ANTI-FERTILITY STUDIES OF HYDROALCOHOLIC RHIZOME EXTRACT OF *TRILLIUM GOVANIANUM* IN FEMALE RATS

ENROLLMENT NO. **131558**

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JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY,

WAKNAGHAT

TABLE OF CONTENT

CERTIFICATE FROM SUPERVISOR

This is to certify that the work titled "**Anti-fertility studies of hydroalcoholic rhizome extract of *Trillium govanianum* in female rats**." submitted by "Parul Sharma and Kritika Jaggi" in fulfillment for the award of degree of B.Tech of Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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DECLARATION

I certify that

- The work contained in this thesis is original and has been done by me under the guidance of my supervisor.
- The work has not been submitted to any other organization for any degree or diploma.
- Whenever, I have used materials (data, analysis, figures or text), I have given due credit by citing them in the text of the thesis.

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SUMMARY

Trillium govanianum commonly known as *Nag chhatri* is a high-value medicinal plant, of the family Trilliaceae, found at Himalayan ranges from Pakistan to Bhutan at an altitude of 2400-3500 m. The rhizome is the desired material of trade containing the medicinal compound trillarin which on hydrolysis yield diosgenin, used in the preparation of steroidal and sex hormones. The plant used in this study is basically meant to facilitate childbirth, and to treat other female problems by the women of many Native American tribes in its folkloric value.

But no report is so far available on its direct usage for any herbal formulation.The plant crude extract has reported to have anti-cancerous properties along with potential for contraception. So present study validates the anti-fertility potential of herbal plant extract of *Trillium govanianum* in female Wistar rats and carried out histopathological studies to see the effect at varying tissue levels. This study involves phytochemical screening and anti-fertility studies of hydroalcoholic rhizome extract of *Trillium govanianum* on female rats. We found that the extract showed significant antiimplantation activities at 125 and 250 mg/kg doses respectively as no implants were observed in the rats administered with the extract. The estrogenic/anti-estrogenic studies concluded that the extract treatment is having a strong estrogenic effect but did not possess any anti-estrogenic effect which might be responsible for the anti-fertility effect of this plant.

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LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
T CHO	Total cholesterol
Tg	Triglycerides
HDL	High density lipoprotein
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
ALP	Alkaline phosphatase

CHAPTER 1

INTRODUCTION

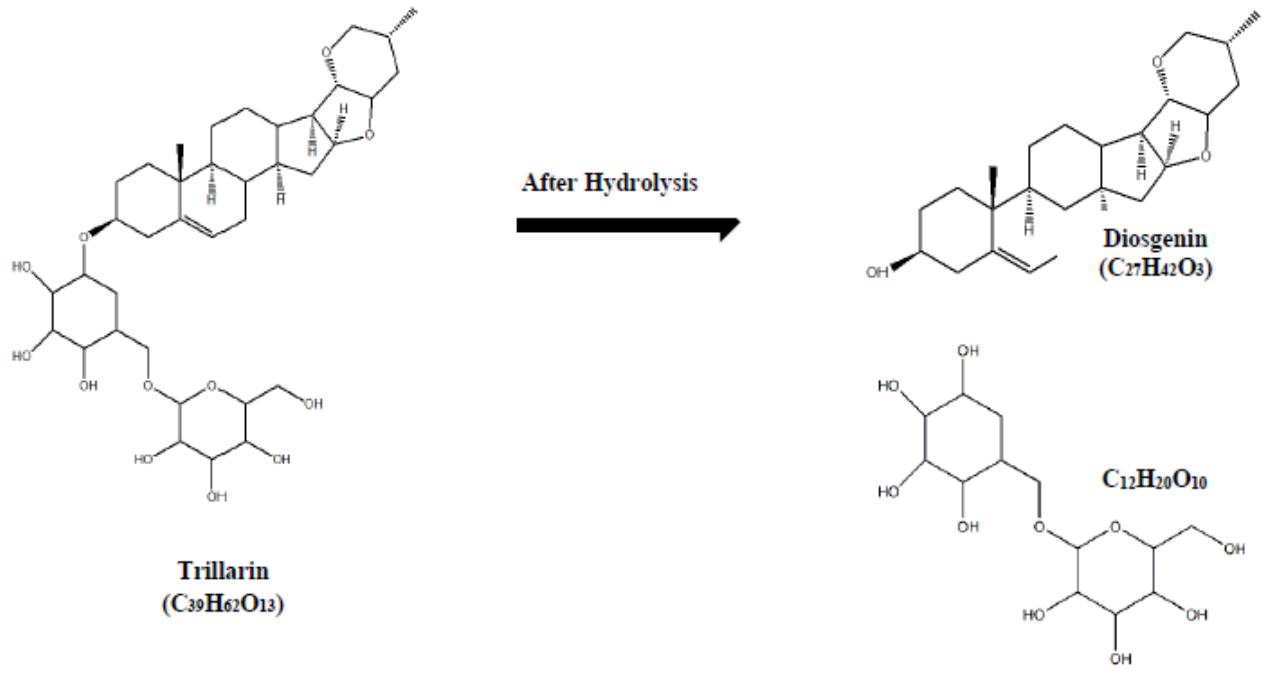
Trillium govanianum which is commonly known as *Nag Chhatri* is a high value medicinal plant that belongs to the family Trilliaceae. This plant is usually found at Himalayan ranges from Pakistan to Bhutan. The plant has stems to 20 cm tall. Leaves shortly stalked, broadly ovate, 3-10cm long (Figure 1A-B). Flowers shortly pedicelled, the sepals and petals similar, brown-purple, l-2cm long, summer. Himachal Pradesh to Kashmir, in woods and stony slopes. A primitive species close to *Faris* and of more interest than beauty. Over exploitation from the natural habitats, to meet the demand of pharmaceutical industry has caused its population depletion to a great extent (Figure 1C to D).

Classification of *Trillium govanianum* is given below :

Kingdom – Plantae
Phylum- Tracheophyta
Class- Liliopsida
Order- Liliales
Family- Melanthiaceae
Genus- *Trillium*
Species- *govanianum*

Fig 1. (A) Plant of Trillium govanianum; B) population; (C) collection of Trillium govanianum and (D) post harvest processing(Shalini Vidyarthi,2013).

The desired material i.e. rhizome is the key material for trade,as rhizome contains trillarin (a medicinal compound).Trillarin on hydrolysis gives diosgenin (a cortico-steroid hormone) which is used in preparation of steroids and sex hormones (Fig. 2).



<u>Fig. 2</u> A schematic hypothesized view for the production of Diosgenin from Trillarin after hydrolysis (Shivam et al 2016)

The plant used in this study is basically meant to facilitate child birth and to treat other female problems by the women of many Native American tribes in its folkloric value.
Diosgenin a raw precursor used as a steroidal drug for production of some hormones such as testosterone, glucocorticoids, progesterone, also used in rheumatism, regulation of menstrual flow, and many more.[Shivam et al 2016].
The phytochemical analysis of the plant reported to have govanoside, new steroidal saponins, and other components such as borassoside and pennogenin, which are explored further for antioxidant activities. Shivam et al also reported the anti-cancer activity of the crude plant extract. Due to its effective medicinal properties, the demand of this drug is high in the international markets. Relentless collection and smuggling of *Trillium govanianum* because of its high price (Rs 2,500- 5,000) have made it a highly prized medicinal herb in Himalayas.

Over exploitation to meet the industry demands have made this a global threat to the population of *nag chhatri* with small geographical niche. Its population in the natural habitat is being threatened by uprooting it from there. Thus state government has banned its collection from natural habitat but even though illegal collection and smuggling are still in process.

So looking upon its usage in the market and its phytochemical analysis we carried out our present study for exploring anti-fertility potential of *Trillium govanianum*. Increasing population

is the growing concern these days. Raise in population level in the developing countries assures the need of effective birth control measures.

There are many synthetic drugs available in markets which are widely used with efficacy. However, these drugs cause various side effects such as hormonal imbalance, hypertension, weight gain, cervical cancer etc. These side effects gives the challenging encouragement for the development of more potent herbal drugs which are less toxic to human and also have least side effects on human body. Such drugs could be made to have contraceptive potential along with anti-cancerous effect. So the search for relatively effective herbal antifertility drug from plant extract might be an alternative for controlling the population.

There are some reports where herbal material was used for contraception like Ruta graveolens L. (Rutaceae) or common rue, native of the Mediterranean region but cultivated throughout Europe and many Asian countries, the whole herb is abortifacient, anthelmintic, antidote, antispasmodic, carminative (Salib et al). *Michelia champaca* commonly known as Champa, is traditionally used for fertility regulation by women of Chhattisgarh state in India (Taprial et al 2013).

As overpopulation has led to serious social and environmental problems such as poverty, overcrowded slums and crime, pollution of air and water and depletion of the protective ozone layer (Greep et al 1998). Family planning has been promoted through several methods of contraception, but due to serious adverse effects produced by synthetic steroidal contraceptives, attention has now been given to indigenous plants for possible contraceptive effects (Ghosh & Bhattacharya 2004) whereby several active chemical constituents accountable for uterotonic effects are discovered in various plant species from time to time. So we carried out present study with following objectives:

1. Preparation of hydroalcoholic rhizome extract *Trillium govanianum* (Nag chhatri) & preliminary phytochemical screening of the extract.

2. To evaluate anti-fertility potential of herbal plant extract of *Trillium govanianum* in female Wistar rats.

CHAPTER - 2
REVIEW OF LITERATURE

Many medicinal plants in India have been checked for their contraceptive potential and anti-fertility effects,as over population is major concern in the country. The probable male/female antifertility effects that are arising from short or long term exposure of certain common and valuable Indian medicinal plants are published in scientific literature. But , the outcomes of research investigations are more often complicated by scientists' compulsions to report positive results.

The site of action of anti-fertility agents in females consists of mainly the following; the hypothalamus, the anterior pituitary, the ovaries, the oviduct, the vagina and the uterus. The Hypothalamus has a major role to control the action of the uterus via follicle stimulating hormone (FSH) and Luteinizing hormone (LH) releasing hormones. Antifertility agents may therefore exert their effort at this level in two ways;firstly by disrupting hormonal function of the hypothalamus or the pituitary, or secondly by interrupting the neural pathway to the hypothalamus that control the liberation of gonadotrophin releasing hormones (Bullock *et al.*, 1995).

Trillium govanianum belong to the Trilliaceae family. This medicinal herb, also called as *Nag Chhatri* is used in traditional system of medicine in various regions of India and Pakistan. The plant is approximately 30cm tall, and has stout rhizome with numerous adventitious roots.

In folk medicine T. govanianum rhizome is of huge importance, basically helps to cure dysentery, backache, healing of wounds, inflammation, skin boils, menstrual and sexual disorders (Rani et al., 2013; Mahmood et al., 2012; Sharma & Samant, 2014).

The illegal uprooting of this medicinal plant from natural habitat, to meet pharmaceutical industry demands has made the made a global threat to the population of*nag chhatri* with small geographical niche.

The plant also possesses analgesic, anti-inflammatory, anticancer as well as antifungal properties. The powdered plant is also used as anthelmintic as reported by Lone et al. in 2013. The plant is recently explored and has found to have a high sell value for its folkloric use (Sher et al., 2014).The rhizomes of this plant species could serve as potential novel source of compounds effective for alleviating pain and inflammation(Shafiq et. al., 2016)

A number of anti-fertility studies have been conducted by researchers on various medicinal plants and herbs till now. One of the antifertility studies included the evaluation of antifertility activity of hydroalcoholic leaves extract of *Michelia champaca L*. in female Wistar rats by Taprial et. al. in 2013.In this study the animals were administered with extract (HAEMC) at different dose level to evaluate the antifertility activity of the plant extract in two experimental models. The two experimental models were anti-implantation activity in female Wistar rats and to study estrogenic/anti-estrogenic activity in the female Wistar rats.So, as a result leaves extract of *Michelia champaca* possess antifertlity effect on female Wistar rats.

Gebrie et.al. reported that the methanolic extract of *Rumex steudelii* root showed antifertility activity in female rats.The results approved that the extract decreased the number of implantation sites significantly. Kachroo et al. in 2011, worked on anti-implantation activity of different extracts of the peels of Citrus medica.

In the year 1971, Arora et. al. studied antifertility activity of Embelia reibes. Kholkute et. al. (1978) and Purandare et. al. (1979) studied contraceptive activity of an indigenous plant Embelia ribes berries. Antifertility effect of Embelia reibes in male rats was studied by Agarwal et. al. in 1986. Gupta et. al. (1989) reported anti-spermetogenic effect of Embelia reibes, a plant benzoquinone on male albino rats in vivo and in vitro.

A study based on antifertility activity arising from *Calotropis gigantea* root has been reported in rats. In one of their study, ethanolic extract was administered to albino rats to investigate its antifertility effect; this extract showed a very strong antiimplantation effect at the dose level of 250 mg/kg(Srivastava et. al.,2007)

Another plant, Hibiscus rosa-sinensis belonging to the family Malvaceae, the active parts of which are believed to be its flowers. Its active principles include quercetin-7-O-galactoside, polyphenolic compounds, kaempferol, and scutellarein and it is used to treat bacterial infection, hyperlipidemia, and depression. Its asserted biological activity is antibacterial, antioxidant, hypolipidemic, and antispermatogenic.

Many plants are mentioned in our ancient literature as well as traditional literature and are used by tribals and by rural communities to prevent pregnancies. Although use of many herbal medicines is well documented in our ancient literature but these are not supported by experimental evidences. In last one or two decades interest of scientific community has developed in medicinal plants and there is much advancement in research in this field. Initially scientific work on medicinal plants was done during 1960s, 70s and 80s. Then enthusiasm of the scientific community declined. But after success of some plant products now global interest in plant products has increased.

CHAPTER – 3

MATERIALS AND METHODS

Plant collection and extraction procedure

The *Trillium govanianum* were procured from the Chamba region at an altitude of 3500m in the state of Himachal Pradesh, in August 2014. The fresh rhizomes of *Trillium govanianum* were washed under running tap water and shade dried. After that, ,rhizomes were grounded and subjected to hydroalcoholic (30:70; water: methanol) Soxhlet extraction for 72 hours (3days). Extracts were filtered while hot, concentrated under reduced pressure using rotary evaporator followed by lyophilization. The extract was stored at 4^0 Celsius until used further (Shivam et al).

Preliminary phytochemical screening

The hydroalcoholic rhizome extract of *Trillium govanianum* was tested for preliminary phytochemical screening as per reported protocols(C.K. Kokate Pharmacognosy).

Animal studies

Female Wistar rats were procured from Central Animal Facility, National Institute of Nutrition, Hyderabad, India, and were housed in the polystyrene cages inside animal house of Jaypee University of Information Technology, Solan, Himachal Pradesh, India. The animals were maintained under standard conditions with 21.5 ± 2^0C temperature, 60±1% humidity and 12h light/dark cycle. Animal were fed standard rat pellets and were having free access to water. Entire experimental procedures were performed after approval from the Institutional Animal Ethical Committee and all the guidelines of CPCSEA were strictly followed. All the necessary efforts were made to minimize suffering to the animals.

Preparation of test sample and dosing

The dose of the extract was selected and administered at 125 and 250 mg/kg doses in the present study. The dose of the extract was reconstituted by suspending the required amount of the extract in the vehicle CMC i.e. Carboxymethyl cellulose (0.3% v/v) and was injected per orally. Control groups received equal volume of vehicle daily.

Antifertility studies

Anti-implantation activity

Animals with proven fertility were used to evaluate anti-implantation effect of plant extract. Esterous cycle in female Wistar rats was regularly monitored and two female rats were caged with one male rat (2:1)on the evening of proestrous stage. Female animals were examined after 12 h next day morning for the evidence of copulation by taking vaginal smears. The rats tested positive for the copulation plug or thick clumps of spermatozoa in vaginal smears were separated and that day was designated as the day 0.5 of the pregnancy. Animals were then divided into three groups; Group 1: control (received vehicle orally, once daily),Group 2: received 125 mg/kg extract (suspended in vehicle, once daily),Group 3: received 250 mg/kg extract (suspended in vehicle, once daily).All the treatments were continued for 11 days and on day 12 animals were sacrificed under anesthesia (90 mg/kg ketamine and 5 mg/kg xylazine), dissected, uterus was weighed and examined to determine the number of implantation sites. Besides, changes in body weight was measured regularly, blood was collected, kept at room temperature for 15 min, centrifuged at 5000 rpm for 10 min and serum was collected. Serum was later used to determine the estrogen levels.

Liver function tests

After the animals were sacrificed blood was collected, kept at room temperature for 15 min, centrifuged at 5000 rpm for 10 min and serum was collected. Serum was later used to test the proper liver functioning. The two aminotransferases that are checked are alanine aminotransferase (ALT or SGPT) and aspartate aminotransferase (AST or SGOT). These tests were performed to check the effect of drug on metabolism.

Estrogenic/anti-estrogenic study

Estrogenic/anti-estrogenic effect of plant extract was evaluated in female rats with proven fertility. Female Wistar rats were ovariectomized under light anaesthesia. Animals were given diclofenac sodium (40 mg/kg) ever 12 h after surgery for 3 days. Animals were allowed to recover for one week after which they were divided into six groups. Group 1-(control) was administered with vehicle(0.3% CMC) daily. Group 2- received a standard drug 17α-Ethinylestradiol (EE;1 μ/rat/day) suspended in olive oil subcutaneously, Groups 3- received 125 mg/kg extract (suspended in vehicle, once daily, Group 4: received 250 mg/kg extract (suspended in vehicle, once daily), Group 5: received 125 mg/kg extract (suspended in vehicle, once daily) along with EE (1μg/rat/day) subcutaneously and Group 6: received 250 mg/kg extract (suspended in vehicle, once daily) along with EE (1 μ/rat/day) subcutaneously. All the

treatments were continued for 12 days. On the 13th day, all animals were sacrificed under anesthesia. The final body weight of all the animals were recorded before anesthesia and blood serum was further processed for the estimation of biochemical parameters such as estrogen level, alkaline phosphates, cholesterol, triglycerides and total proteins.

Statistical analysis

Results are depicted as mean ± SD. Statistical significance was determined using one way ANOVA followed by Dunnett's multiple comparison test, by using Graph pad prizm 6 software. All ten results were compared to control and statistical significance was determined at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

CHAPTER - 4
RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical studies were performed to check the presence different of the components in the rhizome of *Trillium govanianum*. The plant extract revealed the presence of alkaloids, tannins, steroids, flavonoids etc. are shown with **+ve** sign (Table 1).

Table 1 Components present in the plant extract of *Trillium govanianum*

Component Checked	Tests performed	Positive results	Negative results
Acidic compounds	**$NaHCO_3$ test**		**-ve**
Aleurone grains	**Alcoholic iodine**	**+ve**	
Alkaloids	**Dragendroff reagent** **Tannic acid test**	**+ve** **+ve**	
Amino acids	**Ninhydrin test**	**+ve**	
Carbohydrates	**Molisch reagent**		**-ve**
Lignin	**Saffranine reagent**	**+ve**	
Volatile oils	**Sudan III**		**-ve**
Glycosides	**Froth formation test** **Raymon's test** **Baljet's test**	**+ve**	**-ve** **-ve**
Flavonoids	**Alkaline reagent** **Zinc hydrochloride test**	**+ve** **+ve**	
Tannins	**Ferric chloride test**	**+ve**	
Inulin	**αNapthol+H_2SO_4**	**+ve**	
Steroids	**Salkowski test**	**+ve**	
Fats and fixed oils	**NaH Sulphate test**		**-ve**

Anti-implantation study

A dose dependent anti-implantation effect was observed in the table mentioned below. There was not even single implant observed in the two groups with the dose of 125mg/kg and 250 mg/kg. However five implants were observed in the control group animal (administered with no dose).There were 100% inhibition of implants observed in the female rats administered with plant extract.

Table 2 Effect of plant extract on implantation in female rats.

Treatment	Dosage	Anti-implantation effect				
		Body weight change (%)(mean)	No. of implantation sites (mean)	% Inhibition of implants on day 12.5	Estrogen (pg/ml)	Uterus weight (mg)
Control	-	5.48±0.81	7.8±0.84	0%	100.84±5.21	62.3±5.81
Plant Extract	125	1.64±0.3	0.4±0.55	100%	106.72±6.11	89.51±8.11
	250	8.09±0.61	0	100%	216±15.81	112.21±12.11

The effect of the plant extract was checked on various parameters including body weight, uterus weight, foetus weight and the length of foetus. Graphical representation of the same was plotted.

As compared to control group there was significant increase in body weight of the animals who received the extract (Fig. 3(a)). The implants were observed in the control group so the weight of the uterus increased in case of control animal (Fig. 3(b)). 11 foetus were observed in the control animal while no foetus was found in the animals administered with the extract (Fig. 3(c)). The average length of the foetus of control group was 2.91cm. (Fig.3 (d)).

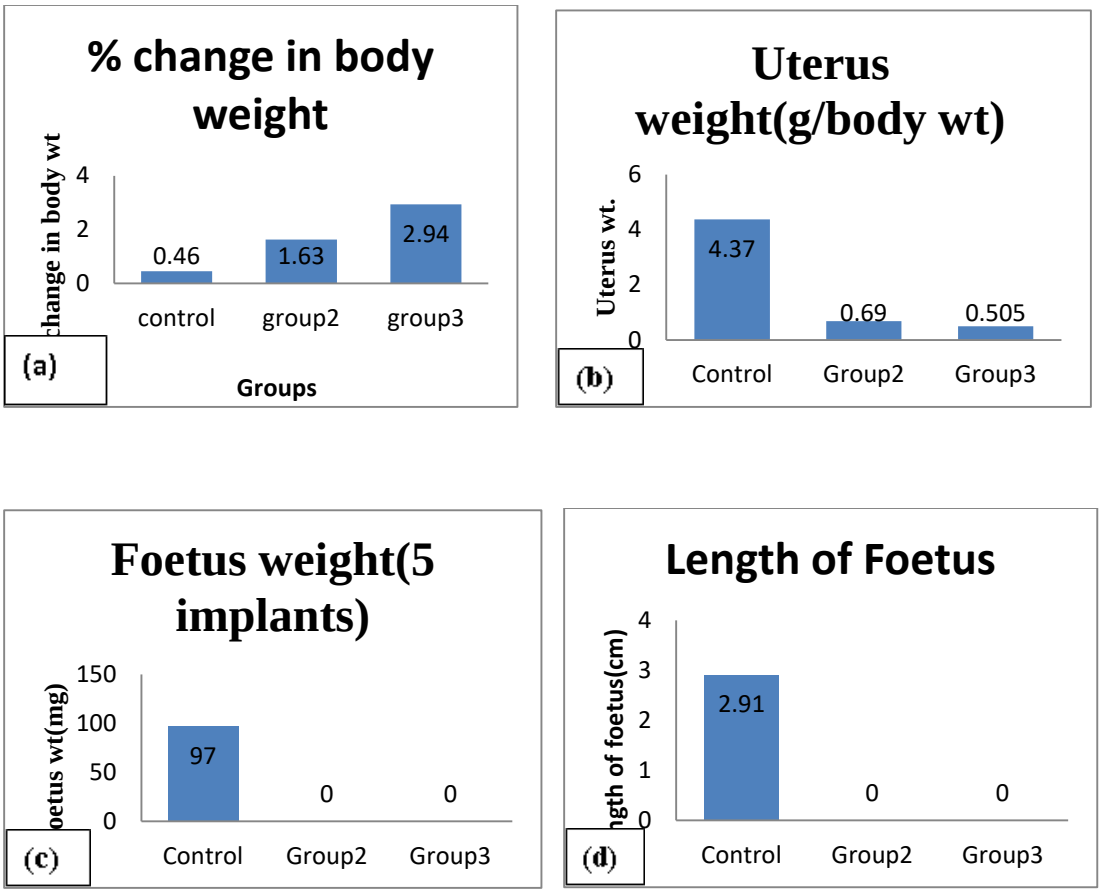


Fig. 3 Effect of plant extract on control v/s experimental female Wistar rats on (a) Body weight (b) Uterus weight (c) Foetus weight (d) Length of foetus

Histopathology results

The effect of plant extract on the uterus and ovary of female Wistar rats was studied. The samples of the respective tissues were sent to MEDICOS CENTRE, CHANDIGARH and the following interpretation was obtained after sectioning.

In the Fig 4 (a) which shows the uterus of the control animal , red arrow shows the endometriun cavity on the left which is of normal physiology and is perfect for the implantation. The black arrow represents the fallopian tube.

In the Fig 4 (b) which shows the uterus of the animal administered with dose of 125 mg/kg, the arrows indicate slight thickening of the endometrium wall.

In the Fig 3 (c) which shows the uterus of the animal administered with dose of 250 mg/kg ,the endometrium lining is very thick in this case because of the increase in the dose but it is of normal physiology.

In the Fig. 5 (a) which shows the ovary of the control animal, the red arrow shows the cystic follicle and the black arrow indicates the corpus luteum of normal physiology.

In the Fig 5 (b) which shows the ovary of the animal administered with dose of 125 mg/kg, ovary with the corpus luteum was seen but the thickening of the wall was observed with normal physiology as a result of the effect of the extract .

In the Fig 5 (c) which shows the ovary of the animal administered with dose of 250 mg/kg , the red arrow indicated the initiation of corpus luteum and black arrow shows the dead cells of corpus luteum. The thickening was more with normal physiology.

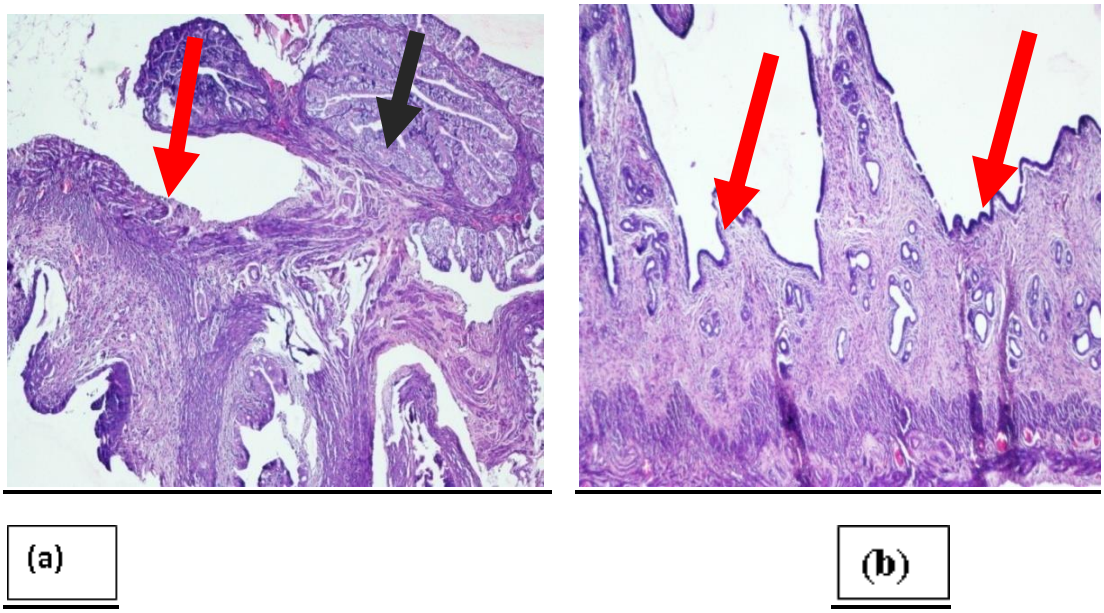
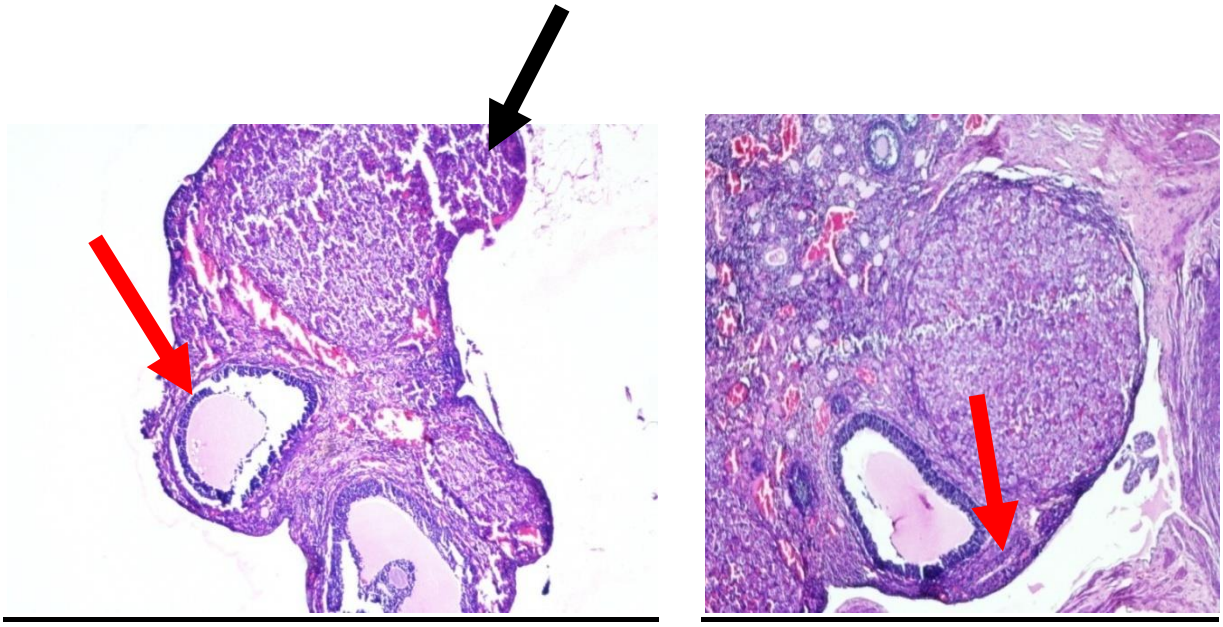
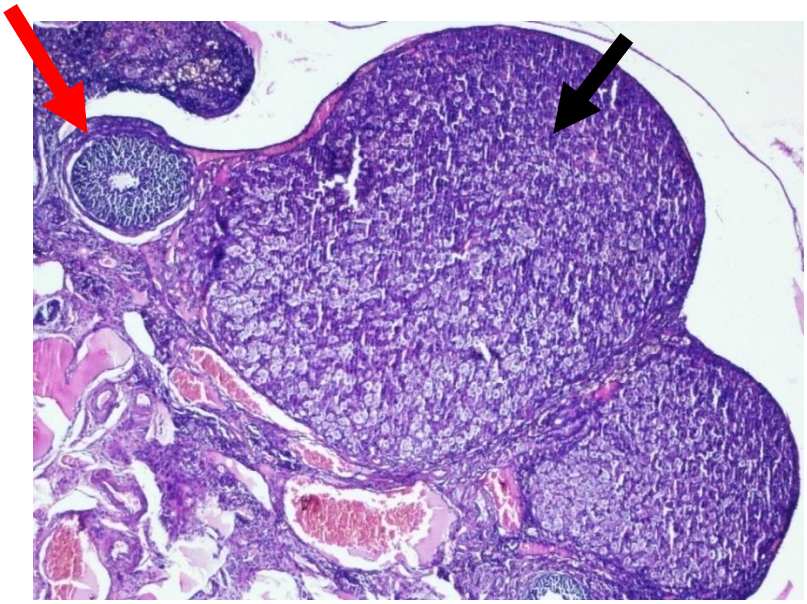


Fig. 4 The uterus sectioning after dosing with plant extract of (a) Uterus of control animal (b) Uterus of animal with 125 mg/kg dose (c)) Uterus of animal with 250 mg/kg dose.



(a)

(b)



(c)

Fig. 5 The ovary sectioning after dosing of plant extract (a) Ovary of control animal (b) Ovary of animal with 125 mg/kg dose (c) Ovary of animal with 250 mg/kg dose.

Toxicity studies

Liver function tests

In order to check the effect of extract on the liver cells SGOT and SGPT was performed and graphs were plotted. As in fig.6 the graphs depict the effect of extract on the metabolism. With the increase in dose more liver enzymes are released as compared to the control group.In both the cases group 3 has the highest absorbance of 614.55 and 705.2 respectively indicating the highest effect of the plant extract.

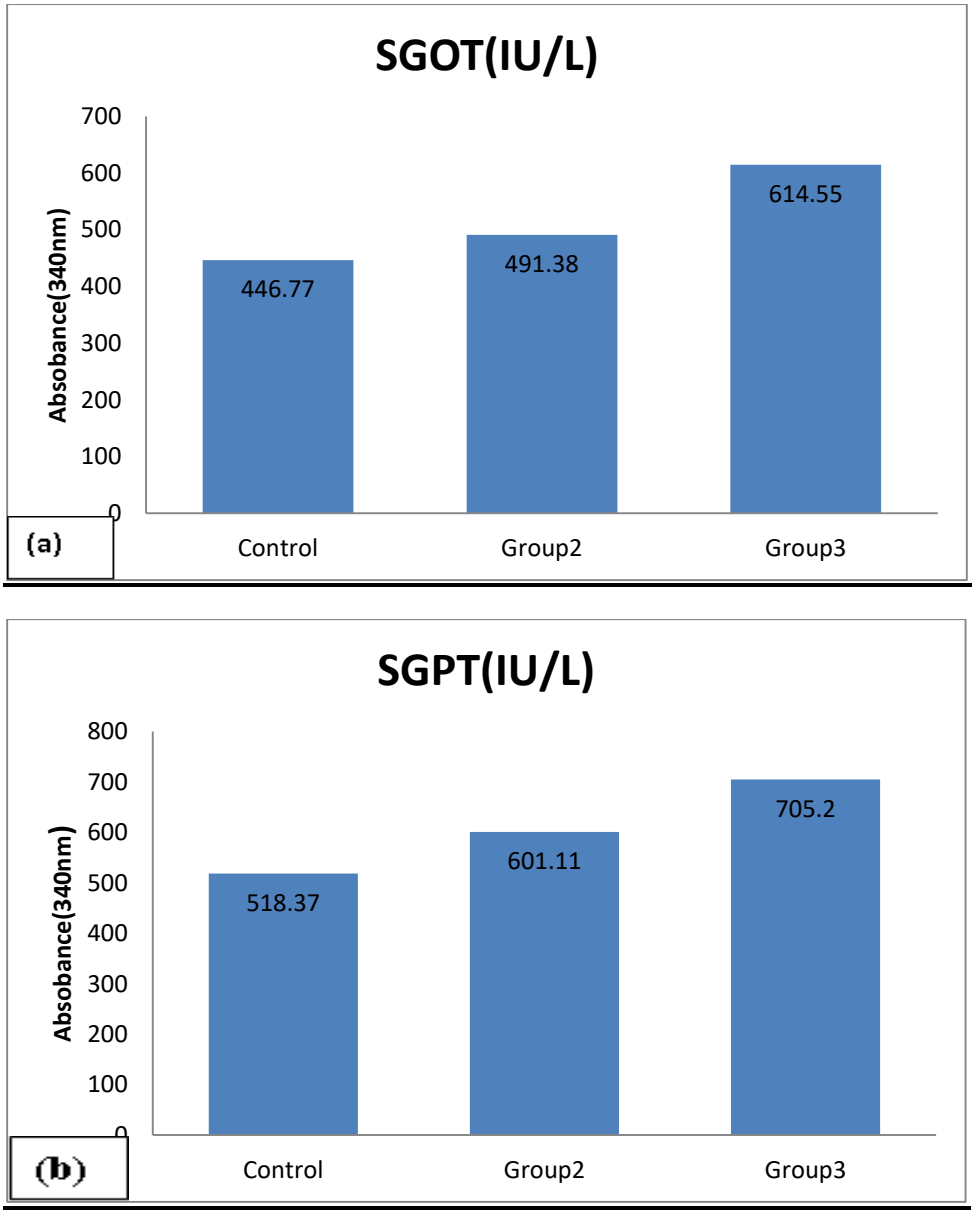


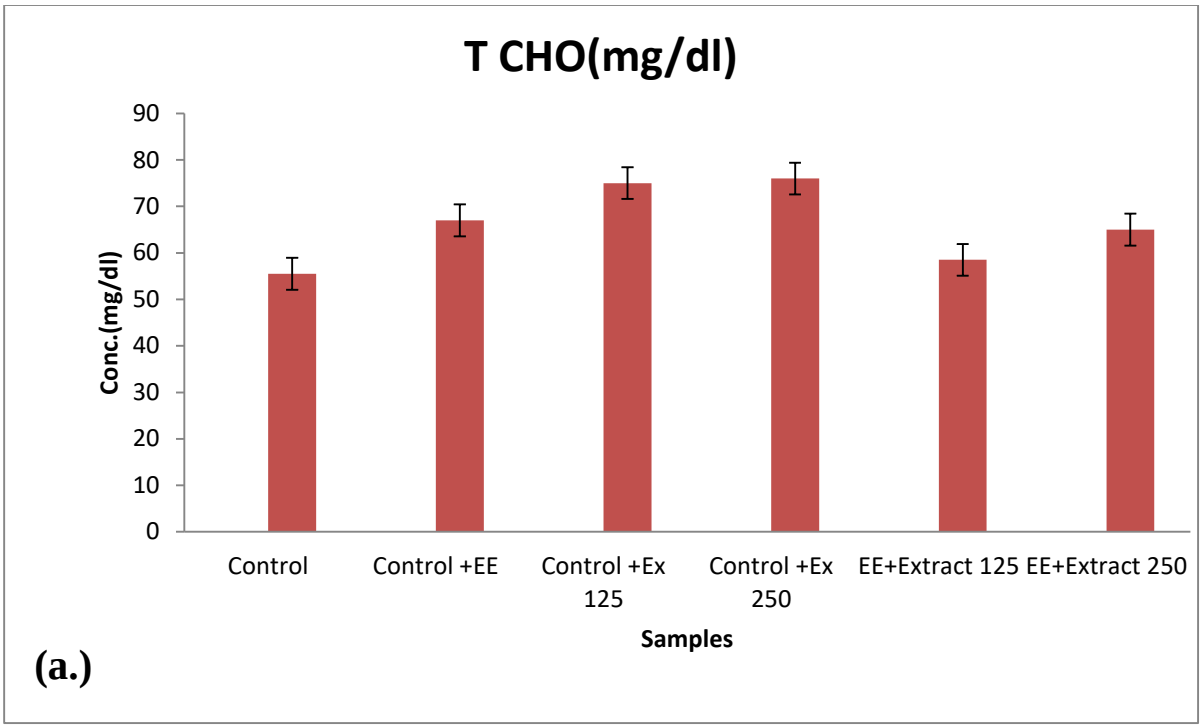
Fig. 6 Effect of plant extract on liver metabolism (a) SGOT (b) SGPT

Estrogenic/anti-estrogenic study

In order to get a proper insight into the anti-fertility mechanism of this plant, we further evaluate its estrogenic and anti-estrogenic effects and evaluated its effect other biochemical parameters. Estrogen levels were found to be significantly higher in animals treated with 1μg EE, when compared to control animals. Our results suggests that plant extract possesses strong estrogenic effect as treatment of animals with plant extract resulted in dose dependent increase in estrogen level, which were found to be significantly higher than control animals. To evaluate anti-estrogenic effect of plant extract, animals were injected EE (1μg) along with oral treatment of plant extract at 125 and 250 mg/kg for 12 days. Results revealed that plant extract did not have any anti-estrogenic effect, rather, estrogen levels were observed to significantly increase after treatments in dose dependent manner. Further, there was no significant effect of EE or plant extract treatment on progesterone levels, suggesting that plant extract is having specificity towards elevating estrogen levels without altering the levels of other crucial hormones for fertilization. We also evaluated the effect of various treatments on the serum levels of total cholesterol (TCHO), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), SGOT, SGPT and alkaline phosphatase (ALP). Treating animals with EE and plant extract significantly elevated the levels of TCHO [fig. 7(a)] and TG [fig. 7(b)], when compared to control animals. Results of extract treatments were comparable to EE treatment and it was observed that increase in TCHO and TG level were more pronounced in animals treated with 125 mg/kg extract than 250 mg/kg treatment. Levels of HDL were significantly increased by extract treatments [fig. 7(c)] and results were comparable to EE treatment. It may be noted that levels of HDL in group 5 and group 6 were comparable and did not differed significantly from group 2, group 3 or group 4, although HDL levels showed slight reduction in EE + extract treated groups. We did not observe any significant changes in for LDL levels and results of all the treatments were comparable to control animals [fig. 7(d)]. Results of VLDL revealed that EE and extract treatment significantly elevated VLDL levels when compared to control [fig. 7(e)]. Our results demonstrated that 125 mg/kg extract treatment demonstrated higher levels when compared to 250 mg/kg treatment, though levels in both the extract treatments were higher than EE treatment. These results suggest that extract treatment is having a strong estrogenic effect and did not possess any anti-estrogenic effect, which might be the cause for the anti-fertility effect of this plant. Further we also demonstrated that ovariectomy altered the serum levels of TCHO, TG, HDL and VLDL, which were attenuated by extract treatments, which might provide additive advantage in imparting anti-implantation effect of the extract (Table 3).

Table 3 Effect of Trillium govanianum rhizome extract on biochemical parameters in mature ovariectomized female rats

Treatment / Parameters	Control	Control+EE	Control+ Extract (125mg/kg)	Control+ Extract (250mg/kg)	EE+Extract (125mg/kg)	EE+Extract (250mg/kg)
TCHO ± SD (mg/dl)	55.5±16.26	67±2.82	75±19.79	76±0	58.5±2.12	65±12.72
Triglyceride ± SD (mg/dl)	67.5±19.09	90±8.48	114±7.07	106±15.55	111±43.84	82±8.48
HDL ± SD (mg/dl)	39±8.48	31±2.82	33±14.14	29±7.07	28±5.65	29.5±3.53
LDL ± SD (mg/dl)	17.8±0	18.5±2.12	20±7.07	26±4.24	8.5±0.70	19.5±10.6
VLDL ± SD (mg/dl)	13.5±3.81	18±1.69	22.8±1.41	21.2±3.11	22.2±8.76	16.4±1.69
ESTROGEN ± SD (pg/ml)	48.255±3.58	2251.38±117.25	446.29± 229.42	436.2±1.27	2922.11±110.15	2138.39±1131.1
PROGESTERON (ng/ml)	23.09	2.69	30.46	33.6	2.59	3.31



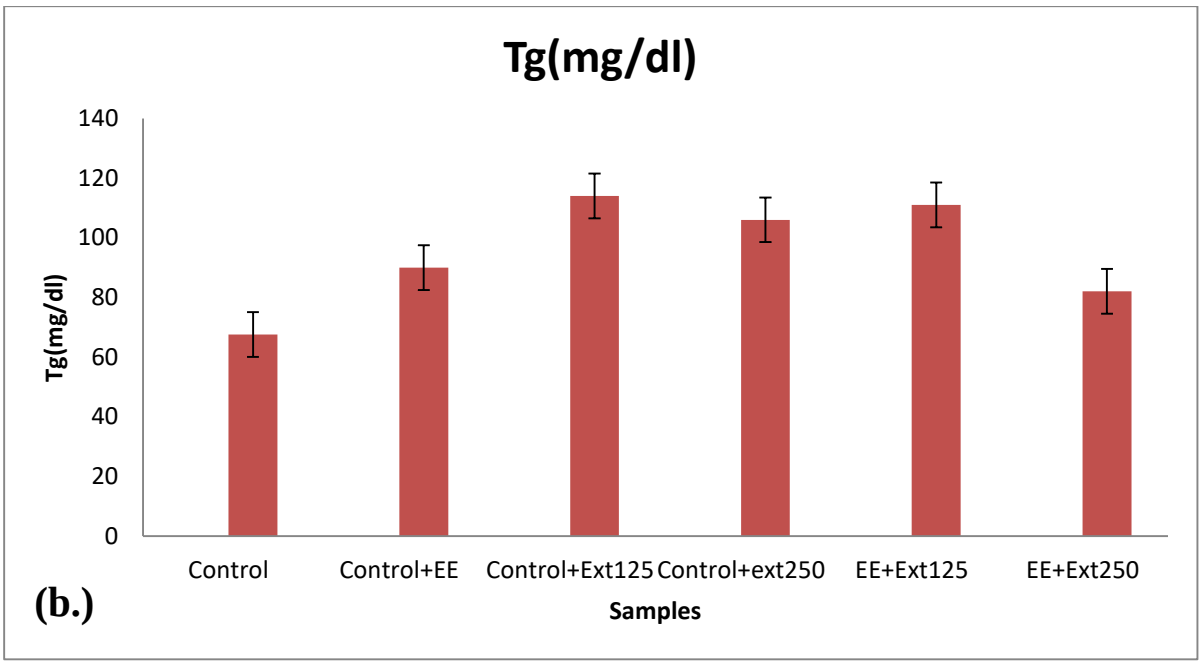
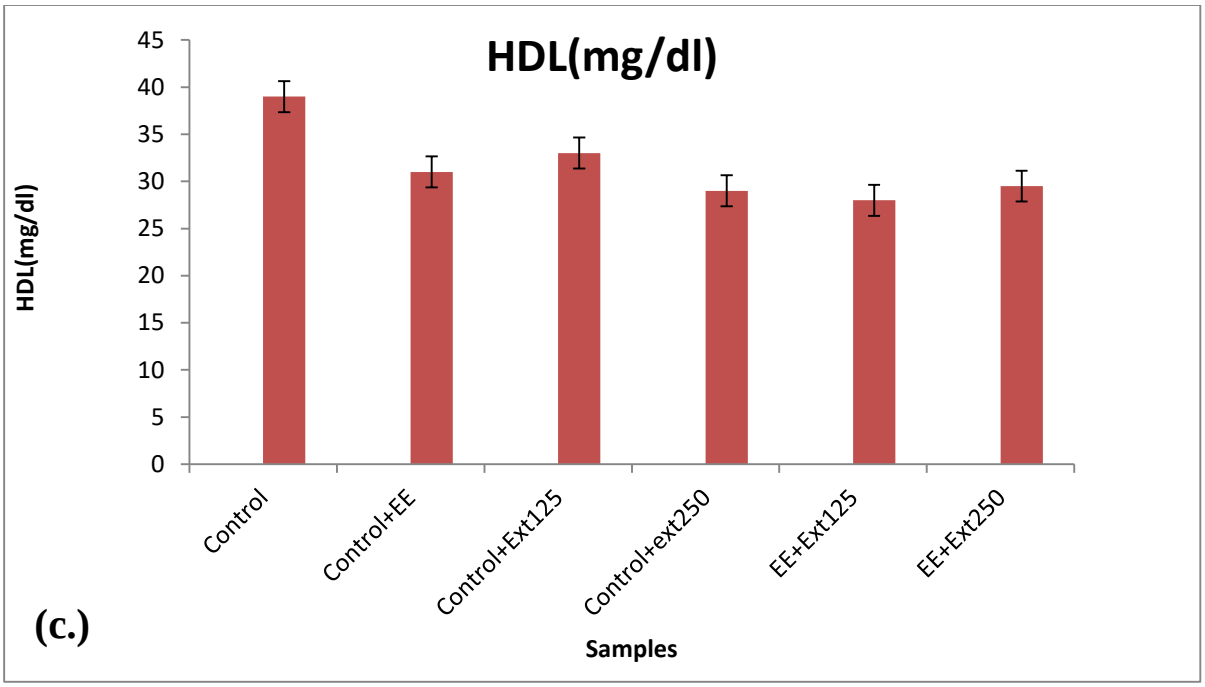


Fig.7 Effect of plant extract on female Wistar rats (a.) T CHO (b.) Tg



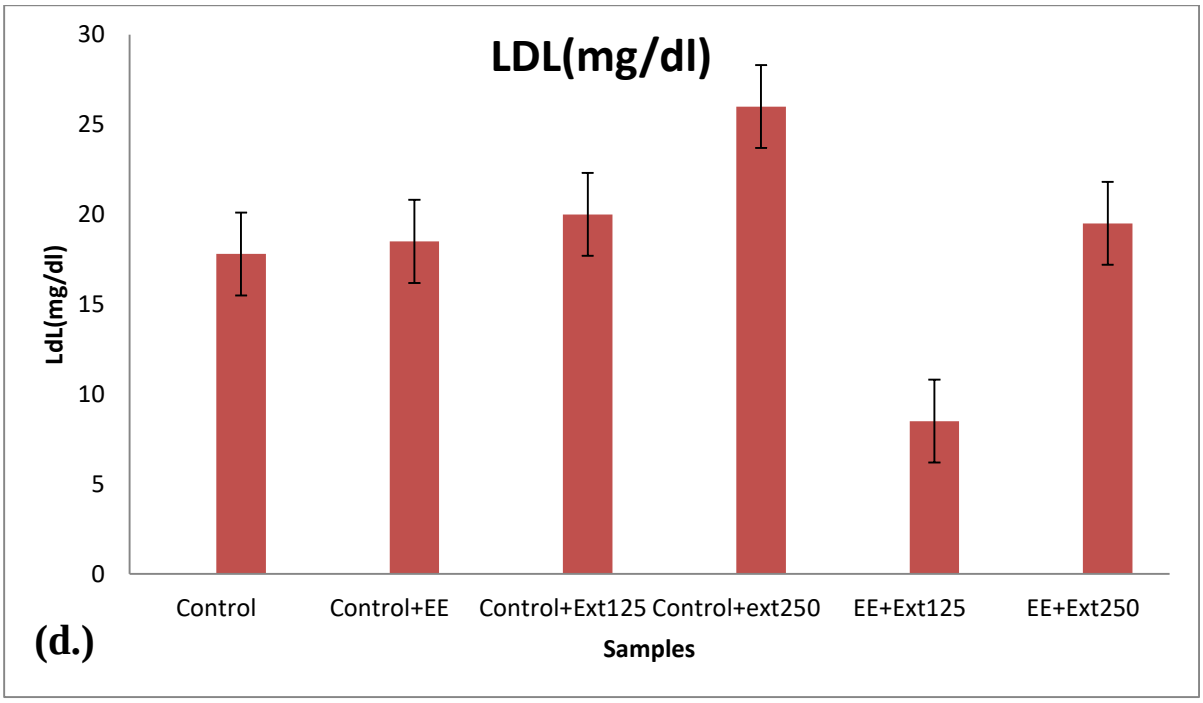
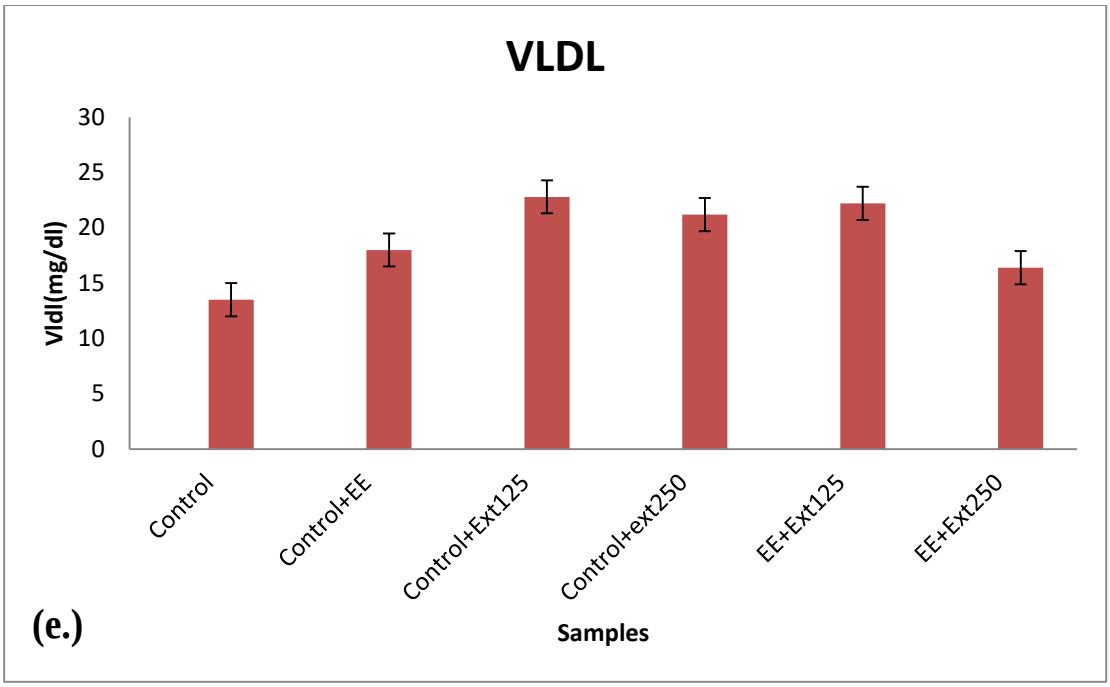


Fig. 7 Effect of plant extract on female Wistar rats (c.) HDL (d.) LDL



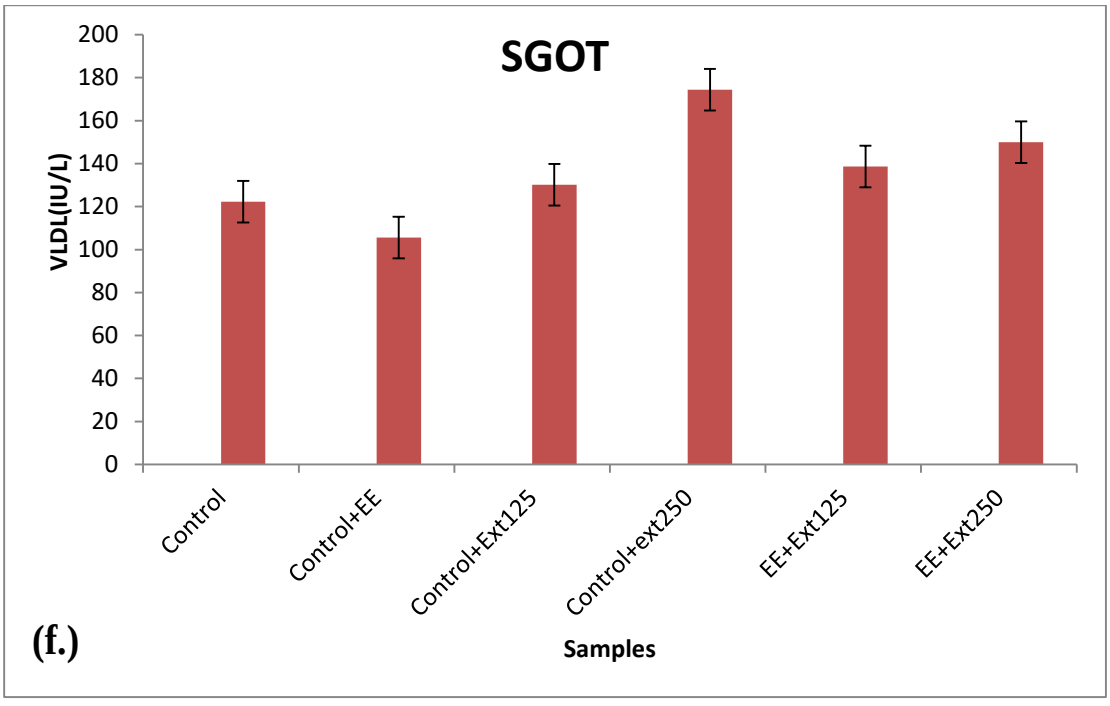
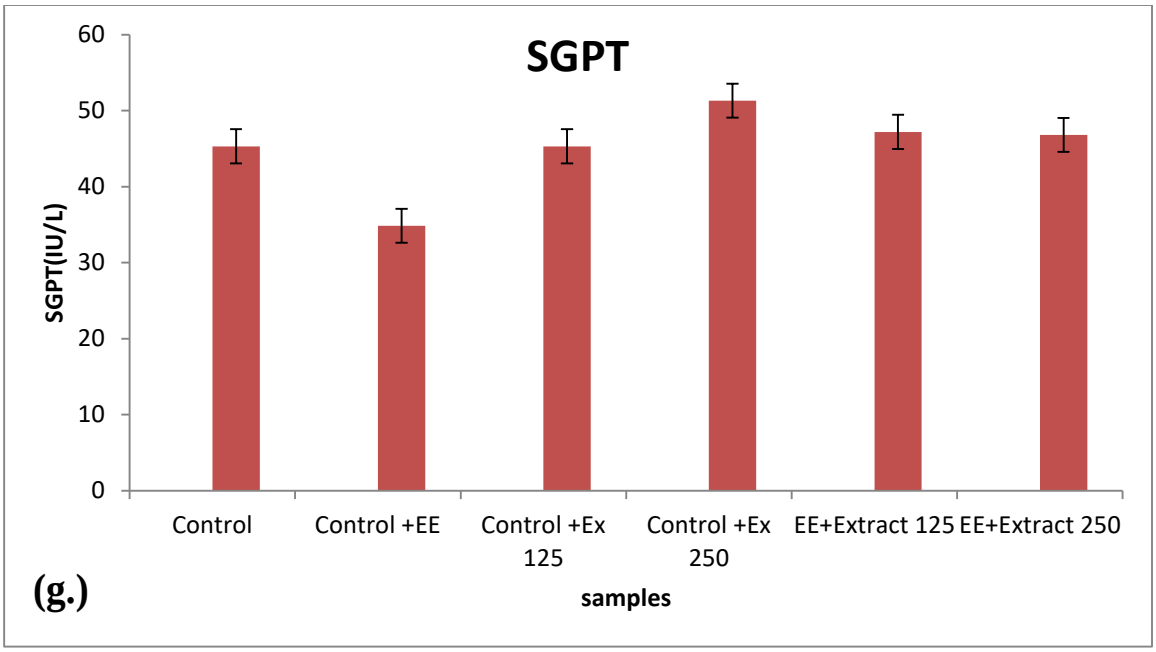


Fig. 7 Effect of plant extract on female Wistar rats (e.) VLDL (f.) SGOT



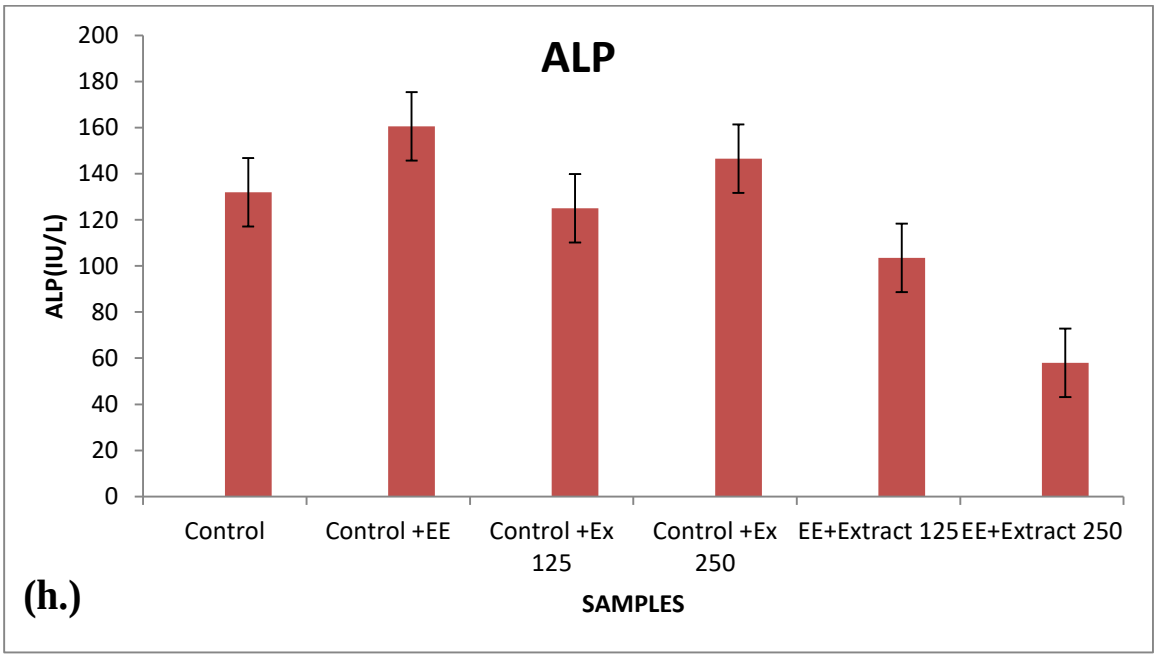
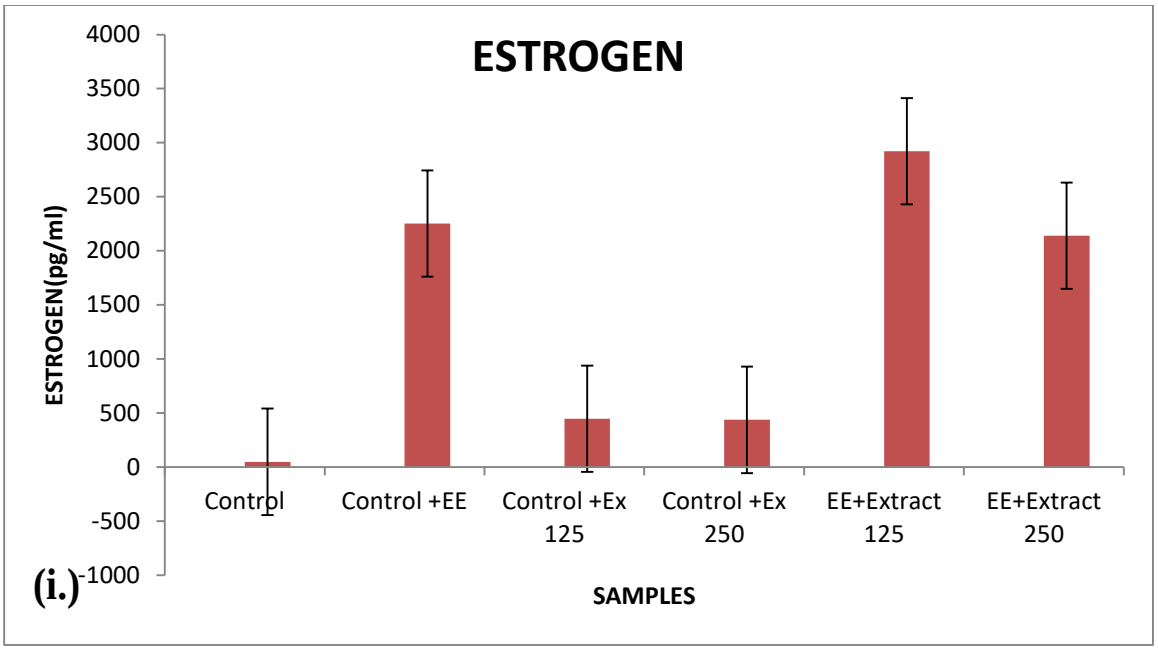


Fig.7 Effect of plant extract on female Wistar rats (g.) SGPT (h.) ALP



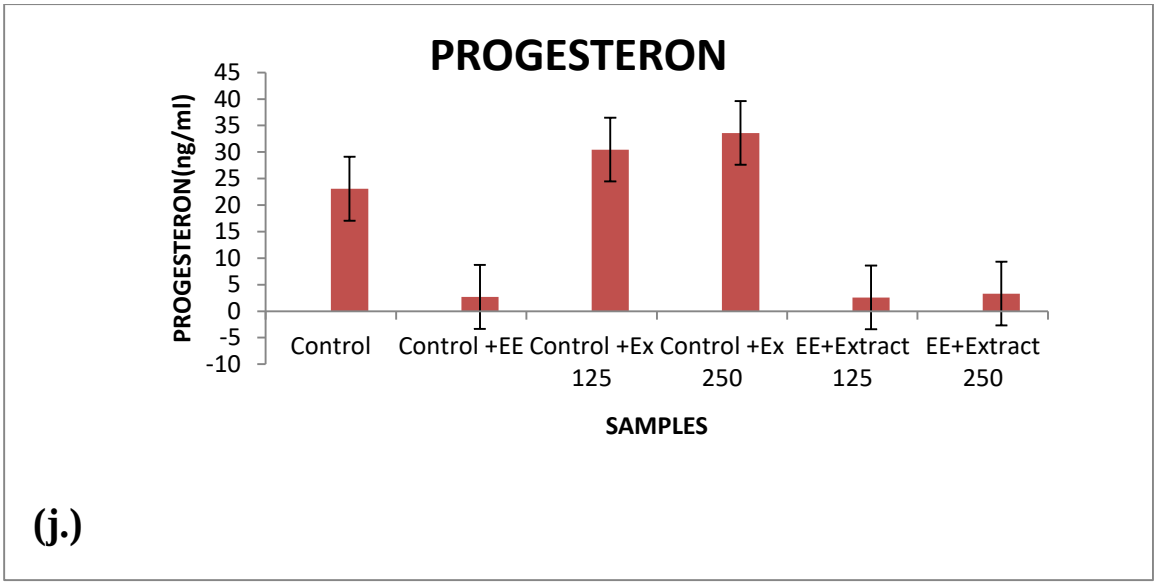


Fig. 7 Effect of plant extract on female Wistar rats on (i.) Estrogen

(j.) Progesteron

Discussion

In the present study we have explored the anti-fertility (anti-implantation & estrogenic/anti-estrogenic) effect of hydroalcholic rhizome extract of ***Trillium govanianum*** in female Wistar rats which was reported by Chauhan et. al.(2016) as a source of steroidal and sex hormones.

The anti-fertility effect of this extract might be attributed to more than one mechanisms. One of the possible mechanism could be the inhibition of the implantation which was shown by significant decrease of implantation sites.

For implantation to occur,especially in rats,the exact equilibrium of estrogen and progesterone hormones is required to create a milieu ideal for implantation. In first study i.e, anti-implantation study,the extract significantly inhibited the implantation in female rats at both the doses. Inhibition of implants was 94.87% and 100% at 125 and 250mg/kg doses respectively. The inhibition of implantation caused by the extract might be due to the unusual thickening of the endometrial lining. An increase in body weight indicated the estrogenic nature of the extract.

The estrogenic substances in the extract causes the expulsion of ova from the fallopian tube and also disrupts the functional equilibrium between the endogenous estrogen and progesterone which may result in failure in fertility (Gebrie et al, 2005).

As reported by Shibeshi et al, 2006 the anti-implantation and abortifacient effects might be mediated through estrogenic activity as estrogens is the main cause to increase the uterine contractibility to expel fertilized egg. For implantation and occurrence of pregnancy exact equilibrium of secretions of both the hormones is necessary.Any disturbance in levels of these hormones may cause anti-implantation or induced abortion (Psychoyos,1996).

It has already been reported that steroids (Natraj et al,2007),flavonoids and alkaloids occurring in variety of plants have shown anti-fertility effect (Anderson et l ,1972) in laboratory animals. Preliminary phytochemical analysis of this plant extract showed the presence of alkaloids, flavonoids, tannins, inulin and steroid. If any of these phytoconstituents is present in the extract,it might be responsible for the anti-fertility activity.

For the exploration of anti-implantation the histopathological examination was carried out in order to confirm its effect at tissue level. Moreover, we have also carried out toxicity studies where we acquired the conclusion that with the increase in dose of the extract , there was an increase in the expulsion of liver enzymes as compared to the control group.

The estrogenic effect of the extract was further observed by significant increase in TCHO,triglycerides and HDL in serum when administered alone or along with EE at higher dose.The estrogen levels were found to be significantly higher in animals treated with EE when compared to control animals.However,significant changes in LDL levels

and result of all the treatments were comparable to control animals.Results of VLDL revealed that EE and extract treatment significantly elevated VLDL levels when compared to control.

As also mentioned in Shivam et al (2016) the Trillium genus possess anticancerous activity as it declines the number of cancerous cells in the body. This anticancerous effect of the this genus gives additional advantage of *Trillium govananium* .

All these results suggest that plant extract treatment is having a very strong estrogenic effect and does not possess any anti-estrogenic effect which might be responsible for anti-fertility effect of the extract.

CHAPTER - 5

CONCLUSION

Due to the effective medicinal properties of *Trillium govanianum* , its demand in the market is very high. Relentless collection and smuggling of this plant because of its high price (Rs 2,500-5,000) have made it a highly prized medicinal herb in Himalayas.

In our present study,we have made our way through the objective of preparing hydroalcoholic (30:70) rhizome extract of *Trillium govanianum* (Nag chhatri) and and have tested the extract for various parameters through preliminary phytochemical screening of the extract. The toxicity of the plant extract was also tested from the serum of female Wistar rats. After its toxological analysis where we find that this extract is not causing any toxicity through liver functioning test i.e SGOT and SGPT.We have also evaluated the anti-fertility effect of *Trillium govanianum* extract on female Wistar rats followed by its biochemical and histopathological evaluation.

Thus, results suggest that plant extract treatment shows strong inhibition of implantation ,having a very strong estrogenic effect and does not possess any anti-estrogenic effect which might be responsible for anti-fertility effect of the extract. So this study could taken as lead for the generation of formulation so that herbal contraceptive which can be available for the masses for the better health and future .

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