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Antidiabetic Study of Ocimum sanctum Linn leaves: Heamatological study and Histological study

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ABSTRACT: The present study was aimed to study the acute and subacute diabetic studies with orally administered aqueous leaf extract of Ocimum sanctum Linn (OSE). In an acute diabetic test, four groups of mice (n = 6/group) were tested, in which the first group was the normal group, the second was injected with 70 mg/kg b.w. alloxan, and the third and fourth groups were orally treated with doses of 100 and 200 mg/kg b.w.of O. sanctum for 28 days, and a hematological study and histopathological study were taken. Subacute treatment with OSE, changes in their body weight and hematological profiles. But in addition, a change was observed in microscopic aspects of liver organs in mice. The result showed that O. sanctum Linn extract could help to relive and be safe for human use.

KEYWORDS: Ocimum sanctum, hematological, histopathological, aqueous, diabetes

I. INTRODUCTION

For plant-based medications and novel pharmaceuticals, medicinal plants are vital and abundant sources. O. sanctum is a fragrant shrub with numerous therapeutic and spiritual uses that is indigenous to Southeast Asia and the Indian subcontinent. It is grown mostly for its essential oil and for its religious and medicinal purposes. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Hunt et al., 2000). There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences (Liu etal., 2009). The World Health Organization has advised in 2008 that more scientific research be done on the harmful side effects of traditional herbs used to treat illnesses since they can be used carelessly without warning of their potential harmful or toxic effects. The leaves of O. sanctum (OS) contain a volatile oil composed of limonene, borneol, copaene, caryophyllene, and elemol, phenolic compounds (rosmarinic acid, apigenin, cirsimaritin, and isothymusin), flavonoids (orientin and vicenin), and aromatic compounds (methyl chavicol and methyl eugenol) (Pattanayak et al., 2010). Eugenol forms the major active constituent of OS, even though other minor constituents like fixed oils and flavones have also been reported to have pharmacological activities (Prakash and Gupta, 2005). According to some paper reviews, there is something missing about the safe dosage of O. sanctum Linn in traditional medicine. So, it was thought to do the acute diabetic (mortality) and subacute diabetic (hematological and histopathological study) in mice.

II. MATERIALS AND METHODS

2.1. Experimental Animals

Mature albino mice were selected for the investigation. There were four groups of mice. Each group consisted of 6 mice individuals. Albino mice were maintained under proper hygienic conditions in a well-ventilated room. They were housed in polypropylene cages and maintained at a controlled environmental temperature in the P.G. department of Zoology, Bhagalpur, animal house, following departmental rules and regulations.

2.2. Plant Material and Preparation of Extract

Aqueous suspension of O. sanctum was prepared each day freshly. 20 g of fresh leaves of Tulsi were washed thoroughly with Double distilled water and then cut into small pieces. These finely cut pieces will mix with 100 ml of doubled distilled water, and this mixture will keep for boiling for a period of 5 minutes. After cooling, it was filtered through Whatman Filter paper no. 1.

2.3. Experimental protocol

A single intraperitoneal (I.P.) injection of a newly prepared solution of alloxan monohydrate

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(70 mg/kg) in normal saline caused diabetes in experimental mice. The mice were given sixteen hours of fasting prior to the administration of alloxan. Eight hours after induction, fasting blood glucose level was measured with Glucospot glucose meter and mice with fasting blood glucose levels above 200 mg/dL were chosen for the antidiabetic study.

2.4. Acute Diabetic Study (with a short period of time)

Adult albino mice of either sex, weighing between 20 and 25 g, fasted overnight and were used for an acute diabetic and antidiabetic study, as per the Organization for Economic Co-Operation and Development (OECD 423) guideline (OECD, 2001). Four groups of mice (n = 6) of both sexes were fasted overnight. The first control group mice received distilled water. The remaining group induced alloxan 70 mg/kg body weight at one time. while the other last two groups received doses of OSE 100 and 200 mg/kg body weight for 28 days regularly. Animals were observed closely for the first 4 hours for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently, observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 28 days, and the number of mice that died within the study period was noted.

2.5. Subacute Diabetic Study (Over a period of 14 to 28 days)

Mice were divided randomly into 4 groups of 6 animals each. After an overnight fast, animals in group I (the control group) were normal. Animals in group II were a little upset and dull due to induced alloxan, whereas mice in groups III and IV (test groups) received OSE at doses of 100 and 200 mg/kg body weight, respectively, for 28 days. Doses of OSE were administered daily by oral gavage in the volume of 0.25 and 0.5 ml/kg body weight, once daily for 28 consecutive days. The animals were observed daily for any abnormal clinical signs and death during the study period. At the end of the study, all animals fasted overnight, and on the last day, the animals were weighed. Blood was collected from the tails of mice and done for hematological and histological analysis, respectively. The animals were sacrificed with an overdose of ether, and body organs were taken out for detailed weight and histopathological changes.

2.6. Hematological Parameters

Hemoglobin, blood glucose, and WBC were determined in control, diabetic and OSE-treated groups.

2.7. Histology

The mice were quickly dissected and the liver were excised and weighed. The specimens for histopathology were fixed in 10% neutral, buffered formalin for 18 h at 4°C. 3-4 µm in thickness of specimen of liver was cut and stained with eosinstain following the standard laboratory procedures. The stained sections were examined under microscope for any cellular damage or change in morphology of that particulartissue.

2.8. StatisticalAnalysis

Statistical comparison was performed using one way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. All statistical analysis was performed using One way ANOVA Calculator, including Tukey HSD. The results were considered statistially significant when $P<0.05$.

III. RESULT

3.1. AcuteToxicityStudy

The limitdose of 100 and 200 mg/kg did not cause death or any toxic signs in treated mice. All six mice were normal throughout the study and survived until the end of the 28-days experimentperiod.

3.2. Subacute Toxicity Study

Oral administration of OSE at doses of 100 and 200 mg/kg body weight daily for 28 days, did not produce any mortality. All the treated and control mice were normal throughout the study. The animals show some dullness in alloxan induce mice and changes in general behavior on that day. After some days they were normal no any other physiological activities change.

3.3. Measurement of fasting body weight

Measurement of body weight: On the 28th day post-treatment, the body weight of animals was measured using a weighing balance (Table1).

3.4. Hematological Studies

Hematological parameters like mean hemoglobin content and WBC, were significantly different with OSE-treated mice from alloxan induced group (Table2).

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3.5. Histopathological Studies

The organs like liver isolated in various groups did reveal some abnormalities in their gross examinations both in treated and control groups (Table 4). The histological studies with liver did reveal some pathological changes after treatment even with higher dose of 200 mg dose of OSE when administered for 28 days (Figures 1).

Figure 1, shows experimental diabetic mice's liver histopathological changes(Figure A) shows that normal mice had hepatic lobules and normally dilated hepatic sinusoids. Diabetic mice (Figure B) exhibited vacuolization, congested nuclei, necrotic foci, infiltration of mononuclear cells, and parenchymatous tissue injuries. Diabetic mice treated with a low dose of O. sanctum showed less histopathological injury (Figure C and D). On the other hand, diabetic mice treated with a high dose of O. sanctum showed less vacuolization and condensed nuclei. OSE administered to diabetic mice showed significant liver protection.

Table 1. Effect on body weight after 28 days oral administration of OSE.

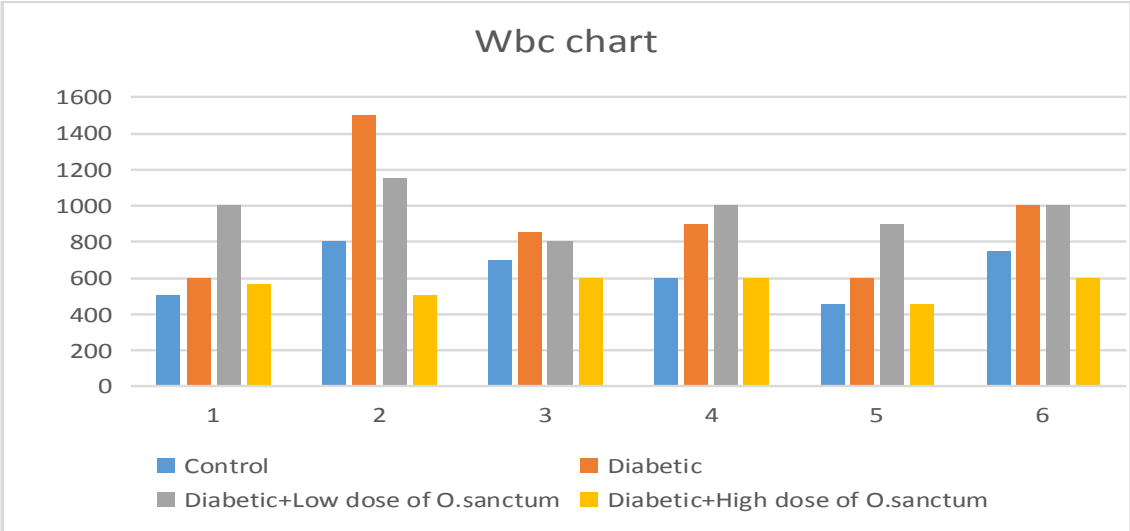
S.I. No.	Control	Diabetic mice	Diabetic+ O. sanctum	Diabetic + O. sanctum
	Weight of mice before administration of alloxan	Weight measured after 28thth day of administration of alloxan	Weight measured after 28th day of administration of alloxan	Weight measured after the end of 28th day
Result	26.33 ± 0.34	25.96 ± 0.37	28.73 ± 0.49	29.26 ± 0.38

Values are expressed as n=6 mean ± sem of 6 mice in each group

Table 2. Effect on Hematological parameters after 28 days oral administration of OSE.

	Control	Diabetic	Diabetic+ Low dose of OS	Diabetic+High dose of OS
Wbc (million/mm3)	633.3± 57.25	908.31± 35.6	908.3± 135.6	566.6± 30.73
Hb (g/dL)	5.88± 0.22	6.11 ± 0.22	6.25± 0.24	7.05 ± 0.29

Values are expressed as n=6 mean ± sem of 6 mice in each group



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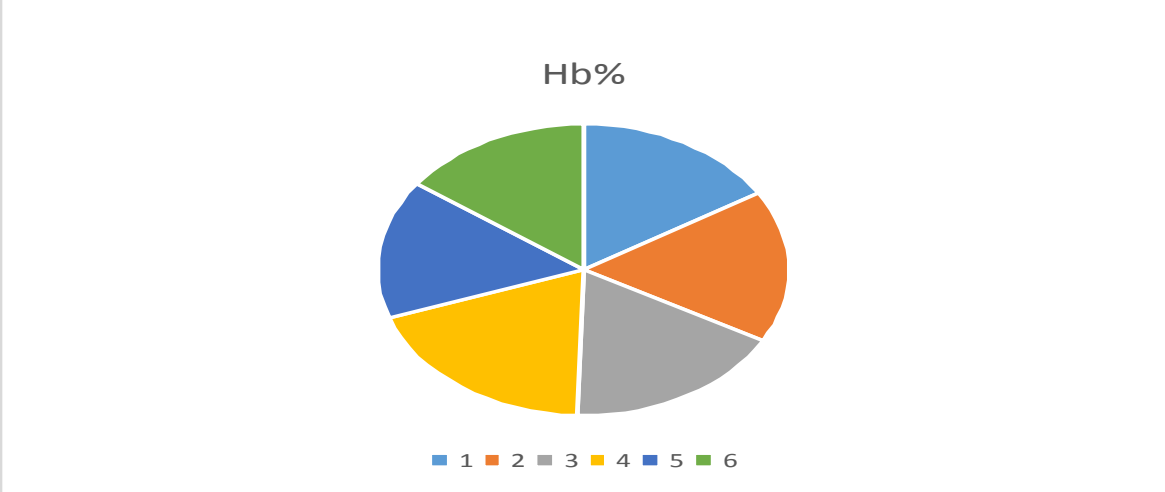

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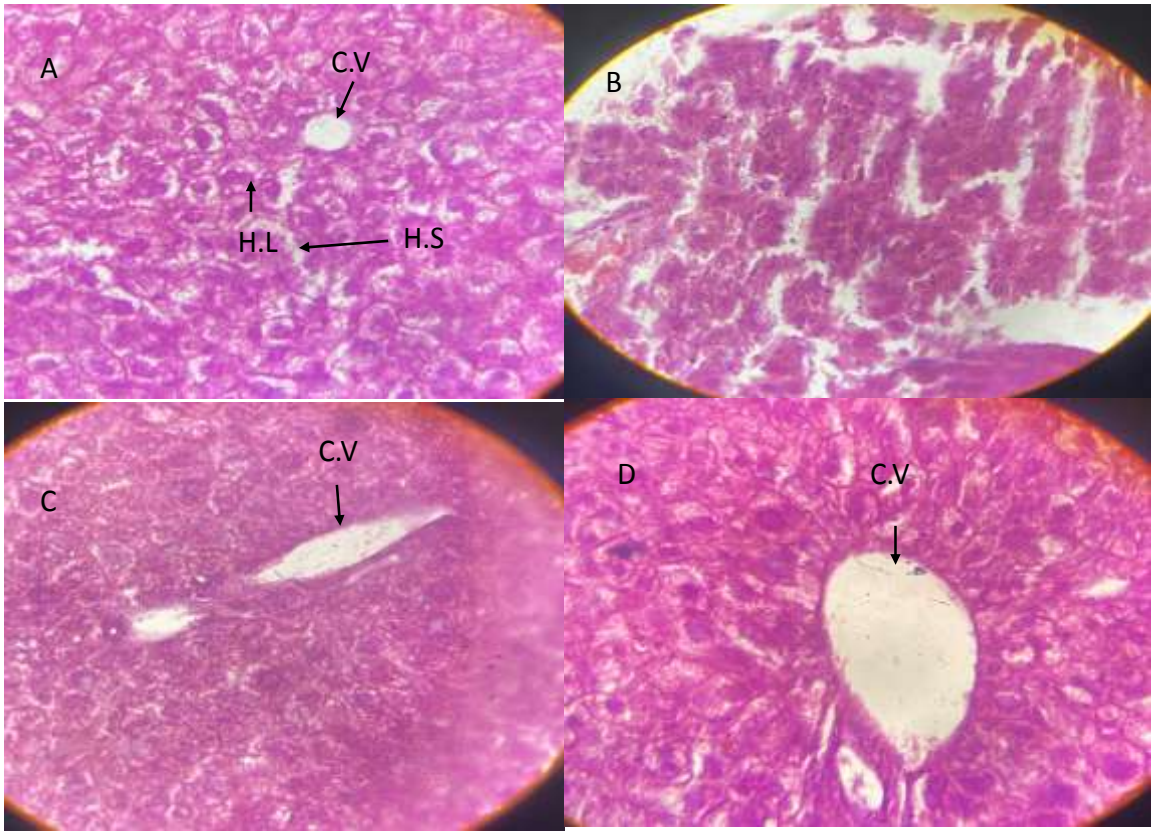

Figure 1. Histological changes in the liver (H&E, x40). Normal group (A), diabetic control (B), diabetic mice treated with low dose of O. sanctum aqueous extract (C), diabetic mice treated with High dose of O. sanctum aqueous extract (D), respectively.

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IV. DISCUSSION

O. sanctum leaves have been used for diabetes treatment. Many studies have backed up the traditional claims of their antihyperglycemic effects (Chattopadhyay, 1993; Rai et al., 1997; Agrawal et al., 1996). Our study used animal models of non-diabetic and diabetic mice to study O. sanctum extracts' acute and subacute antidiabetic effects in order to better understand its effects and therapeutic efficacy in treating diabetes. It is known that diabetics can improve their glucose tolerance by slowing down gastric emptying, increasing intestinal nutrient transit, or altering the release or function of important digestive enzymes (Hannah and Howard, 1994). One of the most significant issues associated with type 2 diabetes is a decreased glucose uptake and utilization by peripheral tissues (Green et al., 2005). Using 3T3 adipocytes, we investigated the impact of O. sanctum leaf extract on glucose uptake both in the presence and absence of insulin cell that has been successfully used in previous research to study how new agents affect glucose uptake (Hannan et al., 2012). O. sanctum extracts significantly improved glucose transport and had more effect when there was insulin. O. sanctum extracts do not work in animal models of type I diabetes, so it is less intriguing. However, further research is needed to understand In vivomolecular mechanisms that help glucose uptake. Although control and O. sanctum treated rats had similar pancreatic insulin levels, the group treated with the plant extract had more hepatic glycogen. Additional research is needed to find out if O. sanctum extracts affect insulin release, glucose uptake and hepatic glycogen deposition in diabetic rats (Lolitkar and Rao, 1996).

V. CONCLUSION

Acquous extracts of O. sanctum leaves improved glucose homeostasis in type 2 diabetes mice by enhancing circulating insulin and delaying carbohydrate digestion. O. sanctum therefore represents a useful source for discovery of novel antidiabetic compounds and as a dietar adjunct for hte management of type 2 diabetes and its dcomplications. By increasing circulating insulin and slowing carbohydrate digestion, aqueous extract of O. sanctum leaves improves glucose homeostasis in type 2 diabetes mice. As a result, O. sanctum serves as a beneficial source for the development of new antidiabetic agents as well as a nutritional supplement for the treatment of type 2 diabetes and its complications.

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