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HAEMATOLOGICAL EFFECTS OF OCIMUM SANCTUM (INFLORESCENCE OF TULSI) ON MICE

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ABSTRACT

Ocimum sanctum commonly called as 'holy basil' in English and 'Tulsi' in Hindi is a many branched, erect, stout and aromatic herb about 70 cm high, small sweet scented herb found throughout India and cultivated at a large scale. *Ocimum sanctum* has been used for a wide range of ailments in traditional medicine. The research study was undertaken to investigate the effect of different dose schedules by orally administration 70% ethanolic inflorescence extract of Tulsi (*Ocimum sanctum)* on haematological changes of chronic poisoning in adult albino mice (85-90 gm) and 10 weeks of age male sex. The experimental mice were divided into three groups the first group C_1 received without the inclusion of the inflorescence of *Ocimum sanctum* (control group), whereas the second group C_2 received a dose containing 150 mg/kg/day and third group C_3 received a dose of 600 mg/kg/week. Doses of *Ocimum sanctum* extract were administered daily by oral gavage in the volume of 10 ml/kg body weight, once daily for 21 consecutive days. There were no statistically significant differences in the studied parameters between two groups. The mean Hb, RBC and Platelet value were reduced significantly ($P<0.05$) in ethanolic extract treated mice, MCV and MCH values were initially increased in group C_2 than after decreased in group C_3 and noticed a significant improvement in both of treated groups C_2 & C_3 ($P<0.05$) PCV and WBC values were found as dose dependent manner.

KEYWORDS: Ocimum Sanctum (Inflorescence), Haematological Alterations, Mice.

INTRODUCTION

Plants have always been an important source of drug. A large number of the world's populations, especially in developing countries depend upon medicinal plants as an alternative and complimentary Drugs therapy for various ailments.[1] Plants produce bioactive compounds which act as defense mechanisms against any disease at the same time, may be toxic in nature. However, the general Acceptability of herbal medicines has been limited by a lack of defined chemical characterization, does regimen, and adequate toxicity data to evaluate their safety.[2]

Ocimum sanctum, holy basil, or *tulsi* ,is an aromatic plant in the family *Lamiaceae*, It is commonly used home remedy and has been advocated for various ailments like cold, fever, dysentery, dyspepsia, chronic conjunctivitis and other eye diseases, as well as gastric and hepatic Disorders in indigenous system of medicine.[3] The plant is endowed with a variety of pharmacological properties including antis tress, antifertility, immunoregulatory, hypoglycemic, antibacterial, antifungal, anti-inflammatory, anticarcinogenic, antioxidant.[4]

Varieties: *Ocimum basilicum* varieties purpurscens is popularly known as Purple Basil. The leaves are as same as that of sweet basil. It is known for its culinary properties and excellent ornamental foliage. *Ocimum basilicum* varieties genovese is also called Genovese Basil This basil has dark green leaves that grows up to 2 inches long. It is used on a large scale in pesto and garlic dishes. *Ocimum basilicum* var. crispum is used largely as garnishing in salad or in sauce, it does not taste much as compared to other green basils and popularly known as Lettuce Leaf Basil. Its leaves are quite wide and large.

Habitat and Distribution: Sweet basil is indigenous to Persia and Sindh and lower hills of Punjab in India.[5] The plant is widely grown as an ornamental and field crop throughout the greater part of India, Burma, Cylone[6] and several Mediterranean countries including Turkey.

Ocimum Sanctum is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. The two main morphotypes cultivated in India and Nepal are green-leaved (Sri or Lakshmi *tulasi*) and purple-leaved (Krishna *tulasi*). The different types of tulsi exhibit vast diversity in morphology and phytochemical composition including secondary metabolites, yet they can be distinguished from other *Ocimum* species by the colour of their yellow pollen, high levels of eugenol and smaller chromosome number.

Chemical constituents of *Ocimum*:- The number of main chemical constituents like, Ursolic acid, Rosamarinic acid, Germacrene-D, Eugenol acid, linalool Oleanolic acid, Beta-caryophyllene Carvacrol, isolated *Ocimum sanctum* extracts.

In vitro studies on animal research, the use of tulsi as part of a polyherbal formulation in humans. However, there are no systematic reviews on the clinical efficacy and safety of tulsi as a single herbal intervention in humans. The human clinical trials of tulsi in order to assess the current evidence on tulsi's clinical efficacy and safety.

MATERIAL AND METHODS

Collection of plant: The Inflorescence of the plant *Ocimum sanctum* wild were collected in the month of March 2017 from Laxmi Narayan puri surajpole, Jaipur city, Rajasthan, India. Plant Inflorescence of *Ocimum sanctum* wild were washed thoroughly with running tap water followed by rinsing with distilled water and then Inflorescence were separated. The Inflorescence were shade dried at room temperature then crushed into powder.

Preparation of extracts: The 20 gm of dried, crushed powdered of Inflorescence of *Ocimum sanctum* were successively dissolved in 1000 ml ethanol. Then kept the dissolved samples on rotary shaker for 5 days. After 5 days the dissolved samples were extracted with ethanol at in Soxhlet assembly. The liquid extract were transferred into the previously tared empty beaker and evaporated at 72^0C, until semi-solid extract were obtained as to remove all the residual traces of the solvent. The ethanolic extract of Inflorescence were converted into semi-solid with the help of hotplate at 75^0. The extracts were stored at 4^0C in refrigerator for further uses.

(A) **(B)**

Fig. 1. (A) 20 gm powder of Inflorescence were dissolved in 1000 ml ethanol. (B) Rotary shaker.

Collection of mice

Experimental Animals: Three group of Albino mice (85–90 gm) of 10 weeks of age male sex were obtained from the animal house of the GT mall, Jaipur. They were kept in the Department of biotechnology lab at 26 ± 20^0C and relative humidity of 44–56%, with light and dark cycles respectively, for three week during the experiments. Animals were provided with standard rodent pellet diet (Manak Chowk, Jaipur) and ethanolic dose. "Principles of laboratory animal care" guidelines were followed. Approval from the Central Animal Ethical Committee of the University were taken prior to the experimental work.

Subacute Toxicity Study: The repeated oral doses for 21 days and toxicity studies were carried out in Albino mice according to the Organization for Economic Co-operation and Development OECD test guideline 407.[7] Mice were divided into 3 groups of animals. The first group C_1 received without the inclusion of the inflorescence of *Ocimum sanctum* (control group), whereas the second group C_2 received a dose containing 150 mg/kg per day and third group C_3 received a dose containing 600 mg/kg per week. Doses of *Ocimum sanctum* extract were administered daily by oral gavage in the volume of 10 ml/kg body weight, once daily for 21 consecutive days. The animals were observed daily for any abnormal clinical signs during the course of study period. Body weights were also measured and recorded at the beginning and then after every week of the experimentation. At the end of the study, all animals fasted overnight (ethanolic extract) and, on 22th day, the animals were weighed. Blood were collected in graded tube containing EDTA to stop it from clotting for haematological analysis.

Haematological Parameters: Blood sample were drawn from normal, control groups of mice for the investigation of haematological parameters, using 10 % EDTA (ethylene di-amine tetra acetate) as an anticoagulant. Haematological parameters such as Haemoglobin, WBCs count by haemocytometer, RBCs count, packed cell volume (PCV) Micro hematocrit method[8], Mean corpuscles volume (MCV), Mean corpuscles Haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Platelets count.

Collection, Preparation of Smear of blood Sample

At the end of experimental period of 21 days. The blood samples from mice were collected from all the groups C_1, C_2, C_3 by making cut on ventral side vein for haematological analysis, blood sample were collected in graded tube containing ethylene di-amine tetra acetate (EDTA) to stop it from clotting as recommended by Dacie and Lewis, 2006.

(A) **(B)**

Fig. 2. (A) Isolation of blood. (B) Collected blood samples of mice.

Haemoglobin (Hb) content in gm % of each animal were estimated by Sahli's haemoglobinometer. Haematological parameters like total red blood cells (RBC), total white blood cells (WBC), differential white blood cells, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, were be estimated on fully automated fluorescence flow cytometry 5-part different analyzers. PCV % were be measured by the use of micro hematocrit method and erythrocyte sedimentation rate (ESR) were determined using Wintrobe's method.

Statistical Analysis: Data are expressed as Means ± S.E.M. Data comparisons were carried out using one-way analysis of variance followed by T- test to compare means between the different treatment groups. Difference between unexposed and exposed (with ethanolic extract treatment) with a p-value <0.05 was considered significant.

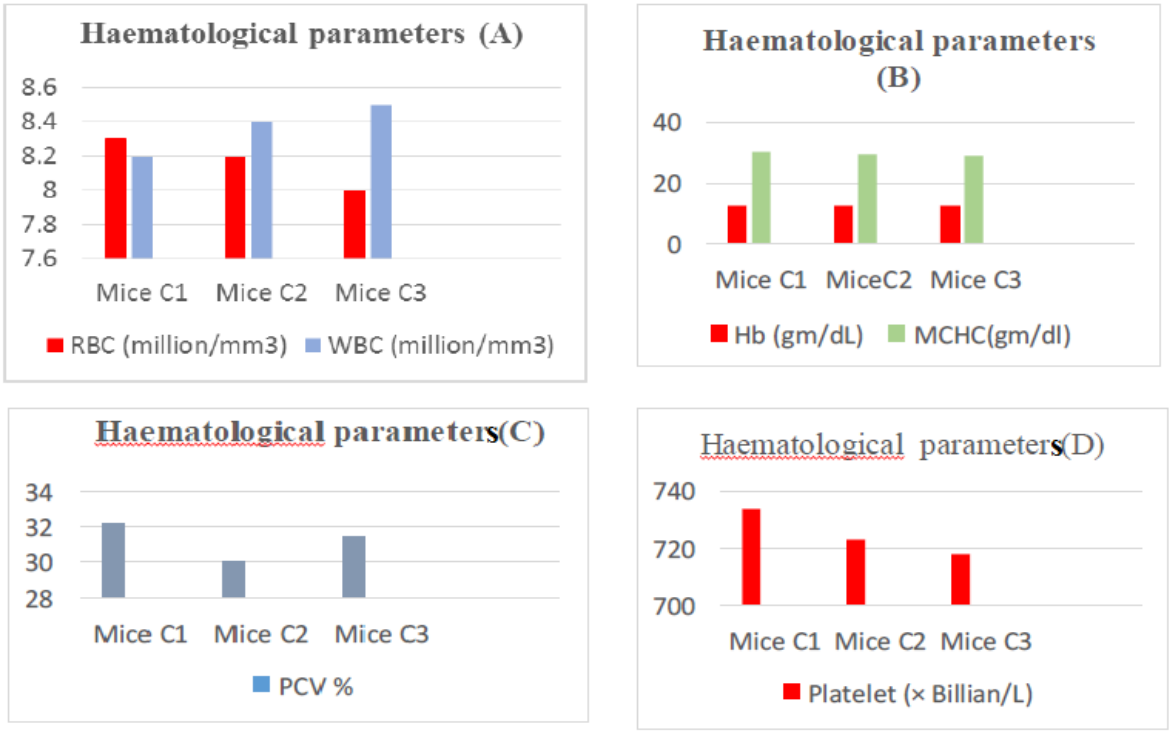
RESULTS

OBSERVATIONS TABLE

Table. 1: Effect of Inflorescence ethanolic extract on haematological parameters after subacute administration.

Parameters	Control (group C_1)	150mg/Kg/day body weight (group C_2)	600 mg/Kg/week body weight (group C_3)
RBC (million/mm3)	8.3 ± 0.8	8.2 ±0.8	8.0 ± 0.6
Hb (gm/dL)	12.6 ± 1.1	12.5 ± 1.0	12.3 ± 0.8
WBC (million/mm3)	8.2 ± 0.6	8.4 ± 0.9	8.5 ± 1.3
PCV (%)	32.20 ± 1.7	33.10 ± 0.65	33.40 ± 0.60
MCV (fl)	51.16 ± 2.05	52.26 ± 3.05	50.35 ± 1.65
MCH (pg)	18.16 ± 0.76	18.87 ± 0.81	17.95 ± 0.72
MCHC(gm/dl)	30.12 ± 1.3	29.26 ± 1.0	29.16 ± 0.8
Platelet (× 10^9/L)	734 ± 25.03	723 ± 28.06	718 ± 23.08

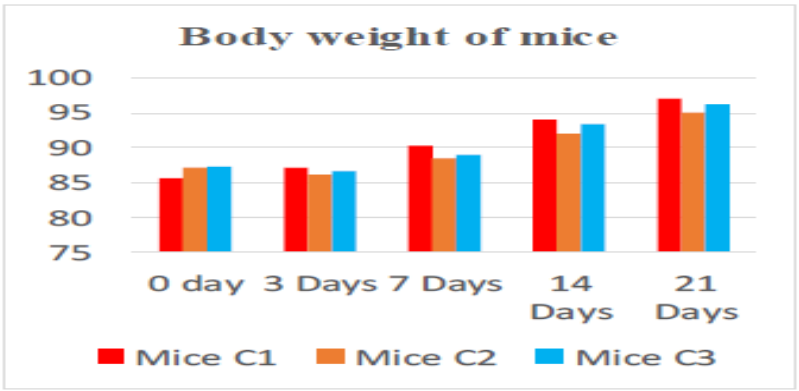
Data are represented as mean ± SEM.



Graph. 1: Effect of Inflorescence ethanolic extract on haematological parameters after Subacute administration.

Table. 2: Effect on body weight after 21 days oral administration of Inflorescence Ethanolic extract.

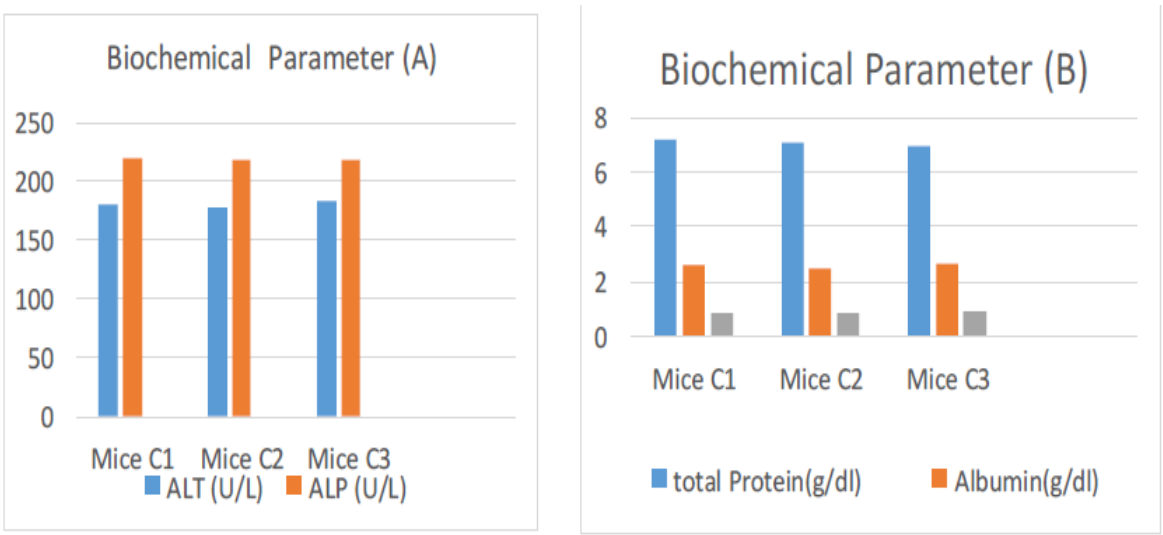
Body weight (gm)	Control (group C1)	150mg/Kg/day body weight (group C2)	600 mg/Kg/week body weight (group C3)
0 Day	85.50	87.00	87.25
3 Days	87.00	86.00	86.50
7 Days	90.25	88.50	89.00
14 Days	94.00	92.00	93.25
21 Days	97.00	95.00	96.25



Graph. 2: Effect on body weight after 21 days oral administration of Inflorescence Ethanolic extract.

Table. 3: Effect of Inflorescence ethanolic extract on *Biochemical* parameters after subacute administration.

Parameters	Control (C1)	Lower dose (150mg/Kg body weight) (C2)	Higher dose (600 mg/Kg body weight) (C3)
Alanine transaminase (ALT) (U/L)	180.40 ± 1.10	178.67 ± 1.30	183.40 ± 0.9
Alkaline phosphatase (ALP) (U/L)	220.16 ± 1.20	218.25 ± 1.40	219.50 ± 1.52
Total protein (g/dL)	7.2 ± 1.5	7.1 ± 1.6	7.0 ± 1.6
Albumin (g/dL)	2.62 ± 0.03	2.48 ± 0.05	2.63 ± 0.06
Creatinine (g/dL)	0.87 ± 0.05	0.85 ± 0.04	0.89 ± 0.05



Graph. 3: Effect of Inflorescence ethanolic extract on haematological parameters after Subacute administration.

Hematological Studies: There are no significant changes were observed in haematological parameters among three groups. The mean values Hb, RBC and Platelets were reduced significantly ($P<0.05$) in ethanolic extract treated mice and a significant improvement were noticed in both of the treated groups C_2 & C_3 ($P<0.05$), PCV and WBC values were dose dependent manner and MCV and MCH were initial increased in group C_2 than reduced in group C_3. The ethanolic extract have the greatest impact on the haematological parameters during the course of highest dose.

Biochemical Analysis: Biochemical parameters for body function test like alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, albumin and total protein did

not showany difference with the doses of inflorecence of *Ocimum sanctum* compared to control group.

Acute Toxicity Study: The limited dose of inflorescence (ethanolic extract) 150mg/kg body wt. /day and 600mg/kg body wt /week did not cause death or any toxic signs in treated male mice. All three mice were shown normal signs thoughtout the study and survived until the end of the 21-day experimental period.

Physical Parameters: Little changes were observed in body weight, food consumption, water intake and ethanolic extract of Inflorescence (150, and 600mg/kg) treated groups compared with control group after 21 days.

DISCUSSION

The research study was conducted to investigate the effect of different schedule of medicinal plants Tulsi (O*cimum sanctum)* leaf powder infusion on, lipid profile, and haematological of broiler chicks. Means Haemoglobin estimation (Hb) level is presented. Treatment T4 receiving 1 % Tulsi (O*cimum sanctum)* leaf powder at the rate were incorporated into the basal diet for six weeks, showed higher ($P<0.05$) Hb level (11.37 g/dl) as compared to other treatment groups. There is similar with the findings of Esonu[9], who observed significant increase in Hb level while feeding herbal plant (tulsi) to the laying hen. Results of our findings is in contrast with the findings of Gautam[10], who noticed that no significant effect on Hb was observed, fed *Withania somnifera* to the animals. Our result is in agreement with the result of Sham[11], who reported significant effect on hemoglobin and red cell count, while feeding *Withania somnifera* to animals.

There was increase in mean PCV and WBC count across the groups relative to the control. While for The RBC count, Haemoglobin concentration, platelets count & MCHC there was a decrease in groups 2 and 3, have been due to a decrease in plasma volume[14] as it has been shown by Effraim[16] that *Ocimum gratissimum* is diuretic. In the present study it is observed that MCV and MCH were an initially increased in group C_2 than decreased in group C_3. And it is recorded that the WBCs and PCV values were slightly advanced.

Subacute studies in mice did not show any change in hematological, liver functions and spleen with 800mg/kg *Ocimum sanctum* when administered for 21 days. The hematopoietic system/bone marrow is one of the most sensitive targets for toxic compounds and an

important index of physiological and pathological status in man and animal.[12] Analysis of blood parameters is relevant to risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the data are translated from animal studies.[13] Subacute exposure of rat to the lower doses of the Ocimum Sanctum extract produced small and transient changes in some hematological parameters. Blood is an important index of physiological and pathological status in man and animals and the parameters usually measured are hemoglobin, total red blood cell and leukocyte count.[15] In our study, The RBC count, Haemoglobin concentration and platelets value were decrease in groups C_2 and C_3, relative to the control C_1.

The increase seen in the white pulp could be correlated from the haematological parameters which showed a significant increase in the lymphocyte count and this is what constitutes the bulk of the white pulp. Since lymphopoiesis in the white pulp contributes to lymphocytes in the circulation[17], it is likely that the plant possibly caused increase in lymphocyte by causing lymphopoiesis in the white pulp of the spleen. Currently we found that, A significant improvement were noticed in PCV and WBC both of treated groups C_2 & C_3 ($P<0.05$).

The idea of using medicinal plants to treat human ailments is not new and in many developing countries their use is still vogue. *Ocimum sanctum* Linn. is a very important drug and is traditionally used to treat a number of health problems. This review provides evidence based scientific validation to some of its action and therapeutic uses described in ethnobotanical literature. But the compounds responsible for these activities have not yet been clearly elaborated so, further studies should be taken into consideration to justify its reported actions through related phyto constituents.

CONCLUSION

➢ There are many herbal plants in the world among which *Ocimum sanctum* to be considered the ruler of herbs due to its great impact in medicinal values. Various medicinal properties of Tulsi are well documented in the Hindu mythology. Collected *Ocimum sanctum* from different geographical origins existed variety of chemical constituents, and the researchers found vide and varied application in traditional health care system.

➢ In conclusion, protection by *Ocimum sanctum*, is currently an important strategy for controlling the process of various diseases including haematology, cold, fever, dysentery, antifertility, and antistress. Therefore, there is a need to explore medicinal plants or other

natural agents that can work as protective agents against haematology. In the present work, our results shown that there is no adverse effects of ethanolic extracts with Inflorescence (*Ocimum sanctum*) on vital body organs of mice.

➢ *Ocimum sanctum* is a natural components might prove to be potentially beneficial but comparatively less toxic. Eventually, plants belonging to *Ocimum* genus could contribute to solve certain economy and future health problems. Thus, essential constituents of *Ocimum sanctum* can be hopefully considered in the future for more clinical evaluations and its applications, and as possible adjuvants to current medication.

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