical scavenging activity in treatment group birds was increased as compared with control group birds at 21 and 42 day. Within the treatment groups, birds in the T3 group represent the highest free radical scavenging activity. MDA concentration was decreased in treatment groups as compared with control group and lowest MDA level was observed in T3 group throughout the experiment (Table 5.8).

Table 5.8: Effect of *P. armeniaca* on oxidative stress parameters like MDA, TAC, and DPPH radical-scavenging activity in broiler chickens (experiment 4)

Groups	0 day	21st day	42nd day
MDA (nmol/mL)			
Control	8.41 ± 0.29	8.06^{d} ± 0.18	8.13^{e} ± 0.19
T1	8.38 ± 0.31	6.47^{b} ± 0.24	4.43^{b} ± 0.17
T2	8.48 ± 0.36	6.60^{b} ± 0.21	5.13^{c} ± 0.16
T3	8.37 ± 0.27	6.07^{a} ± 0.15	4.08^{a} ± 0.18
T4	8.46 ± 0.40	6.84^{c} ± 0.19	4.52^{b} ± 0.08
T5	8.51 ± 0.40	7.02^{c} ± 0.23	5.16^{c} ± 0.09
T6	8.43 ± 0.33	6.90^{c} ± 0.18	5.65^{d} ± 0.15
TAC (µmol/L)			
Control	1098.26 ± 11.21	1189.04^{a} ± 14.63	1167.57^{a} ± 16.87
T1	1098.53 ± 10.06	1313.78^{c} ± 17.48	1686.24^{c} ± 17.61
T2	1097.33 ± 07.78	1392.45^{d} ± 17.36	1487.58^{b} ± 18.24
T3	1098.24 ± 07.92	1496.98^{e} ± 15.47	1785.26^{e} ± 18.08
T4	1096.34 ± 07.56	1380.01^{d} ± 14.63	1741.18^{d} ± 15.86
T5	1096.26 ± 07.60	1300.53^{c} ± 19.58	1668.17^{c} ± 17.29
T6	1097.31 ± 09.94	1224.30^{b} ± 20.03	1474.65^{b} ± 15.83
DPPH radical-scavenging activity (%)			
Control	41.92 ± 0.21	43.14^{a} ± 1.37	42.87^{a} ± 2.11
T1	41.52 ± 0.40	55.94^{c} ± 0.87	64.30^{c} ± 1.87
T2	41.65 ± 0.47	49.26^{b} ± 0.85	63.03^{c} ± 1.41
T3	41.96 ± 0.27	59.61^{d} ± 0.78	66.13^{d} ± 0.63
T4	41.43 ± 0.48	56.67^{c} ± 2.43	61.94^{bc} ± 0.82
T5	41.91 ± 0.12	52.48^{bc} ± 1.23	58.86^{b} ± 2.93
T6	42.03 ± 0.16	48.13^{b} ± 1.03	57.16^{b} ± 1.82

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *P. armeniaca* extract at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c, d, e}$) in a columns differ significantly ($P<0.05$).

5.3.1.9 Cytokine level in chicken plasma in experimental trial 1

In experiment 1, the concentration of IL-1 did not vary among the experimental groups, whereas a significant increase in IL-2 level was observed in T3, T4, T5 and T6 groups as compared with control, T1 and T2 group (Table 5.9). The concentration of inflammatory cytokine IL-6 was decreased in T3, T4, T5, and T6 groups as compared to control T1, and T2 group at 21 and 42 days. Within the treatment groups, the numerically lowest IL-6 level was observed in T5 group (Table 5.9).

Table 5.9: Effect of *R. imbricata* on different inflammatory cytokines (IL-1, IL-2, and IL-6) level in broiler chickens (experiment 1)

Groups	0 day	21st day	42nd day
IL-1 (pg/mL)			
Control	5.21 ± 0.22	5.23 ± 0.19	5.22 ± 0.22
T1	5.20 ± 0.17	5.24 ± 0.24	5.27 ± 0.31
T2	5.23 ± 0.30	5.24 ± 0.26	5.27 ± 0.26
T3	5.21 ± 0.29	5.26 ± 0.35	5.29 ± 0.38
T4	5.22 ± 0.21	5.26 ± 0.30	5.31 ± 0.28
T5	5.20 ± 0.24	5.29 ± 0.27	5.30 ± 0.23
T6	5.21 ± 0.24	5.25 ± 0.21	5.29 ± 0.31
IL-2 (pg/mL)			
Control	8.72 ± 0.37	9.04^{a} ± 0.43	9.26^{a} ± 0.48
T1	8.71 ± 0.42	9.23^{a} ± 0.55	9.39^{a} ± 0.52
T2	8.72 ± 0.34	9.19^{a} ± 0.40	9.27^{a} ± 0.41
T3	8.70 ± 0.29	10.30^{b} ± 0.53	10.32^{c} ± 0.57
T4	8.69 ± 0.33	10.26^{b} ± 0.47	10.42^{c} ± 0.43
T5	8.72 ± 0.40	10.58^{c} ± 0.51	10.60^{d} ± 0.55
T6	8.70 ± 0.37	10.14^{b} ± 0.54	9.92^{b} ± 0.49
IL-6 (pg/mL)			
Control	7.08 ± 0.43	7.27^{b} ± 0.34	7.11^{b} ± 0.27
T1	7.11 ± 0.52	7.30^{b} ± 0.41	7.09^{b} ± 0.35
T2	7.10 ± 0.42	7.27^{b} ± 0.44	7.04^{b} ± 0.29
T3	7.07 ± 0.36	7.00^{a} ± 0.47	6.81^{a} ± 0.38
T4	7.08 ± 0.39	7.01^{a} ± 0.29	6.84^{a} ± 0.32
T5	7.09 ± 0.49	6.98^{a} ± 0.35	6.77^{a} ± 0.40
T6	7.08 ± 0.50	7.04^{a} ± 0.46	6.81^{a} ± 0.39

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received *R. imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a columns differ significantly ($P<0.05$).

5.3.1.10 Cytokine level in chicken plasma in experimental trial 2

In experiment 2, the level of IL-1 did not vary among the experimental groups at 21 and 42 day of experiment whereas the level of IL-2 was increased in the treatment groups as compared with control group (Table 5.10). Within treatment groups, birds in T3 and T4 groups represent the highest IL-2 level. Furthermore, the concentration of IL-6 was recorded significantly lower in all the treatment group birds as compared to control group. Birds in T3 and T4 group represented the lowest IL-6 level (Table 5.10).

Table 5.10: Effect of *H. rhamnoides* on different inflammatory cytokines (IL-1, IL-2, and IL-6) level in broiler chickens (experiment 2)

Groups	0 day	21st day	42nd day
IL-1 (pg/mL)			
Control	5.95 ± 0.23	6.17 ± 0.25	6.39 ± 0.34
T1	5.95 ± 0.27	6.16 ± 0.18	6.37 ± 0.41
T2	5.94 ± 0.31	6.24 ± 0.26	6.33 ± 0.28
T3	5.94 ± 0.24	6.22 ± 0.21	6.28 ± 0.33
T4	5.95 ± 0.26	6.19 ± 0.27	6.26 ± 0.26
T5	5.91 ± 0.22	6.24 ± 0.26	6.27 ± 0.30
T6	5.93 ± 0.31	6.21 ± 0.34	6.29 ± 0.40
IL-2 (pg/mL)			
Control	7.47 ± 0.26	8.20^{a} ± 0.33	8.33^{a} ± 0.38
T1	7.45 ± 0.30	8.55^{b} ± 0.36	8.66^{c} ± 0.43
T2	7.47 ± 0.36	8.63^{b} ± 0.41	8.51^{b} ± 0.49
T3	7.48 ± 0.31	8.77^{c} ± 0.37	8.79^{d} ± 0.55
T4	7.51 ± 0.35	8.82^{c} ± 0.40	8.84^{d} ± 0.51
T5	7.50 ± 0.39	8.61^{b} ± 0.41	8.69^{c} ± 0.47
T6	7.48 ± 0.26	8.69^{bc} ± 0.34	8.71^{c} ± 0.46
IL-6 (pg/mL)			
Control	7.63 ± 0.44	7.71^{c} ± 0.47	7.77^{c} ± 0.50
T1	7.61 ± 0.33	7.59^{b} ± 0.40	7.48^{b} ± 0.43
T2	7.62 ± 0.42	7.57^{b} ± 0.35	7.46^{b} ± 0.31
T3	7.63 ± 0.39	7.44^{a} ± 0.52	7.31^{a} ± 0.47
T4	7.60 ± 0.51	7.47^{a} ± 0.38	7.33^{a} ± 0.44
T5	7.62 ± 0.44	7.60^{b} ± 0.46	7.50^{b} ± 0.50
T6	7.61 ± 0.36	7.49^{a} ± 0.40	7.47^{b} ± 0.40

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received aqueous extract of *H. rhamnoides* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a columns differ significantly ($P<0.05$).

5.3.1.11 Cytokine level in chicken plasma in experimental trial 3

In experiment 3, we observed a significant decrease in the concentration of IL-1 in T4 group birds as compared to control and remaining experimental groups (Table 5.11). The level of IL-2 was recorded significantly higher in a T4 group within the experimental groups, whereas no difference was observed in control, T1, and T2 group. Furthermore, birds in T3, T4 and T5 groups had a lower level of IL-6 as compared with control at 21 day whereas, at 42 day the level of IL-6 was reduced in all treatment group birds as compared with control group. Birds in T4 group represented the lowest IL-6 level (Table 5.11).

Table 5.11: Effect of *S. alba* on different inflammatory cytokines (IL-1, IL-2, and IL-6) level in broiler chickens (experiment 3)

Groups	0 day	21st day	42nd day
IL-1 (pg/mL)			
Control	6.21 ± 0.23	6.34^{b} ± 0.28	6.46^{c} ± 0.36
T1	6.23 ± 0.29	6.32^{b} ± 0.31	6.43^{c} ± 0.33
T2	6.22 ± 0.18	6.28^{b} ± 0.28	6.55^{c} ± 0.42
T3	6.21 ± 0.31	6.29^{b}± 0.40	6.34^{b} ± 0.31
T4	6.24 ± 0.25	6.20^{a} ± 0.35	6.24^{a} ± 0.44
T5	6.20 ± 0.23	6.30^{b} ± 0.33	6.35^{b} ± 0.37
T6	6.20 ± 0.22	6.33^{b} ± 0.27	6.51^{c} ± 0.30
IL-2 (pg/mL)			
Control	8.40 ± 0.39	8.44^{a} ± 0.45	8.51^{a} ± 0.54
T1	8.43 ± 0.32	8.49^{a} ± 0.37	8.55^{a} ± 0.57
T2	8.39 ± 0.28	8.46^{a} ± 0.30	8.55^{a} ± 0.49
T3	8.38 ± 0.26	8.81^{b} ± 0.49	9.16^{c} ± 0.55
T4	8.41 ± 0.37	8.94^{c} ± 0.53	9.22^{d} ± 0.59
T5	8.40 ± 0.30	8.84^{b} ± 0.48	9.08^{c} ± 0.60
T6	8.42 ± 0.35	8.78^{b} ± 0.38	8.93^{b} ± 0.50
IL-6 (pg/mL)			
Control	8.14 ± 0.40	8.19^{c} ± 0.36	8.26^{d} ± 0.48
T1	8.11 ± 0.44	8.15^{c} ± 0.28	8.04^{c} ± 0.46
T2	8.15 ± 0.35	8.14^{c} ± 0.39	8.07^{c} ± 0.38
T3	8.13 ± 0.47	8.01^{a} ± 0.37	7.98^{b} ± 0.41
T4	8.11 ± 0.40	7.97^{a} ± 0.40	7.89^{a} ± 0.44
T5	8.14 ± 0.38	8.09^{b} ± 0.29	8.00^{b} ± 0.32
T6	8.10 ± 0.42	8.15^{c} ± 0.36	8.10^{c} ± 0.38

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received aqueous extract of *S. alba* leaves at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a columns differ significantly ($P<0.05$).

5.3.1.12 Cytokine level in chicken plasma in experimental trial 4

In experiment 4, the concentration of IL-1 did not vary among the experimental groups, whereas a significant increase in IL-2 level was observed in treatment groups that were supplemented with *P. armeniaca* extract as compared with control (table 5.12). Within the treatment groups birds in T3 and T4 groups represent the highest IL-2 level. Furthermore, the concentration of IL-6 was significantly decreased in the treatment group birds as compared to control group (Table 5.12).

Table 5.12: Effect of *P. armeniaca* on different inflammatory cytokines (IL-1, IL-2, and IL-6) level in broiler chickens (experiment 4)

Groups	0 day	21st day	42nd day
IL-1 (pg/mL)			
Control	5.45 ± 0.33	5.48 ± 0.30	5.51 ± 0.34
T1	5.43 ± 0.27	5.46 ± 0.41	5.49 ± 0.38
T2	5.45 ± 0.21	5.48 ± 0.44	5.50 ± 0.49
T3	5.46 ± 0.30	5.45 ± 0.35	5.47 ± 0.42
T4	5.44 ± 0.36	5.43 ± 0.40	5.46 ± 0.50
T5	5.45 ± 0.28	5.47 ± 0.43	5.49 ± 0.46
T6	5.43 ± 0.33	5.45 ± 0.31	5.48 ± 0.40
IL-2 (pg/mL)			
Control	8.56 ± 0.44	8.59^{a} ± 0.51	8.60^{a} ± 0.47
T1	8.55 ± 0.49	8.80^{b} ± 0.56	9.15^{b} ± 0.56
T2	8.54 ± 0.38	8.80^{b} ± 0.50	9.12^{b} ± 0.60
T3	8.57 ± 0.45	8.93^{c} ± 0.62	9.37^{c} ± 0.65
T4	8.56 ± 0.44	8.90^{c} ± 0.56	9.36^{c} ± 0.58
T5	8.58 ± 0.33	8.82^{b} ± 0.42	9.10^{b} ± 0.60
T6	8.55 ± 0.36	8.83^{b} ± 0.50	9.15^{b} ± 0.50
IL-6 (pg/mL)			
Control	8.47 ± 0.24	8.56^{b} ± 0.45	8.61^{b} ± 0.40
T1	8.49 ± 0.32	8.44^{a} ± 0.26	8.39^{a} ± 0.37
T2	8.47 ± 0.26	8.45^{a} ± 0.30	8.39^{a} ± 0.26
T3	8.45 ± 0.20	8.41^{a} ± 0.28	8.35^{a} ± 0.41
T4	8.46 ± 0.36	8.44^{a} ± 0.23	8.37^{a} ± 0.30
T5	8.50 ± 0.38	8.45^{a} ± 0.37	8.37^{a} ± 0.44
T6	8.46 ± 0.38	8.43^{a} ± 0.40	8.34^{a} ± 0.30

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received aqueous extract of *P. armeniaca* in drinking water at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively.

Means bearing the different superscripts ($^{a, b, c}$) in a columns differ significantly ($P<0.05$).

5.3.2 Growth performance, survivability and economic of broiler rearing

5.3.2.1 Growth performance of broilers in experimental trial 1

The effect of a hydro-alcoholic extract of *R. imbricata* on growth performance of broiler chickens is shown in Table 5.13. Birds in the T5 group had significantly higher body weight as compared to control, T1, T2, and T6 groups at 21 day. Moreover, at 42 day we observed a significantly higher body weight in T1, T2, T3, T4, and T5 groups as compared with the control group and birds in the T5 group represented the highest body weight followed by T3, T4, T2, T1, and T6. Furthermore, throughout the experiment cumulative feed and water intake did not differ among the experimental groups. FCR value in the T5 group was found to be significantly improved among the experimental groups.

5.3.2.2 Economics and mortality in broilers during experimental trial 1

Highest mortality rate (13.33%, total 2 birds out of 15) was observed in the control group (Table 5.13) birds followed by T1, T3, and T5 (6.67%, total 1 out of 15). Interestingly no mortality was recorded in T2, T4, and T6 groups during the whole experiment. Post-mortem examination of control group birds showed one mortality induced from ascites and one from coccidiosis whereas single mortality that was observed in T1 group was induced from ascites. We also estimated the economy of the experiment based on the rearing cost of 15 no of birds in each group. Extra price of the extract was added to feed cost whereas other expenses stayed constant. *R. imbricata* decreased the mortality rate in birds and which subsequently increased net return (Table 5.13).

5.3.2.3 Growth performance of broilers in experimental trial 2

In experimental trial 2, birds in T3 group had significantly higher body weight as compared with control and other treatment groups at 21 days (Table 5.14). Whereas, no differences were observed in body weight among the control and other treatment groups at 21 days. Further, at 42 day, we observed a significantly higher body weight in all treatment groups as compared with the control group and within treatment groups birds in the T3 group represented the highest body weight followed by T1, T5, T2, T4, and T6 group. Throughout the experiment cumulative feed and water intake did not differ among the experimental groups. FCR value in the T3 group was found to be significantly improved among the experimental groups (Table 5.14).

5.3.2.4 Economics and mortality in broilers during experimental trial 2

Highest mortality rate (26.67%, total 4 birds out of 15) was observed in the control group (Table 5.14) birds followed by T1, T2, T4, T5 and T6 (13.30%, total 2 out of 15), followed by T3 (6.67%, total 1 out of 15). Post-mortem examination showed 13.30%, 6.67%, 6.67%, 0%, 0%, 0%, and 6.67% mortality in birds induced from ascites and 6.67%, 0%, 6.67%, 0%, 0%, 6.67%, and 0% mortality induced from coccidiosis in control, T1, T2, T3, T4, T5, and T6 groups, respectively.

We also estimated the economy of the experiment based on the rearing cost of 15 no of birds in each group. Extra price of the extract was added to feed cost whereas other expenses stayed constant. *H. rhamnoides* decreased the mortality rate in birds and which subsequently increased net return (Table 5.14).

Table 5.13: Effect of *R. imbricata* on growth performance, survivability of broilers chickens, and cost economics of their rearing at high altitude (experiment 1)

Description	Control	T1	T2	T3	T4	T5	T6
Initial average body weight (gm/chick)	36.40 ± 0.42	36.06 ± 0.55	35.66 ± 0.39	36.86 ± 0.33	36.06 ± 0.45	35.93 ± 0.38	36.53 ± 0.36
Average weight at 21 day (gm/chick)	193.33^{a} ± 4.30	210.93^{b} ± 4.58	210.00^{b} ± 4.22	214.00^{bc} ± 3.83	216.66^{bc} ± 3.63	225.20^{c} ± 4.49	190.46^{a} ± 3.03
Average weight at 42 day (gm/chick)	366.28^{a} ± 6.44	384.26^{b} ± 5.20	408.80^{c} ± 5.45	420.42^{c} ± 6.29	420.26^{c} ± 5.61	461.71^{d} ± 7.80	382.57^{ab} ± 4.93
Cumulative feed intake up to 42 day (gm/chick)	1525.61 ± 6.90	1521.87 ± 7.26	1518.37 ± 5.79	1520.01 ± 6.94	1520.20 ± 6.51	1517.83 ± 5.87	1518.12 ± 6.98
Feed conversion ratio at 42 day	4.62^{d} ± 0.03	4.37^{c} ± 0.06	4.07^{b} ± 0.03	3.96^{b} ± 0.06	3.96^{b} ± 0.04	3.56^{a} ± 0.05	4.39^{c} ± 0.07
Cumulative water intake up to 42 day (mL/chick)	2235.46 ± 15.17	2230.36 ± 14.19	2245.80 ± 20.49	2240.01 ± 18.58	2250.44 ± 19.24	2255.36 ± 16.84	2235.11 ± 13.96
Total mortality (%)	13.33	6.67	0.00	6.67	0.00	6.67	0.00
Mortality by ascites (%)	6.67	6.67	0.00	0.00	0.00	0.00	0.00
Mortality by coccidiosis (%)	6.67	0.00	0.00	0.00	0.00	0.00	0.00
Mortality by other reasons (%)	0.00	0.00	0.00	6.67	0.00	6.67	0.00
Cost of extract/bird (Rs.)	Nil	0.74	1.37	1.79	2.71	3.87	6.67
Cost of feed/bird (@25/Kg Rs.)	38.14	38.04	37.96	38.00	38.00	37.95	37.95
Total feed cost/bird (Rs.)	38.14	38.78	39.33	39.79	40.71	41.82	44.62
Sale of bird at 42 day (@Rs. 200/Kg live weight)*	73.25	76.85	81.76	84.04	84.05	92.34	76.51
Loss due to mortality (Rs.)#	146.50	76.85	0.00	84.04	0.00	92.34	0.00
Total benefit per group (Rs.)##	-	69.65	146.50	62.46	146.50	54.16	146.50

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received hydro-ethanolic extract of *R. imbricata* in drinking water at concentration of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a row differ significantly ($P<0.05$).

* Due to limited availability of fresh chickens at high altitude the rates are very high.

Loss due to mortality = Sale cost per bird × total mortality

Total benefit per group = Loss from mortality in control – loss from mortality in treatment.

Table 5.14: Effect of *H. rhamnoides* on growth performance, survivability of broilers chickens, and cost economics of their rearing at high altitude (experiment 2)

Description	Control	T1	T2	T3	T4	T5	T6
Initial average body weight (gm/chick)	38.86 ± 0.60	39.60 ± 0.58	39.93 ± 0.57	38.20 ± 0.52	38.53 ± 0.58	38.20 ± 0.51	39.33 ± 0.54
Average weight at 21 day (gm/chick)	192.80^{a} ± 3.50	194.26^{a} ± 1.97	192.46 ± 3.45	222.13^{b} ± 5.26	190.26^{a} ± 3.82	189.20^{a} ± 5.99	191.06^{a} ± 8.41
Average weight at 42 day (gm/chick)	356.75^{a} ± 10.06	410.70^{b} ± 10.38	402.53^{b} ± 12.32	470.33^{c} ± 12.68	394.26^{b} ± 6.69	409.20^{b} ± 6.78	392.14^{b} ± 5.55
Cumulative feed intake up to 42 day (gm/chick)	1519.20 ± 9.65	1521.08 ± 9.54	1520.37 ± 8.70	1530.27 ± 14.59	1544.58 ± 16.48	1518.62 ± 11.63	1527.50 ± 11.39
Feed conversion ratio at 42 day	4.78^{d} ± 0.08	4.10^{b} ± 0.06	4.19^{bc} ± 0.02	3.54^{a} ± 0.08	4.34^{c} ± 0.06	4.09^{b} ± 0.05	4.33^{c} ± 0.04
Cumulative water intake up to 42 day (mL/chick)	2138.76 ± 17.37	2140.71 ± 11.67	2145.69 ± 12.92	2140.39 ± 11.39	2155.02 ± 16.36	2150.71 ± 11.86	2155.43 ± 11.96
Total mortality (%)	26.67	13.30	13.30	6.67	13.30	13.30	13.30
Mortality by ascites (%)	13.30	6.67	6.67	0.00	0.00	0.00	6.67
Mortality by coccidiosis (%)	6.67	0.00	6.67	0.00	0.00	6.67	0.00
Mortality by other reasons (%)	6.67	6.67	0.00	6.67	13.33	6.67	6.67
Cost of extract/bird (Rs.)	Nil	0.69	1.02	1.58	1.96	2.74	5.40
Cost of feed/bird (@25/Kg Rs.)	37.98	38.02	38.00	38.26	38.61	37.97	38.18
Total feed cost/bird (Rs.)	37.98	38.71	39.02	39.84	40.57	40.71	43.58
Sale of bird at 42 day (@Rs. 200/Kg live weight)*	71.35	82.14	80.50	94.06	78.85	81.84	78.43
Loss due to mortality (Rs.)#	285.40	164.28	161.00	94.06	157.70	163.69	156.87
Total benefit per group (Rs.)##	-	121.12	124.40	191.34	127.70	121.70	128.53

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received aqueous extract of *H. rhamnoides* in drinking water at concentration of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a row differ significantly ($P<0.05$).

* Due to limited availability of fresh chickens at high altitude the rates are very high.

Loss due to mortality = Sale cost per bird X total mortality

Total benefit per group = Loss from mortality in control – loss from mortality in treatment

5.3.2.5 Growth performance of broilers in experimental trial 3

In experiment 3, birds in T4 group had significantly higher body weight as compared with the control and other treatment groups at 21 day whereas, no difference was observed in body weight among the control, T1, T2, T5, and T6 groups (Table 5.15). Further, at 42 day, body weight was found to increase in T3 and T4 groups as compared with control, T1, T2, T5, and T6 groups. Among the T3 and T4 groups, the highest body weight was observed in T4 group. Throughout the experiment cumulative feed and water intake did not differ among the experimental groups. FCR value in the T4 group was found to be significantly improved among the experimental groups (Table 5.15).

5.3.2.6 Economics and mortality in broilers during experimental trial 3

Highest mortality rate (53.33%, total 8 birds out of 15) was observed in the control group (Table 5.15) birds followed by T2 and T6 (33.33%, total 5 out of 15), followed by T3 (26.67%, total 4 out of 15), and followed by T1, T4, and T5 groups (20%, total 3 out of 15). Post-mortem examination showed 26.67%, 0%, 6.67%, 6.67%, 6.67%, 0%, and 0% mortality in birds induced from ascites and 20.33%, 6.67%, 13.33%, 6.67%, 0%, 13.33%, and 6.67% mortality induced from coccidiosis in control, T1, T2, T3, T4, T5, and T6 groups, respectively.

We also estimated the economy of the experiment based on the rearing cost of 15 no of birds in each group. Extra price of the extract was added to feed cost whereas other expenses stayed constant. Addition of *S. alba* extract decreased the mortality rate in birds and which subsequently increased net return (Table 5.15).

5.3.2.7 Growth performance of broilers in experimental trial 4

In experiment 4, we did not observe any difference in the body weight of chickens among the experimental groups at 21 day (Table 5.16) whereas, at 42 day we observed an increased body weight in all treatment groups as compared with the control group and birds in the T3 group represented the highest body weight followed by T4, T2, T1, T5, and T6 group. Furthermore, throughout the experiment cumulative feed and water intake did not differ among the experimental groups. FCR value in the T3 group was found to be significantly improved among the experimental groups (Table 5.16).

Table 5.15: Effect of *S. alba* on growth performance, survivability of broilers chickens, and cost economics of their rearing at high altitude (experiment 3).

Description	Control	T1	T2	T3	T4	T5	T6
Initial average body weight (gm/chick)	38.86 ± 0.45	38.66 ± 0.39	38.53 ± 0.42	38.20 ± 0.36	38.26 ± 0.47	38.13 ± 0.51	38.80 ± 0.45
Average weight at 21 day (gm/chick)	190.33^{ab} ± 3.78	186.42^{a} ± 2.56	197.38^{ab} ± 3.54	201.45^{b} ± 4.82	224.23^{c} ± 5.99	196.25^{ab} ± 3.75	184.72^{a} ± 6.09
Average weight at 42 day (gm/chick)	360.80^{a} ± 7.49	350.91^{a} ± 5.80	368.72^{a} ± 7.50	415.11^{b} ± 9.91	476.10^{c} ± 10.97	362.16^{a} ± 9.33	343.20^{a} ± 7.66
Cumulative feed intake up to 42 day (gm/chick)	1521.88 ± 6.20	1519.49 ± 4.58	1526.76 ± 3.40	1516.59 ± 4.38	1524.49 ± 4.22	1528.97 ± 4.38	1514.62 ± 4.09
Feed conversion ratio at 42 day	4.73^{c} ± 0.07	4.87^{cd} ± 0.07	4.62^{c} ± 0.06	4.02^{b} ± 0.04	3.48^{a} ± 0.04	4.72^{c} ± 0.09	4.98^{d} ± 0.06
Cumulative water intake up to 42 day (mL/chick)	2245.07 ± 6.35	2250.89 ± 4.64	2246.37 ± 6.24	2255.40 ± 4.76	2260.90 ± 6.11	2250.10 ± 6.78	2245.00 ± 7.06
Total mortality (%)	53.33	20.00	33.33	26.67	20.00	20.00	33.33
Mortality by ascites (%)	26.67	0.00	6.67	6.67	6.67	0.00	0.00
Mortality by coccidiosis (%)	20.33	6.67	13.33	6.67	0.00	13.33	6.67
Mortality by other reasons (%)	6.67	13.33	13.33	13.33	13.33	6.67	26.67
Cost of extract/bird (Rs.)	Nil	0.60	0.95	1.38	2.26	2.49	4.82
Cost of feed/bird (@25/Kg Rs.)	38.04	37.99	38.17	37.91	38.11	38.22	37.87
Total feed cost/bird (Rs.)	38.04	38.59	39.12	39.29	40.37	40.71	42.69
Sale of bird at 42 day (@Rs. 200/Kg live weight)*	72.16	70.18	73.74	83.02	95.22	72.43	68.64
Loss due to mortality (Rs.)#	577.28	210.54	368.70	332.08	285.66	217.29	343.20
Total benefit per group (Rs.)##	-	366.74	208.58	245.20	291.62	360.00	234.08

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received aqueous extract of *S. alba* in drinking water at concentration of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a row differ significantly ($P<0.05$).

* Due to limited availability of fresh chickens at high altitude the rates are very high.

Loss due to mortality = Sale cost per bird X total mortality

Total benefit per group = Loss from mortality in control – loss from mortality in treatment

Table 5.16: Effect of *P. armeniaca* on growth performance, survivability of broilers chickens, and cost economics of their rearing at high altitude (experiment 4)

Description	Control	T1	T2	T3	T4	T5	T6
Initial average body weight (gm/chick)	37.20 ± 0.40	37.46 ± 0.55	38.00 ± 0.37	38.13 ± 0.63	37.13 ± 0.52	37.60 ± 0.66	38.06 ± 0.52
Average weight at 21 day (gm/chick)	184.71 ± 7.69	195.46 ± 4.76	203.14 ± 8.04	209.20 ± 9.46	204.28 ± 8.32	198.00 ± 3.98	200.40 ± 10.78
Average weight at 42 day (gm/chick)	348.53^{a} ± 10.41	410.13^{b} ± 8.30	417.57^{bc} ± 9.03	450.14^{d} ± 8.59	440.28^{cd} ± 8.70	408.80^{b} ± 10.41	398.15^{b} ± 8.30
Cumulative feed intake up to 42 day (gm/chick)	1511.21 ± 6.26	1514.12 ± 6.00	1524.41 ± 5.77	1519.33 ± 5.26	1519.40 ± 4.80	1525.46 ± 4.70	1518.48 ± 5.92
Feed conversion ratio at 42 day	4.86^{d} ± 0.04	4.06^{b} ± 0.05	4.02^{b} ± 0.06	3.69^{a} ± 0.05	3.77^{a} ± 0.05	4.11^{b} ± 0.04	4.22^{c} ± 0.04
Cumulative water intake up to 42 day (mL/chick)	2245.60 ± 6.27	2250.52 ± 5.44	2240.84 ± 5.31	2255.56 ± 6.72	2250.35 ± 5.39	2250.19 ± 6.45	2240.15 ± 5.59
Total mortality (%)	20	6.67	6.67	0.00	6.67	0.00	6.67
Mortality by ascites (%)	13.33	0.00	6.67	0.00	0.00	0.00	0.00
Mortality by coccidiosis (%)	6.67	6.67	0.00	0.00	0.00	0.00	0.00
Mortality by other reasons (%)	0.00	0.00	0.00	0.00	6.67	0.00	6.67
Cost of extract/bird (Rs.)	Nil	0.88	1.48	1.89	2.80	3.54	6.98
Cost of feed/bird (@25/Kg Rs.)	37.78	37.85	38.11	37.98	37.99	38.14	37.96
Total feed cost/bird (Rs.)	37.78	38.73	39.59	39.87	40.79	41.68	44.94
Sale of bird at 42 day (@Rs. 200/Kg live weight)*	69.70	82.02	83.51	90.02	88.05	81.76	79.63
Loss due to mortality (Rs.)#	209.10	82.02	83.51	0.00	88.05	0.00	79.63
Total benefit per group (Rs.)##	-	127.08	125.59	209.10	121.05	209.10	129.49

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens received aqueous extract of *P. armeniaca* in drinking water at concentration of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively.

Means bearing the different superscripts ($^{a, b, c, d}$) in a row differ significantly ($P<0.05$).

* Due to limited availability of fresh chickens at high altitude the rates are very high.

Loss due to mortality = Sale cost per bird X total mortality

Total benefit per group = Loss from mortality in control – loss from mortality in treatment

5.3.2.8 Economics and mortality in birds during experimental trial 4

Highest mortality rate (20.00%, total 3 birds out of 15) was recorded in the control group (Table 5.16) birds followed by T1, T2, T4, and T6 (6.67%, total 1 out of 15). Interestingly, no mortality was recorded in T3 and T5 group during the entire period. A post-mortem examination showed 13.30%, and 6.67% mortality in birds induced from ascites in control and T2 group respectively whereas 6.67% mortality was recorded each in control and T1 group induced from coccidiosis.

We also estimated the economy of the experiment based on the rearing cost of 15 no of birds in each group. Extra price of the extract was added to feed cost whereas other expenses stayed constant. Addition of *P. armeniaca* extract decreased the mortality rate in birds and which subsequently increased net return (Table 5.16).

5.4 Discussion

Blood biochemical constituents are important indicators of bird's health status and their physiological responses to different stress conditions. In this study, we assessed the efficacy of *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca* extracts on different blood biochemical parameters of broilers. The results revealed that supplementation of the phytoextracts as a feed additive in broiler diet enhanced the plasma total protein, albumin, and globulin concentration in treatment group birds. The increased protein level in birds might be due to the higher nutritional content of these phytoextract [5, 81, 89] which causes greater amino acid absorption in intestinal tissues, increased protein synthesis, and decreased muscle protein catabolism. Albumin protein is a negative acute phase protein which is a useful marker of inflammation [174] and elevated level of albumin in treatment group birds could have been due to the anti-inflammatory activity of polyphenols, flavonoids and carotenoids content present in these phytoextract [93, 175-177]. Globulins are the globular proteins and are produced by the immune system cells and increased globulin content in treatment groups birds might be because of immune stimulating activity of bioactive phytomolecules exist in these phytoextract [55, 64, 76, 92]. A/G was also altered among the treatment groups and this might be due to increase globulin concentration, which also indicates improved immunity in birds.

LDL is mentioned as bad cholesterol and its main task is to transport the cholesterol from the liver to peripheral tissues while HDL is mentioned as good cholesterol as it transports extra cholesterol from peripheral tissues to the liver for its transformation into bile

acid [178]. The lower level of LDL and increased level of HDL in treatment group birds in this study might be due to the ability of plant bioactive phytomolecules to decrease the secretion of apolipoprotein B (apoB) and increase the secretion of apolipoprotein A-1(apoA-1), respectively [179]. The lower level of cholesterol and triglyceride in treatment groups probably because of the inhibitory effect of polyphenolic compounds on the activity of HMG-CoA reductase and acetyl coenzyme acetyltransferase, key regulatory enzymes in cholesterol biosynthesis [180, 181].

Higher concentration of blood glucose in birds has been reported under stress environment due to increased glucocorticoids secretion [182]. Glucocorticoids are steroid hormones that induce gluconeogenesis from non-carbohydrate precursors such as amino acids and glycerol. In the present study, supplementation of plant extracts reduced the glucose level in birds and this might be because of decreased glucocorticoid secretion by plant polyphenols which could regulate protein and lipid catabolism with decreased gluconeogenesis [183]. There was no change in the plasma creatinine and uric acid level which exhibits the non-toxic and non-pathological effect of plant extracts on the kidney. ALT and AST are intracellular enzymes accruing in liver and heart, and their enhanced activity is the indication of liver and heart damage [184]. In this present study, all the plant extracts altered the ALT and AST level in treatment groups and which indicated the hepatoprotective activity of these phytoextracts inside birds liver cells [51, 77, 144, 185].

In the present study, *R.imbricata, H. rhamnoides, S. alba*, and *P. armeniaca* extracts were supplemented to broiler birds as a source of antioxidant and these extracts were found to possess higher TAC and free radical scavenging capacity, as determined by reduced DPPH and ABTS activity. Additionally, antioxidant parameters such as MDA, FRAP, and DPPH were analysed too in blood plasma samples of chickens for determining the effect of phytoextracts on antioxidant enhancement. Results indicated that supplementation of these phytoextracts as a feed additive in broilers enhanced the level of TAC and free radical scavenging activity while reduced the level of MDA in plasma samples. This increased in the antioxidant defense level and deceased in oxidative stress marker MDA might probably due to the synergistic effect of polyphenols, flavonoids, and carotenoids content that was present in these phytoextracts. These phytomolecules provides protection from oxidative stress due to their higher antioxidative activity with their ability to scavenge free radicals and their ability to inhibit the caspase-3 activation and intercellular Ca^{2+} production, downregulating the

expression of pro-apoptotic genes Bax, upregulating the expression of anti-apoptotic genes Bcl-2 and activation of transcriptional factor Nrf2 [186-192].

It was earlier reported by Calcabrini et al. [193] and Xu and Li [194] that administration of salidroside reduced the oxidative stress in laboratory animals by eliminating the production of free radicals. Earlier studies of Purushothaman et al. [195] and Zhou et al. [196] in laboratory animals revealed a marvellous depletion in MDA concentration and higher antioxidant defense level in animals after addition of *H. rhamnoides* extract. Ishikado et al. [191] also indicated a reduction in oxidative stress and increased antioxidant defense level after administration of *S. alba* extract. Earlier studies of Ozturk et al. [185] and Yilmaz et al. [197] also revealed marvellous depletion in the free radicals and higher antioxidant defence level in laboratory animals with the administration of *P. armeniaca* extract. In this study, TAC was positively associated with free radical scavenging activity and both of these activities were negatively associated with MDA level in treatment group birds.

Cytokines are proteins or signalling molecules that act as a regulator of host responses to inflammation, immune responses and infection. Interleukin-1 is proinflammatory cytokine which is secreted by macrophages, monocytes, dendritic cells and B lymphocytes and controls stimulation of acute phase response and initiation of both cellular and humoral immune responses [198]. IL-6 is another proinflammatory cytokine, secreted by T cells and macrophages which play a key role in inflammatory reactions, differentiation of B cells into plasma cells, and control regulation of immune responses [199]. IL-2 is secreted by activated T helper cells 1 (Th1) and play a central role in cell-mediated immunity by promoting differentiation of T cell into effector T cells and memory T cells [200]. Therefore, these parameters were studied to know the phytoextracts role in stress-induced inflammation, which is prevalent at high altitude.

It is well known that hypoxic conditions stimulate the production of proinflammatory cytokines through inducing the expression of transcriptional factor nuclear factor-*k*B (NF-*k*B) (a transcriptional factor controlling IL-1, IL-6, TNF-α production) and reduces the cellular immunity mediated by T cells [201, 202]. In the present study supplementation of *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca* extracts as a feed additive to broiler decreased the level of proinflammatory cytokines in treatment groups which might be because of anti-inflammatory activity of the polyphenols, flavonoids and carotenoids content present in these phytoextracts through the downregulation of the NF-*k*B signaling pathway

and C-reactive proteins expression and inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes leading to inhibition of prostaglandin synthesis [176, 177, 203-208]. In the present study, all the phytoextracts stimulated the production of IL-2 in treatment group birds which might be due to the immunomodulatory property of antioxidant-rich phytoextracts. This suggests that, supplementation of *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca* extracts exerts immunomodulatory effects in broilers by mediating both cellular and humoral immunity.

Our present findings of low body weight in broilers in all the experimental trials support the hypothesis that high altitude reduces the growth performance in broiler chickens [116]. Our results are in agreement with reports of Li et al. [49] and Balog et al. [116] who found a similar reduction in body weight of broilers reared at 2986 m and 2900 m altitude, respectively. However, the final body weight of broilers in those two studies was much higher than that of our present findings. The reason behind the low body weight could be attributed to the difference in altitude of experimental site and types of poultry breed/strains. We performed our study at 3500 meters above MSL, and used dual purpose poultry breed/ strain (RIR cross-bred), while in those studies experimental sites were located in between 2900 to 2980 m. Moreover, the decrease in body weight might be due to the reduction in energy intake and increase in energy expenditure at high altitude under hypobaric-hypoxic condition. This misbalance in energy utilization leads to decrease in body mass through poor intestinal malabsorption and increase in the catabolism which ultimately reduces the overall growth [172, 209, 210]. Therefore, energy intake is the supreme factor of body weight loss under hypoxic and cold conditions.

However, in the present study supplementation of plant extracts at different dose concentrations increases the live body weight of broilers in treatment groups as compared to control group birds under the same environmental conditions. This increase in the body weight could have been due to the synergistic effect of polyphenols, flavonoids, and carotenoids content present in *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca* extracts which might help in the higher utilization of feed by stimulating increased digestion in birds and which results in the enhanced growth in birds [211-213]. Improved growth performance in broilers can also be implicated with effective antioxidative properties of the plant extracts that could stimulate the protein synthesis by bird enzymatic system and

eliminates the production of free radicals which ultimately reduces the oxidative stress in poultry birds and improves their growth performance [77, 85, 144, 214].

Moreover, treatment group birds had improved FCR value as compared to control group in all experimental trials and this might be due to enhanced nutrient availability to birds that were supplemented with plant extracts. Authors strongly believe that supplementation of these plant extracts enhanced the anabolic activities and reduced the catabolism of muscular proteins and body tissue in broilers. Furthermore, water intake of birds did not vary among the groups and this implies that supplementation of plant extracts did not create any significant changes in order and taste of the water when mixed with drinking water for administration. The net economic return also disclosed higher profit in the treatment groups due to a reduction in the mortality rate from ascites and coccidiosis in birds. Hence, these phytoextracts have a beneficial effect on nutrient digestibility and scavenging of free radical under high altitude stress condition and therefore, these could be useful as a feed additive for better growth rate and for better survivability of broilers at high altitude.

5.5 Conclusion

The *in-vivo* efficacy of *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca* extract was evaluated which indicated favorable changes in physio-biochemical indices, growth performance and survivability of broilers chickens. Further, cost economic of broiler rearing at high altitude indicated significant increase in farm income. The beneficial effect of these plant extracts on these parameters at different dose concentrations might be due to the synergistic effect of polyphenols, flavonoids, and carotenoids present in the extract. Efficacy study indicated that among the different dose level of phytoextracts, 400 mg/kg body weight of chicken of *R. imbricata*, 200 mg/kg body weight of chicken of *H. rhamnoides*, 300 mg/kg body weight of chicken of *S. alba*, and 200 mg/kg body weight of chicken of *P. armeniaca* has shown better effect as compared to other dose regime. Therefore, considering the beneficial effects of these phytoextracts, a new feed additive may be prepared/formulated using individual extracts in certain ration or preparation.

CHAPTER 6

TO FORMULATE THE PHYTOGENIC FEED ADDITIVE FOR BROILER CHICKENS AND A COMPARATIVE STUDY ON THEIR EFFICACY WITH OTHER COMMERCIALLY AVAILABLE FEED ADDITIVES

Abstract

In the present study, a new phytogenic feed additive was prepared and its efficacy was compared with commercially available feed additives for their effect on growth performance of broiler chickens at high altitude. To prepare the feed additive, aqueous extract of *H. rhamnoides* fruits, *S. alba* leaves and *P. armeniaca* seeds were mixed together and three formulations were prepared to have different concentration of extracts. The prepared formulations were F1 (contains, *H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken), F2 (*H. rhamnoides* @ 100mg/kg body weight of chicken, *S. alba* @ 300mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken), and F3 (*H. rhamnoides* @ 100mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 200mg/kg body weight of chicken). Thereafter, efficacies of these prepared formulations were compared with available commercial feed additives. A total of 175 one day old RIR cross-bred broiler chicks were distributed into 7 experimental groups as per complete randomized design. There were 5 replications per experimental group with 5 chickens per replicate pen. Experimental groups included control (fed the basal diet), T1 (basal diet + F1 formulation), T2 (basal diet + F2 formulation), T3 (basal diet + F3 formulation), T4 (basal diet + commercial probiotic), T5 (95% basal diet + 5% Lucerne powder), and T6 (basal diet + commercial herbal feed additive), respectively. Body weight was significantly increased at 21 day in T2 and T3 group as compared to other experimental groups. However, at 42 day, body weight was increased significantly in the T1 group and followed by T2 and T3 groups. Moreover, no differences were recorded in average body weight among the control, T4, T5, and T6 groups. FCR value was improved significantly in T1 group. Further, net economic return also revealed a higher profit in T1, T2, and T3 groups. In conclusion, the F1 formulation was recorded best among the other feed additives

and therefore, identified as novel phytogenic feed additive product for broiler chickens at high altitude.

6.1 Introduction

Leh-Ladakh region of trans-Himalayan possesses adverse climatic conditions where native human inhabitants and livestock are adapted to live with the adverse conditions. In traditional *'Amchi'* system of medicine plants found in the high-altitude Himalayas are widely used for curing high-altitude maladies as prophylactic and therapeutic agent and these diverse phytomolecules show protecting effect against acute disease while proceeding synergistically instead of individually [42, 43]. Therefore, in this study, we aimed at the preparation of a phytogenic feed additive formulation comprising of extract part of *Hippophae rhamnoides*, *Salix alba*, and *Prunus armeniaca* and which may be capable of fulfilling necessary nutritional requirements of poultry birds under hypoxic conditions. The plants which were selected for the preparation of feed additive are widely used in a traditional medicinal system for treatment of common ailments and the bioactive phytomolecules, medicinal values, and nutritional properties of these plants have been extensively studied by previous researchers [58, 75, 84]. The objective of this study was to examine the effect of feed additive formulation on growth performance of broiler chickens at high altitude. In addition, we compared the efficacy of phytogenic feed additive formulation with some of the feed additives that are commercially available in the market and are generally used for improvement in the growth performance of broilers.

6.2 Materials and Methods

6.2.1 Preparation of feed additive formulations

To prepare the phytogenic feed additive formulations, aqueous extracts of *Hippophae rhamnoides* fruit, *Salix alba* leaves, and *Prunus armeniaca* seeds, at their respective dose concentrations (at which it exhibited maximum beneficial effect in broiler, as explained in chapter 5) of 200 mg/kg, 300 mg/kg, and 200 mg/kg body weight of chicken, were selected and further three different formulations were made by mixing the three extract in saline water at different dose concentration ratio. Three formulations were F1 (contains, *H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken), F2 (*H. rhamnoides* @ 100mg/kg body weight of chicken, *S. alba* @ 300mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken), and F3 (*H. rhamnoides* @ 100mg/kg body weight of

chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 200mg/kg body weight of chicken). The compositions of three formulations are described in table 6.1.

Table 6.1: Composition of feed additive formulations:-

Formulations	*H. rhamnoides* fruit extract	*S. alba* leave extract	*P. armeniaca* seed extract
F1	200 mg/kg body weight of chicken	200 mg/kg body weight of chicken	100 mg/kg body weight of chicken
F2	100 mg/kg body weight of chicken	300 mg/kg body weight of chicken	100 mg/kg body weight of chicken
F3	100 mg/kg body weight of chicken	200 mg/kg body weight of chicken	200 mg/kg body weight of chicken

6.2.2 TAC

TAC was determined as described earlier in the section 4.2.4.3.

6.2.3 Total phenolic content

Total phenolics content was determined as described earlier in the section 4.2.4.4.

6.2.4 Total flavonoid content

Total flavonoid content was determined as described earlier in the section 4.2.4.5.

6.2.5 Determination of carotenoids

Total carotenoids content was determined as described earlier in the section 4.2.4.6.

6.2.6 Location and experimental design

To determine the efficacy of feed additive formulations their effects were compared with some of the commercially available feed additives. The study was conducted at DIHAR, Leh. A total of 175 one day old RIR cross-bred broiler chicks were distributed into 7 experimental groups as per complete randomized design. There were 5 replications per experimental group with 5 chickens per replicate pen. Experimental groups included control (fed the basal diet only), T1 (fed the basal diet + F1 formulation), T2 (fed the basal diet + F2 formulation), T3 (fed the basal diet + F3 formulation), T4 (fed the basal diet + commercial probiotic), T5 (fed 95% basal diet + 5% Lucerne powder), and T6 (fed the basal diet + commercial herbal feed additive), respectively. Commercial broiler feed (SMPOORNA feed, Phagwara; starter code No. 08; finisher code No. 07) was used as basal diet. All other *in vivo* experimental parameters remain the same as described in section 5.2.1.

6.2.7 Statistical analysis

As described earlier in the section 3.2.4. For growth performance, 5 replicates pen per group (5 broiler chickens per replicate pen) served as an experimental unit.

6.3 Results

6.3.1 TAC

TAC of feed additive formulations is presented in Table 6.2 and it was recorded to be 473.82 ± 18.11, 459.34 ± 19.07, and 455.11 ± 18.32 µM Fe (II)/g of extract in F1, F2, and F3, respectively.

6.3.2 Total phenolic, flavonoid, and carotenoid contents

The content of total phenolic in feed additive formulations F1, F2, and F3 is presented in Table 6.2 and was recorded to be 269.16 ± 3.17, 241.26 ± 2.90, and 239.45 ± 3.05 mg GAE/g of extract, respectively. Similarly, the content of flavonoids in F1, F2, and F3 was recorded to be 94.67 ± 1.12, 88.14 ± 0.96, and 88.01 ± 1.15 mg QE/g of extract, respectively. Moreover, carotenoid content in F1, F2, and F3 was recorded to be 4.56 ± 0.32, 4.30 ± 0.27, and 4.34 ± 0.40 mg/100g extract, respectively (Table 6.2). Total phenolic, flavonoid, and carotenoid content was higher in F1 formulation as compared to F2 and F3 formulations.

Table 6.2: Level of total antioxidant capacity, total phenolic, flavonoids, and carotenoid in freshly prepared feed additive formulations

Formulation	FRAP (µM Fe (II)/g of extract)	Total phenolic (mg GAE/g of extract)	Flavonoids (mg QE/g of extract)	Carotenoids (mg/100g extract)
F1	473.82 ± 18.11	269.16 ± 3.17	94.67 ± 1.12	4.56 ± 0.32
F2	459.34 ± 19.07	241.26 ± 2.90	88.14 ± 0.96	4.30 ± 0.27
F3	455.11 ± 18.32	239.45 ± 3.05	88.01 ± 1.15	4.34 ± 0.40

6.3.3 Growth performance

Comparative effect of feed additive formulations and commercial feed additives on growth performance of broilers is presented in Table 6.3. At 21 day of age, birds in T2 and T3 groups that were supplemented with formulations F2 and F3 respectively had significantly higher body weight as compared to control and remaining experimental groups. In addition to this, we did not observe any difference in the body weight among the control, T1 and T6 groups at 21 day. Further, at 42 day, body weight was increased in T1 (supplemented with formulation F1) group followed by T2 and T3 group. Moreover, no difference was recorded in the body weight among the control, T4, T5, and T6 groups at 42 day. Cumulative feed intake was decreased in T5 group as compared with other experimental groups. FCR value was significantly improved in T1 group. Highest mortality rate (16%, total 4 birds out of 25) was

recorded in the control group (Table 6.3) birds followed by T6 (8%. Total 2 out of 25), which is followed by T4 and T5 (4%, total 1 out of 25). Interestingly, no mortality was recorded in T1, T2, and T3 groups which subsequently results increased in the net return.

6.4 Discussion

A wide range of plant phytomolecules such as polyphenol, flavonoids, carotenoid etc. are derived from plant products and these diverse phytomolecules show a protective effect against acute disease while proceeding synergistically instead of individually [43]. Therefore, in this study, we prepared phytogenic feed additive formulations by using different plant part extracts, which could be beneficial for growth performance of broiler chickens at high altitude. The main constituents of the formulations were extracted parts of *H. rhamnoides*, *S. alba* and *P. armeniaca* plants, which are habituated to grow in stressful environmental surroundings. Therefore, many secondary metabolites are synthesized in the plants which could be useful in animal and human health improvement. In this study, the feed additive formulations were found to be rich in the total antioxidant capacity as well as rich in polyphenols, flavonoids and carotenoids content. As the total antioxidant capacity of plant extract is mostly associated with the polyphenolic content and from our results, it is apparent that the antioxidant capacity of the feed additive formulation could be attributed mainly due to total polyphenols, flavonoids and carotenoids content of integral plant extracts. *H. rhamnides*, as well as *S. alba* and *P. armeniaca*, also found to possess a diverse array of bioactive phytomolecules in this study and these phytomolecules probably acting together synergistically and elevated the antioxidant level of the prepared feed additive.

In this study, we also validated the efficacy of our feed additive formulations with some of the feed additives that are commercially available in the market and used for the better growth performance of poultry birds. Among the additives, we comparatively tested probiotic, Lucerne mix, and one of the commercially available herbal feed additives with our phytogenic feed formulation. Lucerne or alfalfa is a rich source of protein and fibres with low energy content and is widely used in animal feeding [215]. In this study body weight of chickens was improved in treatment groups that were supplemented with feed additive formulations as compared to control and other groups that were supplemented with probiotic *Lactobacillus sporogenes*, Lucerne mix, and herbal feed additive. This increase in the body weight might be possibly due to synergistic effect of polyphenols, flavonoids, and carotenoids content in the feed additive which could stimulate digestion and metabolism of

nutrients causing higher efficiency in the utilization of feed which results in enhanced growth in birds [146, 216, 217].

Table 6.3: Comparative study on the efficacy of feed additive formulations with other commercially available feed additives at high altitude

Description	Control	T1	T2	T3	T4	T5	T6
Initial average body weight (gm/chick)	42.63 ± 0.71	42.80 ± 0.46	43.00 ± 0.61	42.38 ± 0.57	42.90 ± 0.50	42.85 ± 0.59	42.23 ± 0.59
Average weight at 21 day (gm/chick)	361.16^{b} ± 4.18	369.20^{b} ± 6.75	401.40^{c} ± 5.15	406.12^{c} ± 6.83	337.20^{a} ± 3.06	333.16^{a} ± 3.36	360.52^{b} ± 4.58
Average weight at 42 day (gm/chick)	769.44^{a} ± 18.18	926.48^{c} ± 20.20	879.04^{bc} ± 19.51	842.92^{b} ± 19.15	773.44^{a} ± 9.34	779.62^{a} ± 9.13	781.44^{a} ± 8.64
Cumulative feed intake up to 42 day (gm/chick)	2132.92^{b} ± 4.34	2137.40^{b} ± 4.19	2139.80^{b} ± 5.90	2135.64^{b} ± 3.75	2138.04^{b} ± 5.00	2067.88^{a} ± 5.11	2137.63^{b} ± 6.24
Feed conversion ratio at 42 day	2.93^{c} ± 0.03	2.42^{a} ± 0.02	2.56^{b} ± 0.02	2.67^{b} ± 0.02	2.93^{c} ± 0.02	2.81^{c} ± 0.02	2.90^{c} ± 0.03
Total mortality (%)	16.00	0.00	0.00	0.00	4.00	4.00	8.00
Mortality by ascites (%)	4.00	0.00	0.00	0.00	0.00	0.00	4.00
Mortality by coccidiosis (%)	8.00	0.00	0.00	0.00	0.00	4.00	4.00
Mortality by other reasons (%)	4.00	0.00	0.00	0.00	4.00	0.00	0.00
Cost of feed additive/bird (Rs.)	Nil	3.84	3.83	3.55	2.00	0.58	1.40
Cost of feed/bird (@26/kg Rs.)	55.46	55.57	55.63	55.53	55.59	53.76	55.58
Total feed cost/bird (Rs.)	55.46	59.41	59.46	59.08	57.59	54.34	56.98
Sale of bird at 42 day (@Rs. 200/Kg live weight)*	153.88	185.30	175.80	168.58	154.69	155.92	156.29
Loss due to mortality (Rs.)#	615.52	0.00	0.00	0.00	154.69	155.92	312.58
Total benefit per group (Rs.)##	-	615.52	615.52	615.52	460.83	459.60	302.94

Treatments: 100% basal diet (Control); 100% basal diet + F1 formulation supplemented in drinking water (T1); 100% basal diet + F2 formulation supplemented in drinking water (T2); 100% basal diet + F3 formulation supplemented in drinking water (T3); 100% basal diet + commercial probiotic supplemented in drinking water (T4); 95% basal diet + 5% Lucerne powder (T5); 100% basal diet + commercial herbal feed additive supplemented in drinking water (T6).

Means bearing the different superscripts ($^{a, b, c, d}$) in a row differ significantly ($P<0.05$).

Moreover, among the three formulated feed additives, birds supplemented with formulation 1 (*H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken) has shown better growth performance. This might be probably due to the appropriate dose concentration of integral plant extracts that could be sufficient to reduce the catabolic activities in broilers under oxidative stress conditions. Furthermore, we did not observe any beneficial effect of probiotic on growth performance of broilers at high altitude which might be due to an

insufficient number of *Lactobacillus sporogenes* strain used in this study [119]. Similarly, Lucerne mix supplementation has no improvement in performance of broilers and it might be due to the presence of antinutritive compound such as saponin which interferes with absorption of nutrients in the gastrointestinal tract [215]. Interestingly, commercial herbal feed additive has no beneficial effects on growth performance of broilers. However, previous coworkers [218, 219] reported significant improvement in the broilers growth after supplementation of herbal feed supplements. The contradictory findings of the present study may be attributed to the non-nutritional composition of a commercial feed additive that was used in this study to meet the maintenance and productive requirement of poultry birds at high altitude. FCR was also improved in the birds supplemented with feed additive formulations which could have been due to the enhanced nutrient availability due to the synergistic effect of present phytomolecules.

6.5 Conclusion

A phytogenic feed additive formulation has been developed for broilers comprising phytoextract of different parts of herbal plants grow in high altitude Himalaya, that are rich in bioactive phytomolecules having stress-ameliorating and antioxidant properties. Feed additive formulation was comparatively tested with commercially available feed additives and it was established that feed additive formulation 1 (*H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken) contained a considerable amount of diverse phytomolecules and therefore supplementation of this formulation to broilers improves their growth performance. As a whole, it can be concluded that this feed additive formulation could be an aid for poultry birds in the stressful environment of high altitude where there is marked deficiency of nutritious poultry feed and fodder.

CONCLUSIONS

1. Identification of suitable broiler strains having more adaptability under hypoxic conditions at high altitude is more important to overcome unsatisfactory growth performance in Leh-Ladakh. Among the three broiler strains, RIR cross-bred has shown better growth and survivability, which may be due to less metabolic rate.

2. These RIR cross-bred performs as dual purpose breed, which are more hardy and grow better withought stress. Hence, dual purpose poultry birds and slow growing broiler strains/breeds would be more suitable to this region.

3. Therefore, efforts should be given on development of these kinds of poultry birds for Leh-Ladakh.

4. Commercially available probiotics has no beneficial effect on weight gain, however has reduced mortality rate at Leh-Ladakh.

5. Hence, before the selection of probiotics, more studies are required to know the dose, microbial population, and kinds of gut microflora species prevalent in this farming system. This could be helpful for weight gain and reducing the mortality.

6. Hydro-ethanolic extract of *Rhodiola imbricata* roots and aqueous extract of *Hippophae rhamnoides* fruits, S*alix alba* leaves and *Prunus armeniaca* seeds were phytochemically characterized which revealed rich in polyphenols, flavonoids, and carotenoids content. Moreover, *in-vitro* studies indicated enhanced antioxidative and cytoprotective activities of these phytoextracts on chicken peripheral blood lymphocytes. Therefore, considering the pharmacological antioxidant properties of these four extracts their nutraceutical value might be evaluated for their utilization in feed additive as performance enhancer.

7. The *in-vivo* efficacy of *R. imbricata*, *H. rhamnoides*, *S. alba*, and *P. armeniaca* extract was evaluated which indicated favorable changes in physio-biochemical indices, growth performance and survivability of broilers chickens. Further, cost economic of broiler rearing at high altitude indicated significant increase in farm income. The beneficial effect of these plant extracts on these parameters at different dose concentrations might be due to the synergistic effect of polyphenols, flavonoids, and carotenoids present in the extract. Efficacy study indicated that among the different dose level of phytoextracts, 400 mg/kg body weight

of chicken of *R. imbricata*, 200 mg/kg body weight of chicken of *H. rhamnoides*, 300 mg/kg body weight of chicken of *S. alba*, and 200 mg/kg body weight of chicken of *P. armeniaca* has shown better effect as compared to other dose regime. Therefore, considering the beneficial effects of these phytoextracts, a new feed additive may be prepared/formulated using individual extracts in certain ration or preparation.

8. Total three phytogenic feed additive formulation has been developed for broilers comprising extract parts of different herbal plants grow in high altitude Himalaya, that are rich in bioactive phytomolecules having stress-ameliorating and antioxidant properties. Feed additive formulation was comparatively tested with commercially available feed additives and it was established that feed additive formulation 1 (*H. rhamnoides* @ 200mg/kg body weight of chicken, *S. alba* @ 200mg/kg body weight of chicken, and *P. armeniaca* @ 100mg/kg body weight of chicken) contained a considerable amount of diverse phytomolecules and therefore supplementation of this formulation to broilers improves their growth performance. As a whole, it can be concluded that this feed additive formulation could be an aid for poultry birds in the stressful environment of high altitude where there is marked deficiency of nutritious poultry feed and fodder.

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(B) Research Publications

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(C) Conference Publications

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