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EFFECT OF ETHANOLIC EXTRACT OF Urtica dioica ON MICE MODEL OF STRESS INDUCED DEPRESSION

By:

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Submitted in partial fulfillment of the Degree of

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DEPARTMENT OF BIOTECHNOLOGY, BIOINFORMATICS AND PHARMACY

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY WAKNAGHAT

Learning Resource Center JUIT Waknaghat

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CERTIFICATE

This is to certify that the work entitled **"Effect of ethanolic extract of *Urtica dioica* on mice model of stress induced depression"** submitted by **Ms. Anjali sharma** and **Ms. Jhanvi mohanta,** in partial fulfillment for the award of Degree of Bachelor of Pharmacy of Jaypee University of Information Technology, Waknaghat (Solan), 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.

Signature of Supervisor

Name of Supervisor **Dr. Udayabanu .M**

Designation **Sr. Lecturer**

Date 27/5/13

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Name of students

Janhavi Mohanta (091761)

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Date: 27-5-2013

SUMMARY

Urtica dioca was extracted by using continuous hot extraction method in absolute ethanol and phytochemical test for 5-HT determination was done. Qualitative test was carried out for coumarine glycosides, tannis, flavonoids, carbohydrates, steroid, proteins which came out to be positive with ethanol and negative with methanol. The assay of catalase enzyme activity was performed. The plant extract did not show any alteration in the catalase activity, which is known to be decreased in stress condition, resulting in elevated free radical formation.

CHAPTER 1

INTRODUCTION

Depression is the most common of the affective disorders; it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. [1]

The symptoms of depression include emotional and biological components.

Emotional symptoms: misery, apathy and pessimism low self-esteem: feelings of guilt, nadequacy and ugliness Indecisiveness, loss of motivation.

Biological symptoms: retardation of thought and action loss of libido Sleep disturbance and loss of appetite.

There are two distinct types of depressive syndrome, namely unipolar depression, in which the mood swings are always in the same direction, and bipolar affective disorder, in which depression alternates with mania. Unipolar depression is commonly (about 75% of cases) non-familial, clearly associated with stressful life events, and accompanied by symptoms of anxiety and agitation; this type is sometimes termed *reactive depression.* Bipolar depression, which usually appears in early adult life, is less common and results in oscillating depression and mania over a period of a few weeks. There is a strong hereditary tendency, but no specific susceptibility genes have been identified either by genetic linkage studies of affected families, affected individuals.[2]

STRESS HORMONES

The corticotrophin-releasing hormone (CRH) is released from the hypothalamus in response to the psychological stress by cortical brain regions. This hormone induces the secretion of pituitary corticotrophin, which stimulates the adrenal gland to release cortisol into the plasma. The physiologic response to stress is partly gender-specific: women show generally greater stress responsiveness than men, which is consistent with the greater incidence of major depression in women. Altered stress hormone secretion is most prominent in depressed subjects with a history of childhood trauma. Elevated cortisol may act as a mediator between major depression and its physical long-term consequences such as coronary heart disease, type II diabetes, and osteoporosis. CRH produces a number of physiological and behavioural alterations which resemble the symptoms of major depression, including decreased appetite, disrupted sleep, decreased libido, and psychomotor alterations. [3]

Extract of stinging nettle

Stinging nettle or common nettle, *Urtica dioica*, is an herbaceous perennial flowering plant, native to Europe, Asia, northern Africa, and North America, and is the best-known member of the nettle genus Urtica. The plant has many hollow stinging hairs called trichomes on its leaves and stems, which act like hypodermic needles that inject histamine and other chemicals that produce a stinging sensation when contacted by humans and other animals. [4]

SCIENTIFIC CLASSIFICATION[5]

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Rosales
- Family: Urticaceae
- Genus: Urtica
- Species: U. dioica
- Binomial Name : *Urtica dioica*

CONSTITUENTS

It contains on average 22% protein, 4% fats, 37% non-nitrogen extracts, 9-21% fiber, and 19-29% ash. The leaves contain about 4.8 mg chlorophyll per gram of dry leaves, depending on whether the plant was grown in the sun or shade. The dried leaf of nettle contains 40% protein. They are one of the highest known sources of protein in a leafy green, and of superior quality than many other green leafy vegetables The leaves are also noted for their particularly high content of the metals selenium, zinc, iron, and magnesium. The fresh leaves contain vitamins A, C, D, E, F, K, P, and b-complexes as well as thiamin, riboflavin, niacin, and vitamin B-6, all of which were found in high levels, and act as antioxidants [6].

Phytochemicals:

Histamine, acetylcholine, serotonin, flavonol glycosides, sitosterol, lectin, coumarins, hydroxysitosterol, tannins, lignans, scopoletin [7]

DISTRIBUTION:

Stinging nettles are abundant in northern Europe and much of Asia.In North America it is widely distributed in Canada and the United States also can be found in northernmost Mexico. It grows in abundance in the Pacific Northwest, especially in places where annual rainfall is high.[8]

USES:

The stinging nettle has stimulating action on the kidneys and bladder. Nettle shoots, eating during spring, helps to clean the body of toxins. Stinging nettle is used to treat inflammation of the urinary tract. Stinging nettle improves the excretion of uric acid thereby reducing the symptoms of gout and arthritis. Stinging nettle leaves have diuretic properties. Nettle root is also used for the treatment of urinary retention caused by prostate enlargement [9][10].

APPLICATIONS:

Aerial part: INFUSION – This herbal form of the nettle remedy can be used to stimulate the circulatory system in people suffering from impairment in the flow of blood and it can also be used as a detoxification agent to cleanse the system of toxins in individuals afflicted by disorders such as arthritis, it can be used to treat rheumatism, to treat symptoms of gout, and to treat symptoms of eczema. The herbal infusion made from the nettle also helps in increasing the flow of milk in nursing mothers with lactation issues. A revitalizing spring tonic can be produced from the fresh shoots of the nettle.

TINCTURE - The herbal tincture form of the nettle is utilized in combination with other beneficial herbs in the treatment of various disorders such as arthritic conditions, to treat various skin problems, and in the treatment of heavy uterine bleeding in women suffering from menstrual diseases.

WASH – The herbal remedies made from the nettle can also be used as a healing salve and herbal wash and applied to burns, to insect bites, and to wounds.

JUICE – The herbal nettle remedy can also be used in the form of a nettle juice and this can be prepared by liquefying the whole fresh plant to make a good herbal tonic for the

treatment of debilitating conditions and cases of anemia, this same tonic can be used to soothe the stings of the nettle hairs.
The nettle based tonics are also often prescribed for the treatment of cardiac insufficiency coming along with disorders such as edema.

POWDER – Herbal remedies made from the powdered leaves of the nettle can be inhaled as a snuff for the treatment of nosebleeds.

Root: HAIR RINSE – The nettle roots can also be used to make an herbal decoction, which can be used as a rinse for the treatment of dandruff, to stem the causes of falling hair, and as a general conditioner for a healthy scalp.

OINTMENT – As an herbal nettle ointment, the nettle is used to topically treat cases of hemorrhoids, the ointment is directly applied to the affected region of the body.

COMPRESS – The herbal remedies made from the nettle can be used to make a herbal compress by soaking a pad in the herbal tincture of the nettle[11]

TOXICITY STUDIES[12]

Toxicity is a property of matter.

- It is a biological property.
- It is the ability of a material to injure a living organism by other than mechanical means.
- The degree to which something is poisonous

Toxicity has two main components: the effect caused and the level of exposure (dose) at which the effect is observed. Some tests are designed specifically to detect a particular effect (such as skin and eye irritancy, skin sensitisation and mutagenicity studies). Other tests (suchas sub-chronic and chronic studies) are designed to detect a wider range of less-specific effects on organs or body systems and the dose range over which the effect develops. Whenever we administer a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. The types of toxicity tests which are routinely performed by pharmaceutical manufactures in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 the dose which has proved to be lethal (causing death) to 50% of the tested group of animals. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds.

AIM OF ACUTE TOXICITY TEST[13]

To determine the therapeutic index, i.e. ratio between the lethal dose and the pharmacologically effective dose in the same strain and species (LD50/ED50).
The greater the index, safer is the compound. LD50 with confidence limits is to be established on one common laboratory species such as mouse/rat using the standard method. The LD50 dose thus found was administered to guinea pigs, rabbits, cats or dogs on weight basis (on basis of relative surface area gives better results).
To determine the absolute dose for a species in the column, the absolute dose given to the species in a row was multiplied by the factor given at intersection of the relevant row and column (Table 1). Because of species variation, several species of animals (one rodent and one non-rodent) were used to determine LD50. When a clearly different response was observed in any of these species, a larger number of that species needs to be tested to establish theapproximate LD50 value.

Table 1: Surface area ratios of some common laboratory species and man

	20 g Mouse	**200 g Rat**	**400 g Guineapig**	**1.5 kg Rabbit**	**2 kg Cat**	**4 kg Monkey**	**12 kg Dog**	**70 kg Man**
20 g Mouse	1.0	7.0	12.25	27.8	29.7	64.1	124.2	387.9
200 g Rat	0.14	1.0	1.74	3.9	4.2	9.2	17.8	56.0
400 g Guineapig	0.08	0.57	1.0	2.25	2.4	5.2	10.2	31.5
1.5 kg Rabbit	0.04	0.25	0.44	1.0	1.08	2.4	4.5	14.2
2 kg Cat	0.03	0.23	0.41	0.92	1.0	2.2	4.1	13.0
4 kg Monkey	0.016	0.11	0.19	0.42	0.45	1.0	1.9	6.1
12 kg Dog	0.008	0.06	0.10	0.22	0.24	0.52	1.0	3.1
70 kg Man	0.0026	0.018	0.031	0.07	0.076	0.16	0.32	1.0

ACUTE TOXICITY TEST

Acute toxicity tests are generally the first tests conducted. Acute toxicity is produced after administration of a single dose or multiple doses in a period not exceeding 24 hours, up to a limit of 2000 mg/k g. Objective of acute toxicity studies is to identify a dose causing major adverse effects and an estimation of the minimum dose causing lethality.
Standardized tests are available for oral, dermal and inhalation exposure.[14]

Table 2 Basic parameters of acute toxicity tests:

Species	**Rats, rabbits**
Age	Young adults
No. of animals	5 of each sex per dose level
Dosage	3-5 dose levels recommended, exposures are single doses or fractional dose up to 24hours for oral and dermal studies and 4hours exposure for inhalation studies
Observation period	14 days

CLASSIC ACUTE TOXICITY TESTING

A variety of factors should be considered when planning and organizing a study for the determination of LD50, not all of which are associated with the calculation of 95% confidence limits. Most preparations among the different routes of administration are similar although some changes are required as shown below.

Oral LD50:Table summarizes some of the parameters and factors that are monitored as a classic oral LD50 study commences. Such Factors include but are not limited to (1) randomization of animals, (2) maintenance of a narrow range of body weights, (3) appropriate number of animal per group, (4) identification of individual test subjects, (5) fasting, and (6) availability of water. Preliminary range-finding experiments are initiated to minimize the extent of no lethality or 100% lethality, thus reducing the number of groups in

the total study. This method also improves the precision of the LD50 determination.

Table 3: Consideration during preparation for oral LD50 study

Parameter	Factor for consideration
Randomization of animals	Unbiased distribution into groups
Narrow range of body weights	Uniform distribution of similar sized animals
Number of animals	For classic LD50 average of 10 per treatment group
Identification of individual animals	Ensure individual observation and monitoring: allows for group Housing
Fasting (16 to 24 hr)	Optimal GI absorption
Water ad libitum	Prevention of dehydration

Dermal LD50

Dermal LD50 studies are conducted on toxicants if the probable exposure route is through skin absorption. As with the oral LD50, lethality is generally assessed in two species, one of which is non-rodent. Also, the test substance is applied to shaved skin in increasing doses to several groups of experimental animal, one dose per group. The parameters involved with the dermal LD50 determination are the same as those described for the oral LD50. Some of the factors that are unique to dermal studies are summarized in table

Table 4 Consideration during preparation for Dermal LD50 study

Parameter	**Factor for consideration**
Formulation	Solids dissolved in water or inert oil-based vehicle
Application to skin	Shave for 24 hr prior to test, uniform application
Absorption of toxicant	Depend on water- soluble or lipid- soluble properties

Variability of results	High degree of variability; determine LC50

Most of the variability in dermal LD50 studies arises from these parameters. Incomplete absorption of a toxicant due to poor vehicle solubility, inability to penetrate intact skin, and lack of uniformity in the application method are largely responsible for the inconsistency seen with dermal LD50 studies. Because of known or suspected inadequate absorption, an LC50 (median lethal concentration 50%) is determined after absorption and may substitute for the lack of a reliable dosage determination. Dermal toxicity testing is required to determine this.

Skin

In order to evaluate the degree of skin irritation that may be exerted by a potentially toxic substance, it is necessary to examine the effect in human subjects. Due to enormous variability in the response of the skin of different animal species to toxic chemicals, there is little value in skin irritancy testing that requires extrapolation of finding from one species to another.

Eyes

Any chemical with irritant properties when applied to the skin is also likely to be irritant to the cornea and conjunctiva, and ocular irritancy tests need not be carried out. The most widely used predicting test for ophthalmological irritancy is still the Draize test in rabbits.

Mucosal surface

Irritancy testing of mucosal surfaces is necessary when substances are designed for application to particular surfaces such as the vagina, where local factors such as pH have to be considered. There is little difference between species, and between individuals, in mucosal responses to toxic injury.

Inhalation LD50

Air-borne toxic materials that are transported via gases, aerosol, smoke, or ventilation necessitate the determination of acute inhalation LD50. for the classic LD50 , rodents and non-rodents are exposed for 4 to 24 hr to a test substance in increasing concentrations (one concentration per group, at least four doses plus a control group).

Well controlled inhalation studies incorporate a negative pressure dynamic inhalation system with programmable airflow settings. Currently used systems are capable of delivering precise test material concentration, continuously monitoring toxicant in the

exposure chamber. The range of doses is capable of producing a corresponding series of toxic effects and mortality rates to facilitate assessment of acute toxicity for LD50 or LC50 determination.

Table 5:Consideration during preparation for Inhalation LD50 study

Parameter	Factor for consideration
Concentration of delivered test Agent	Air flow rate into chamber; Air temperature and humidity; monitoring concentration in chamber; check integrity of exposure chamber
Particle size	Determines distribution to target organ
Control group	Determines distribution to target organ

SUB-ACUTE TOXICITY TEST

Subacute toxicity tests are employed to determine toxicity likely to arise from repeated exposures of several weeks to several months.Standardized tests are available for oral, dermal,and inhalation exposures. Detailed clinical observations and pathology examinations are conducted. Subacute toxicity tests-in which animals (usually rats and dogs) are dosed daily, starting at around expected therapeutic level and increasing stepwise every 2 or 3 days until toxic signs are observed.[15]

Table 6 Basic parameters of subacute toxicity tests:

Species	**Rodents, Rabbits, Non-rodents**
Age	Young adults
No. of animals	10 of each sex for rodents, 4 of each sex for non-rodents per dose level
Dosage	3-5 dose levels + a control groups
Observation period	90 days

CHRONIC TOXICITY TEST

Chronic toxicity tests determine toxicity from exposure for a substantial portion of a subject's life. They are similar to the subchronic tests except that they extend over a longer period of time and involve larger groups of animals.

Table 7 Basic parameters of chronic toxicity tests:

Species	**Two species – rodent & non-rodent(rat and dog)**
Age	Young adults
No. of animals	20 of each sex for rodents, 4 of each sex for non-rodents per dose levels
Dosage	3-5 dose levels; include a toxic dose level
Observation period	12-24 months(1 month, 3 months, 6 months, 1 year)

The objective of these chronic toxicity studies is to characterize the profile of a substance in a mammalian species (primarily rodents) following prolonged and repeated exposure. The Test Guideline focuses on rodents and oral administration. Both sexes should be used. For rodents, at least 20 animals per sex per group should normally be used at each dose level, while for non-rodents a minimum of 4 per sex per group is recommended. At least three dose levels should be used in addition to the concurrent control group. Frequency of exposure normally is daily, but may vary according to the route chosen (oral, dermal or inhalation) and should be adjusted according to the toxicokinetic profile of the test substance. The duration of the exposure period should be 12 months. The study report should include: measurements (weighing) and regular detailed observations (haematological examination, urinalysis, clinical chemistry), as well as necropsy procedures and histopathology.[16]

SUB-CHRONIC TOXICITY TESTS- it is similar to chronic tests except that they extend over a longer period of time and involve larger groups of animals

Basic Ninety-Day Oral Toxicity Study (Rodent)

- Groups of 10 males and 10 females are treated with the test substance by gavage at 3 dose levels for 90 days; a vehicle control group is included (40 animals)
- Study measurements include daily clinical observations, weekly body weights and feed consumption, and ophthalmological examination pretest and at study termination
- Hematology and serum clinical chemistry at termination
- Necropsies are performed on any animals that die or upon termination; weights are recorded for major organs
- Full histopathological examinations are conducted on the control and high-dose groups and on any unscheduled deaths
- Target organs are examined in the intermediate groups[17]

TOXICITY STUDY PROTOCOL[18]

Design of acute toxicity test

The test substance was administered orally/intraperitoneal in graduated doses to several groups of experimental animals, one dose being used per group.

Dose selection: This is based on the results of a range finding test. Animals showing severe and enduring signs of distress and pain were killed after anesthesia.

Assignment of animals– Each animal was assigned a unique identification number. A system to assign animals to test groups and control groups randomly is required.

Housing –Animals were group-caged by sex, but the, number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g. morbidity, excitability, etc.) may indicate the need for individual caging

Dose levels and dose selection

The substance used in the toxicity tests should be as pure as the material eventually to be given to humans.At least three to four dose levels were used, spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data should be sufficient to produce a dose-response curve and permit an acceptable estimation of LD50.If the lethality of the groups is such that only one group has a lethality falling between 4 and 6 probits, more groups may be required.

Solvent: Where necessary, the test substance was dissolved or suspended in a suitable solvent.

Volume: This depends on size of the test animal. In rodents it should not exceed 1 ml/100 g body weight maximum of 50 ml/kg. Injection was given slowly and uniformly. This will avoid undue killing.

Maintenance of experimental rats

The animals were maintained in 12 hours light and dark cycle at 28°C ± 2°C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India. All animal experiments were performed according to the ethical guidelines suggested by the institutional animal ethics committee (IAEC). Paddy husk was used as beding material and changed twice a week.[19]

Route of administration

The LD50 value depends on the route of administration. Usually the values are found to increase with the following sequences of routes: intravenous, intraperitoneal, subcutaneous and oral. The intravenous route is preferable to the intraperitoneal route (because many drugs get detoxified by the liver if the intraperitoneal route.[17]
is employed).

Weekly body weight

The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.[20]

Signs recorded during acute toxicity studies:

During the four-week dosing period, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and up to 4 hour after dosing. These are increased motor activity, anesthesia, tremors, arching and rolling, clonic convulsions, ptosis, tonic extension, lacrimation, Straub reaction, exophthalmos, pilo-erection, salivation, muscle spasm, opisthotonus, writhing, hyperesthesia, loss of righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis and analgesia. After the test the animal is the sole occupant of the cage, with free access to food and water during the observation period of 1–2 h, and thereafter at intervals. At the end of the test surviving animals were weighed and sacrificed. The relative organ weight of each animal was then calculated as follows,

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

A gross necropsy was performed; all gross pathology changes were recounted. If necropsy cannot be performed immediately after the death of the animal it should be refrigerated to minimize autolysis. Necropsies must be performed no later than 16 h after death.[20]

Catalase[21]

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human catalase is approximately 7, and has a fairly broad maximum (the rate of reaction does not change appreciably at pHs between 6.8 and 7.5). The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species.

Objectives

- Ethanolic extraction the *Urtica dioca* by continuous hot percolation method.
- Phytochemical analysis of the ethanolic extract.
- To study the effect of *Urtica dioica* extract on erythrocyte catalase activity.

CHAPTER 2

EXTRACTION

Urtica dioca was extracted by using continuous hot extraction method in absolute ethanol.

5-HT DETERMINATION TEST

Material required-Aqueous 5% sodium carbonate, folin Cio calteu reagent, UV chamber

Methodology

1 ml sample solution(urtica solution) was taken and 10 ml water was added to it. 2 ml folin Cio calteu reagent(diluted 1:5 with water) was added to the above made solution. Then 2 ml sodium carbonate solution of the above prepared 5% solution was added and volume was made upto 25 ml with water. Thereafter the above prepared solution was taken and separately added to blank (no urtica) and to sample solution (urtica).Then the solutions were kept at room temperature for 60 minutes and absorbance was taken at 736 nm.

QUALITATIVE TESTS

Coumarin glycoside

Material required- test tube, filter paper moistert-C, hot water bath, UV chamber.

Methodology :

Small amount of extract was taken in test tube and covered with a filter paper moistert-C with dilute sodium hydroxide solution. Covered test tube was placed on water bath for several minutes. Paper was removed and exposed to ultraviolet (UV) light, green fluorescence indicates the presence of coumarin glycoside.

Tannins (phenolic compound)

Ferric chloride test

Extract was treated with ferric chloride solution, blue colour indicates presence of hydrolysable tannins and green colour indicates presence of condensed tannins.

Flavonoids

Alkaline reagent test

Extract was treated with few drops of sodium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops dilute acid indicate presence of flavonoids.

Carbohydrates

Molisch' test

To the extract, few drops of alcoholic α-naphthol and then few drops of concentrated sulphuric acid through sides of test tube was added. Purple to violet rings appear at the junction shows the presence of carbohydrate.

Volatile oil

To a thin section of drug sudan III solution was added,red clour was obtained by globules indicated presence of volatile oil.

Steroid

Libermann-burchard test

To the extract few drops of acetic anhydride was added,boiled and cooled. Then concentrated sulphuric acid was added from the side of the tube,brown ring was formed at the junction two layers and upper layer turned green which showed the presence of steroids and formation of deep red colour indicates presence of triterpenoids.

Proteins

Test with Trichloroacetic acid

To the test solution trichloroactic acid was added,precipitate was formed.

Erythrocyte catalase

Material required- human blood, 10% sodium citrate, 0.9% sodium chloride, phosphate buffer (pH 7.4), hydrogen peroxide, UV chamber.

Preparation:

Sr.n	solution	concentration	volume	supernatant	extract	ethanol	H2O2
Sr.n	solution	(µg/µl)	(ml)	(µl)		(µl)	
1	Blank		3	3000	-	-	-
2	extract	10	3	1990	10	-	1
3		20	3	1980	20	-	1
4		50	3	1950	50	-	1
5		100	3	1900	100	-	1
6		200	3	1800	200	-	1
7		500	3	1500	500	-	1
8	ethanol	10	3	1990	-	10	1
9		20	3	1980	-	20	1
10		50	3	1950	-	50	1
11		100	3	1900	-	100	1
12		200	3	1800	-	200	1
13		500	3	1500	-	500	1

Table 8

Methodology

3ml human blood was collected. 10% sodium citrate was added to the blood as an anticoagulant. Mixture was centrifuged for 10min at 3,000 rpm and the plasma was removed. The erythrocytes were washed three times with 5 ml 0.9% sodium chloride and lysed in 10 volumes of cold water. The whole mixture was centrifuged further for 10min at 3,000 rpm. The cell debris was removed and the clear hemolysate was diluted 100 times phosphate buffer (pH- 7.4). Catalase decomposes hydrogen peroxide (H2O2) to form water and molecular oxygen. In the ultra violet range, H2O2 shows a continual increase in absorbance with decreasing wavelength. At 240nm, H2O2 absorbs maximum light. When H2O2 is decomposed by catalase then the absorbance decrease. The decreased absorbance was measured at 240nm at 30 second intervals up to 1min and the difference in absorbance (A at 240nm) per unit time was measured.

RESULT

5-HT determination test

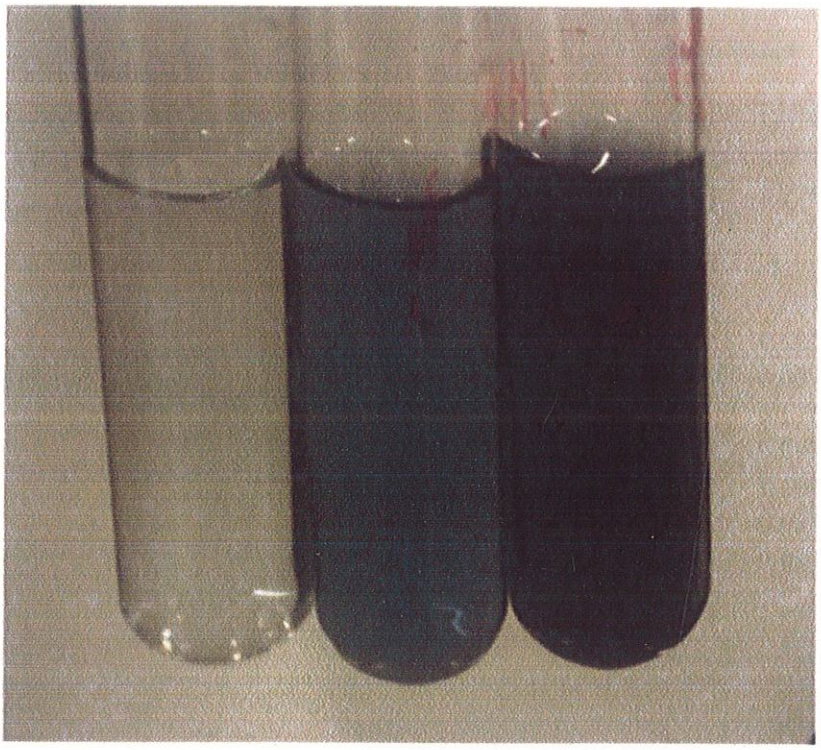

Fig 2.1 right most: blank; middle: ethanolic extract; left: methanolic extract

Qualitative tests

Result

Table 9

Tests	**Ethanol**	**Methanol**
Volatile oil	Positive	Negative
Tannin	Positive	Negative
Flavonoids	Positive	Negative
Proteins	Positive	Negative
Steroid	Positive	Negative
Carbohydrate	Positive	Negative
Coumarin	Negative	Positive

Erythrocyte catalase

Table 10

solution	concentration µg/µl)	Absorbance 0 sec	30 sec	60 sec	90 sec
Blank	-				
Urtica solution	1o	0.002	-0.0971	-0.0971	-0.0971
	20	-0.0971	-0.0971	-0.1762	-0.1762
	50	-0.1251	0.002	-0.222	-0.0971
	100	-0.222	-0.0793	-0.222	-0.222
	200	-0.0971	-0.1251	-0.0971	-0.1762
	500	0.002	-0.1251	0.002	0.002
Ethanol solution	10	0.002	-0.1762	-0.0971	-0.1762
	20	-0.222	-0.222	-0.1762	-0.0793
	50	-0.0971	-0.0971	-0.0971	-0.1762
	100	-0.0793	-0.0971	0.002	-0.1762
	200	-0.0971	-0.1762	-0.0971	-0.222
	500	-0.0793	-0.0971	-0.0971	-0.0793

CONCLUSION

Urtica dioca was extracted by using continuous hot extraction method in absolute ethanol and phytochemical test for 5-HT determination was done. 5-hydroxytryptophan was absent in the ethanolic extract but present in high content in methanolic (methanol:water; 1:1) stinging nettle extract. Phytoconstituents such as volatile oil, tannin, carbohydrate, flavanoids, steroid except coumarin are present in the stinging nettle extract. The plant extract did not show any alteration in the catalase activity, which is known to be decreased in stress condition.

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