

HEPATOPROTECTIVE ACTIVITY OF *OCIMUM SANCTUM* LINN. AGAINST LEAD INDUCED TOXICITY IN ALBINO RATS

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ABSTRACT

Background: Recently man has started depending on natural resources even for his healthcare. Problems with drug resistant microorganisms, side effects of the modern medicine and the increasing number of emerging diseases for which no cure is available in modern medicine has renewed the interest in plants as a significant source of therapeutics.

Primary Study Objective: Liver is a versatile organ that regulates the internal environment of the body and hence becomes prone to toxicity. Despite the tremendous strides in modern medicine there is still a need for a drug that stimulates liver function. Hence the present study focuses on the study of the hepatoprotective potentials of *Ocimum sanctum* Linn. in lead induced toxicity.

Methods and Design: Aqueous extract of *O. sanctum* was prepared as per protocol. Wistar strains of Albino rats were used as the experimental models. Animals were grouped into six comprising of six rats each. Hepatotoxicity was induced using lead. The selected plant drug was administered to the animals orally for a period of 21 days. The Hepatic serum markers AST, ALT, ALP, GGT, Serum Protein, Serum Bilirubin and Tissue Glycogen were analyzed. The antioxidant status of the animals was also assessed in the animals by measuring the activity of GSH and SOD. The extent of lipid peroxidation (LPO) was also measured.

Results: The results obtained depicted the protective nature of the selected drug source. The induction of liver injury with lead resulted in significant raise in the serum marker enzyme level along with an increase in the serum bilirubin content. The inactivation of the liver was evident from the lowered levels of serum protein and tissue glycogen levels. The antioxidant status was very low thus causing an accumulation in lipid peroxides in the hepatic tissues. All the parameters studied were restored to near normal when treated with the aqueous extract of *O. sanctum*. Values, $p < 0.05$ were considered statistically significant.

Conclusion: The present study clearly depicts the hepatoprotective nature of *O. sanctum* in lead induced toxicity. The protective activity may be attributed to the antioxidant activity of the plant.

Keywords: Hepatotoxicity, Hepatoprotective activity, Lead, Antioxidants

INTRODUCTION

The liver is a vital organ with a wide range of functions such as detoxification, protein synthesis and production of biochemicals necessary for digestion. In the fast developing world men are exposed to various foreign chemicals (xenobiotics) – drugs, food additives and pollutants etc. Most of these compounds are subjected to metabolism (chemical alteration) in the human body, with the liver being the main organ involved. Acetaminophen, alcohol, ibuprofen, CCl₄, lead and contraceptives are some drugs that cause hepatic injury¹.

Exposure to lead causes simple biochemical and structural alterations to molecular characterization of hepatic hyperplasia or liver cell proliferation have demonstrated pathologic changes indicative of liver toxicity. Further the mild to moderate dysfunction in hepatic drug metabolism associated with lead toxicity may also have greater impact on general public health².

Herbalism is a practice of using traditional medicine or folk medicines which are basically prepared from plant extracts. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. Herbs from a part of our culinary and have a good therapeutic value. Many plants have been used traditionally in treating liver disease.

Ocimum sanctum a common medicinal plant has a wide range of therapeutic potentials. Tulsi's extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning, and malaria. Traditionally, tulsi is taken in many forms: as herbal tea, dried powder, fresh leaf, or mixed with ghee³. The present study was designed to validate the hepatoprotective effect of *Ocimum sanctum* Linn. on lead induced hepatotoxicity.

MATERIALS AND METHODS

Collection of plant material

The whole plant (aerial) *Ocimum sanctum* Linn. was collected in and around Trichy identified with the Flora of Presidency of Madras and

authenticated with the voucher specimen deposited at the Rapinet herbarium of St. Joseph's college, Trichy.

Preparation of aqueous extract

The plant was shade dried and coarsely powdered. The powder was mixed though roughly with 6 times the volume of water and stirred continuously until the volume reduced to 1/3rd. The extract was filtered with muslin cloth. The residue was re extracted. The filtrate was mixed and evaporated in a water bath till it reaches a thick consistency. The extract was stored in refrigerator till further use.

Animal models

Wistar strains of Albino rats of both sexes weighing 150-200 g were selected and used for the present study. Animals were housed in well ventilated cages in the CPCSEA approved animal house. The protocol was approved by the Institutional Animal Ethics committee. Animals were allowed to take standard laboratory feed and water. They were acclimatized to the laboratory conditions for a week before experiments.

Experimental design

The rats were divided into 6 groups, consisting of six rats each.

Group 1: Untreated animals and served as Normal control.

Group 2: Disease control (lead is administered orally at a dosage of 2.10 mg / 150 g body weight for 3 days)

Group 3: Induced with lead (as above mentioned dose) and aqueous extract of *Ocimum sanctum* Linn. (100mg/kg body weight) orally administered for 21 days.

Group 4: Induced with lead (as above mentioned dose) and aqueous extract of *Ocimum sanctum* Linn. (200 mg/kg body weight) orally administered for 21 days.

Group 5: Induced with lead (as above mentioned dose) and aqueous extract of *Ocimum sanctum* Linn. (300 mg/kg body weight) orally administered for 21 days.

Group 6: Induced with lead (as above mentioned dose) and treated with silymarin at a dose of 25mg/kg body weight.

At the end of experimental period the animals were sacrificed by cervical decapitation. The blood was collected and serum separated. The liver was washed in ice cold saline and homogenized. It was used for various experiments.

BIOCHEMICAL PARAMETERS STUDIED

Aspartate transaminase ⁴

The assay mixture containing 1ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37°C. To the control tubes serum was added after the reaction was arrested by the addition of 1 ml of DNPH. The tubes were kept at room temperature for 30 min. Added 0.5 ml of Na OH and the color developed was read at 540 nm. The activity of AST was expressed as μ moles of pyruvate formed /min/mg of protein

Alanine transaminase ⁴

The assay mixture containing 1ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37°C. Added 1 ml of DNPH and kept at room temperature for 20 min. Serum was added to the control tubes after the reaction was arrested by the addition of 1 ml of DNPH. Added 5 ml of Na OH and the color developed was read at 540 nm.

The activity of ALT was expressed as μ moles of pyruvate formed /min/mg of protein.

Alkaline phosphatase⁴

The reaction mixture containing 1.5 ml carbonate buffer, 1 ml Di sodium phenyl phosphate, 0.1 ml Magnesium Chloride and 0.1 ml of serum was incubated at 37 °C for 15 min. The reaction was arrested by the addition of Folin's phenol reagent. Control tubes were also treated similarly but serum was added after the reaction was arrested with Folin's phenol reagent. Added 1ml of Sodium Carbonate. The color developed was read after 10 min at 640 nm.

The activity of ALP was expressed as μ moles of phenol liberated /min/mg of protein.

Serum bilirubin ⁵

For the determination of total bilirubin 0.2 ml of serum was taken and made up to 2 ml with water. Then added 0.5 ml of diazo reagent, 2.5 ml of methanol. To the blank 0.2 ml serum was added and made up to 2 ml with water and added 0.5 ml of diazo blank and 2.5 ml methanol. The color developed was read at 540nm. The values were expressed as mg/dl.

Total protein ⁶

Aliquots of the suitably diluted serum (0.1ml to 10ml by two serial dilutions) was made up to 1.0 ml with water and 4.5 ml of alkaline copper reagent was added to all the tubes including blank, containing 1.0ml water and standards containing aliquots of standard BSA and made up to 1ml with water. The tubes were incubated for 10 min at room temperature. 0.5 ml was added to all the tubes and incubated for 20 min at room temperature. The blue color developed was read at 640nm. The protein content was expressed as g/dl.

Liver Glycogen ⁷

A weighed amount of the tissue was subjected to alkali digestion in a boiling water bath for 20 min. The tubes were cooled and 3ml of absolute ethanol and a drop of ammonium acetate were added. The tubes were then placed in a freezer overnight so as to facilitate precipitation of glycogen. The precipitated glycogen was collected after centrifugation. The precipitate was washed thrice with alcohol and dissolved in 3 ml of water. Aliquots were taken and made upto 1 ml with water. 4ml of anthrone was added to the tubes kept in an ice bath, mixed and heated in a boiling water bath for 20 min. The green colour developed was read at 640 nm.

Superoxide dismutase⁸

0.1 ml of tissue homogenate was added to tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant added 0.5 ml EDTA solution and 1 ml of buffer. The reaction was initiated by the addition of 0.5 ml of epinephrine and the increase in absorbance was measured at 480 nm. The enzyme activity was expressed as 50% inhibition of epinephrine auto oxidation.

Reduced Glutathione⁹

One ml of homogenate/blood was precipitated with 1 ml of TCA and the precipitate was removed by centrifugation. To 5ml of the supernatant added 2ml of DTNB and the total volume was made up to 3 ml with phosphate buffer. The absorbance was read at 412 nm. The concentration of glutathione was expressed as μ g/mg protein.

Lipid peroxide¹⁰

0.1 ml of tissue homogenate was mixed with 4 ml of 0.85N H₂SO₄ and mixed gently. 0.5 ml of phosphotungstic acid was added and stirred well. The contents were centrifuged for 10 min. The supernatant was discarded and the sediment mixed with 2.0ml of N/12 H₂SO₄ and 0.3 ml of 10% phosphotungstic acid. The mixture was centrifuged for 10 min. The sediment was suspended in 4.0 ml of distilled water and 1 ml of TBA reagent. The tubes were kept in a boiling water bath for 1 hr. After cooling 5 ml of butanol was added to each tube and the color extracted in the butanol phase was read at 532 nm. The lipid peroxide content was expressed as nanomoles of TBA reactants/mg protein.

STATISTICAL ANALYSIS

The data obtained was expressed as mean \pm SEM. The data were subjected to one way ANOVA. The p value < 0.05 was considered statistically significant.

RESULTS

In the evaluation of liver response to the aqueous extract of *Ocimum sanctum* Linn. ALP and serum transaminases - AST and ALT were estimated along with the serum bilirubin, total protein and antioxidants. The results obtained were tabulated in table 1, 2 and 3 and discussed.

Table 1: Shows the effect Of *Ocimum Sanctum* Linn on the Biochemical Parameters in Lead Induced Toxicity.

Groups	Bilirubin (mg/dl)	Total Protein (mg/dl)
GpI Normal Control	0.98 \pm 0.045	7.2 \pm 0.03
GpII Disease control (lead induced)	5.46 \pm 0.15*	2.5 \pm 0.022 *
GpIII (100mg/kg body wt)	3.6 \pm 0.43	4.6 \pm 0.032
GpIV (200mg/kg body wt)	2.03 \pm 0.033	6.78 \pm .002#
GpV (300mg/kg body wt)	1.0 \pm 0.005#	7.4 \pm 0.056#
GpVI (Silymarin 25mg/kg body wt)	1.01 \pm 1.06#	6.90 \pm 0.12#

Values are mean \pm S.E.M (n=6), * p<0.05 statistically significant when compared with normal control, # p< 0.05 statistically significant when compared with lead treated group.

Table 2: The effect of *Ocimum sanctum* Linn on Enzyme markers in lead induced toxicity

Groups	SAST (U/L)	SALT (U/L)	ALP (U/L)
GPI Normal Control	7.34 ± 0.7	41.7 ± 0.66	597.2 ± 0.8
GPII Disease control (lead induced)	145 ± 1.67*	228 ± 0.65*	1003 ± 0.88*
GPIII(100mg/kg body wt)	103.17 ± 6.5 #	47.8 ± 4.5 #	278.8 ± 5.0#
GPIV(200mg/kg body wt)	64.33 ± 7.5	20.6 ± 1.7#	113.2 ± 6.93 #
GPV(300mg/kg body wt)	6.7 ± 1.8 #	8.7 ± 1.25*	24.3 ± 2.44 *
GPVI(Silymarin 25mg/kg body wt)	46.32 ± 1.01#	3.33 ± 1.82#	2.19 ± 1.11#

Values are ± SEM, n=6, * p<0.05 statistically significant when compared with normal control, # p<0.05 statistically significant when compared with lead treated group, S AST- Serum Aspartate Transaminase, S ALT - Serum Alanine Transaminase, S ALP - Serum Alkaline Phosphatase.

Table 3: Antioxidant Status of the Models in the Lead Induced and Plant Drug Treated Groups

Groups	GSH (nmoles/min/mg)	SOD (U/mg prot)	LPO (nmoles/mg prot)
GPI Normal Control	43.63 ± 0.74	2.77 ± 0.11	2.09 ± 1.09
GPII Disease control (lead induced)	23.22 ± 0.50*	0.83 ± 0.11*	6.77 ± 0.14*
GPIII(100mg/kg body wt)	37.73 ± 0.82	1.56 ± 0.33	5.11 ± 0.003
GPIV(200mg/kg body wt)	40.31 ± 0.23	1.79 ± 0.33#	3.33 ± 0.22#
GPV(300mg/kg body wt)	47.3 ± 0.27#	2.44 ± 0.23#	2.76 ± 0.12
GPVI(Silymarin 25mg/kg body wt)	46.32 ± 1.01#	3.33 ± 1.82#	2.19 ± 1.11#

Values are mean ± S.E.M (n=6), * p<0.05 statistically significant when compared with normal control, # p<0.05 statistically significant when compared with lead treated group, GSH - Reduced Glutathione, SOD - Superoxide Dismutase, LPO - Lipid peroxides.

DISCUSSION

Hyperbilirubinemia seen in liver injury can result from impaired hepatic uptake of unconjugated bilirubin¹¹. From Table 1 it is evident that the elevated serum Bilirubin levels due to lead induced toxicity was restored within the normal levels on treatment with the aqueous extract of *Ocimum sanctum* Linn. The restoration of the bilirubin levels indicates regeneration of the hepatic tissues and improved hepatic efficiency.

Serum protein level is a gross measure of protein status and reflects major changes in the liver functions. Liver is the site of synthesis and storage of many proteins. Hepatotoxins impair the capacity of liver to synthesize protein. Liver is the major site of protein metabolism and a healthy functioning liver is required for the synthesis of the serum proteins except for the γ globulins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis¹². The biochemical studies of blood samples of lead induced animals showed a significant decrease in total protein level reflecting liver injury, while the blood samples treated with aqueous extract of *Ocimum sanctum* Linn showed an increase in total protein content.

AST, ALT and ALP are located in the cell cytoplasm under normal circumstances. Due to lead induced hepatotoxicity the membranes of hepatocytes become damaged releasing the enzymes into circulation causing elevation¹³. It is noteworthy, that the aqueous extract of *Ocimum sanctum* Linn. has a profound effect in restoration of AST, ALT and ALP levels towards their respective normal values.

Accumulation of significant amounts of lead in liver tissue was implicated in the induction of an oxidative stress response in the liver. Although lead is considered to be a poor inducer of oxidative stress, lipid peroxidation with concomitant inhibition of several antioxidant enzymes such as superoxide dismutase, glutathione reductase has been reported¹⁴. In the present study the lead induced animals showed a decrease in the activity of SOD and GSH. After treating the animals with aqueous extract of *Ocimum sanctum* Linn., Superoxide dismutase and Glutathione reductase activity was restored to near normal level.

Oxidative stress in lead induced hepatotoxicity was also noted to play a significant role in the regression phase of hepatic hyperplasia with the generation of Lipid peroxide (LPO)¹⁵. GSH is an abundant tripeptide non-enzymatic biological antioxidant present in the liver.

It is a critical determinant of tissue susceptibility to oxidative damage and depletion of hepatic GSH has been shown to be associated with enhanced lipid peroxidation¹⁶. The reduction in the GSH levels resulted in the accumulation of LPO in the hepatic tissues of the disease control group. Administration of the aqueous extract of *Ocimum sanctum* Linn. produced a significant reduction in the LPO levels. In the present study the restoration of the hepatic activity by the aqueous extract of *Ocimum sanctum* Linn. was found to be profound with the maximal activity at the dose level of 300 mg/kg BW.

CONCLUSION

The study suggests that the aqueous extract of *Ocimum sanctum* Linn. controls the damage caused by lead on hepatocytes membrane and provides a prognostic value with hepatoprotection.

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