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Research Article

Hepatoprotective Role of Flavonoids from Ocimum Sanctum Against Halothane-Induced Liver Toxicity in Rats

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ABSTRACT

Liver is one of the main sites of metabolism and also has a significant role through its various roles. It can split fats and proteins into smaller substances so that the cells of other tissues can use them for energy or for synthesizing specially needed biomolecules. Liver also synthesizes particles needed for blood coagulation, transport of fats conferring immunity to infection and many other purposes. Liver is dexterous of storing large quantity of fats, carbohydrates and even proteins and later releasing these nutrients into the tissue, which needs them. Any liver disease can cause measure physiological problem with grave consequences. The liver synthesizes about 50 gm of protein each day, primarily in the form of albumin. Liver cells also chemically convert amino acids to produce keto acidosis and ammonia, from which urea is formed and excreted in the urine. Digestive fat is converted in the intestine to triglycerides, fat, phospholipids and lipoproteins. These substances are converted in the liver into glycerol and fatty acids, through a process known as ketogenesis. Lever failure is continuously associated with cellular necrosis cellular necrosis, increased tissue lipid peroxidation and depletion in the flesh GSH levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated. Hence in the present study, the best effort is made to evaluate the caring effect of methanolic extract of Ocimum sanctum leaves against various hepato toxic agents in validated rat models. Additional study is required on the bioactive elements of OS that give rise to its varied therapeutic characteristics and their molecular actions. Furthermore, pharmacological characteristics of the ethno pharmacological information of OS for pharmaceutical therapeutic applications must be supported by its bioactive components.

INTRODUCTION

Around 18,000 individuals every year pass away from liver illnesses, according to the WHO.

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Cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, Fulminant hepatic failure, and specific tumours like Hepatoma are among the frequent liver diseases[1]. Hepatitis B infections affect two billion people worldwide, according to estimates. Of them, the chronic form of the disease affects about 350 million people. This alarming data and puzzling findings call for urgent research at any level, either to confirm the efficacy of current formulations or to investigate novel herbal treatments to lower the morbidity and mortality rates associated with hepatic problems. In the allopathic branch of medicine, corticosteroids and immune suppressants are frequently used to treat liver disease. But these medications are associated with opposing effects such as immune suppression and bone marrow depression[2]. Further, the success rate of treating liver diseases is disappointing. Around the world, efforts are being done to obtain scientific proof for these often reported herbal medicines. There is a growing emphasis on following systematic research practices and evaluating scientific justification for the use of traditional herbal medicines that are allegedly capable of exerting hepatoprotective activity because of the severe unfavourable side effects of synthetic agents and the lack of effective liver protective drugs in modern medicine. About 70–80 percent of people worldwide use traditional medicine, which is primarily composed of plant-based ingredients. Ayurveda, Siddha, Homeopathy, and Unani are only a few of the natural health care philosophies included in the term "traditional medicine." Ocimumsanctum is a member of the Labiateae family and is significant for its medicinal potential (Image 1and 2). Tulsi is also known as Ocimium sanctum (OS), extracts are used in ayurvedic remedies for antioxidant, antidiabetic, antiulcer, anticancer, antifungal, antibacterial and various forms of poisoning. Traditionally, *Ocimum sanctum* L. is taken in many forms, as herbal tea, dried power or fresh leaf. Several recent investigations using these extracts have indicates anti-inflammatory, antioxidant and immune-modulatory and antistress properties. Phytoconstituents present in OS are Flavonoids, Alkaloids, Saponins, Tannins, Phenols, Terpinoids and sterols[17].

Image: 1 *Ocimum sanctum* L

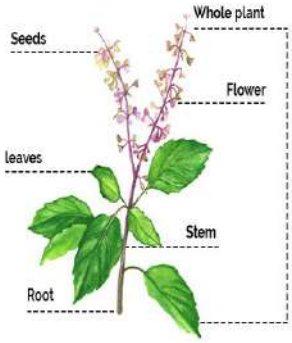

Image: 2 *Ocimum sanctum* L

MATERIALS AND METHOD

Collection of plant material:

The leaves of *Ocimum sanctum* used for the current studies were collected from Hyderabad district of Telangana. The plant was known, confirmed and authenticated by comparing with voucher specimen available at Review of medicinal plants & collection unit, Department of AYUSH, Ministry of Health & Family Welfare, Govt. of India, and Emerald by Field Botanist Dr. Anjani kumar S. A copy of certificate is attached. The plants were cut into small pieces and shade dried. The dried physical was then pulverized separately in to coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

Preparation of Methanolic Extract:

The powdered drug was dry and packed well in Soxhlet apparatus and removed with1500 ml of methanol for seven days. The extract was focused and dried using Rotary flash evaporator. It was kept in dessicator until used.

Experimental Animals:

Albino rats (Wistar strain) of either sex weighing among 150-200 g are procured from vivo biotech labs Turkapally, Genomevally, Telangana state. The animals were adjusted for seven days under laboratory conditions. The animals were fed with commercially available rat pelleted diet (vivo biotecs labs & foods, Hyderabad).Water was allowed *ad libitum* under strict clean conditions. Studies were performed in accordance with the CPCSEA guidelines (CPCSEA registration number 21/5H/SDRCP/IAEC). The Methanolic extract of *Ocimum sanctum* leaves was endangered to the following investigations:

Determination of acute toxicity studies LD_{50}:

Number of animals required: 6 rats, number of groups required:2 groups (6 animals each group) dose level 1800mg /kg body Wight of animal, study duration 14 days.

Methanolic extract of *OS* leaves was suspended in 3%CMC, to prepare a dose of 1800 mg/kg body weight of animal, and administered 1ml/100 gm body weight of the animal.

Procedure:

The procedure was separated into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female rats (each set of 6 rats) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water reserved before 4 hours of the dosing, body weights were noted before and after dosing with Methanolic leaf extract of *Ocimum sanctum* (1800mg/kg) orally. Individually animals were observed for 4 hours to see any clinical symptoms, any change in behavior or death. 6 hours post dosing again body weights noted. Form the next day on wards, each day 1hourthebehaviouralchange, clinical symptoms or mortality was observed in the same creatures for next 14 days and animal body weights were recorded on 8^{th} and 14^{th} day. The same process was repeated with another set of animals to nullify the errors.

Selection and preparation of dose for pharmacological screening

The Methanolic extract was suspended in 0.3% CMC solution to make two dose levels, 100 and 200 mg/kg body weight of the animals.

Halothane induced Hepatoprotective activity:

Group A - Normal control (10 ml/kg distilled water, p. o)

Group B - Toxicant (halothane 500mg/kg, p. o)

Group C - Standard (halothane + silymarin 25 mg/kg, p. o)

Group D - Halothane +Methanolic extract of OS (leaves 100mg/kg twice daily p. o)

Group E - Halothane + Methanolic extract of *Ocimum sanctum* leaves (200mg/kg twice daily p. o)

Experimental procedure:

Wistar rats of either sex considering between 150-200 g were divided into five groups of six rats each. Group A (control), Group B (halothane treated), Group C halothane +Silymarin (25mg/kg p .o), group D halothane and Methanolic extract of OS leaves (100mg/kg) and Group E halothane + Methanolic extract of OS leaves (200mg/kg). for the first seven days of study Group A and B were fed with normal lab feed and water[19]. Group C animals were treated with silymarin (25 mg/kg) and Group D and E animals were treated orally with ethanolic extract of *OS leaves* (100mg/kg & 200mg/kg) respectively for seven days. On the seventh and eighth day animals of Group B, C, D & E were administered orally with a single dose of Halothane with 5% acacia combination (600mg/kg/ day). After thirty minutes of

Halothane administration Group C, D & E rats were treated with MLMP100mg/kg, MLMP 200mg/kg and Silymarin 25mg/kg correspondingly. On day 8, thiopentone sodium (40 mg/kg, i.p) was injected and the sleeping time recorded in all the animals. The same animals were then under using anesthetic ether, 1 hour after complete recovery from thiopentone sodium effect and blood calm by retro orbital puncture and biochemical parameters like ALT,AST,ALP, Direct Bilirubin, Total Bilirubin, Triglycerides, Cholesterol, Total Proteins and Albumin were estimated. The animals were sacrificed by overdose of ether and autopsied. Livers from all animals were removed, washed with ice-cold saline, considered and measure the wet liver volume. Small piece of liver tissue collected and preserved in10%formalin solution for histo pathological trainings. Livers of some animals were homogenized with ice-chilled 10% KCl solution and centrifuged at 2000rpm for 10 minutes. Then collect the supernatant liquid and the antioxidant parameters like Catalase, Super oxide Dismutase and Thio barbiturate were estimated.

Physical Parameters:

Determination of wet liver weight:

Animals were for went and livers were isolated and washed with saline and weights determined by using an electronic balance. The liver weights were spoken with respect to its body weight i.e. gm/100gm.

Determination of Wet Liver Volume:

After recording the weight all the livers were dropped individual in a measuring cylinder containing a fixed volume of refined water or saline and the volume displaced was recorded.

Functional parameters:

On the last day, Thiopentone sodium (40 mg/kg, i.p) was injected and the asleep time recorded in all the animals.

Biochemical parameters:

The biochemical limits were estimated as per the standard procedure prescribed by the manufacturer's instruction manual providing in the kit. (Coral clinical systems, Verna Goa, India) using Semi Autoanalyser (ARTOS).

Estimation Of Serum SGPT (UV- Kinetic method):

Principle:

SGPT catalyses the transfer of amino group from L-Alanine to 2-oxo glutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The amount of this reaction is monitored by an indicator reaction coupled with LDL in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a reducing in the absorbance of NADH at 340 nm, which is proportional to SGPT activity.

L- alanine +2-oxoglutarate $\xrightarrow{ALT}$ pyruvate+Lglutamate

Pyruvate +NADH $\xrightarrow{LDH}$ L- Lactate +NAD

Where:

ALT: Alanine amino transferase

LDH: Lactate dehydrogenase

Procedure:

Pipette	Sample(µl)
Working reagent	1000
Sample	100

Mix well and aspirate

Estimation Of Serum SGOT (UV-kinetic method):

Principle:

SG0T catalyses the transfer of amino group from L- Aspartate to 2-oxo glutarate with the formation of oxalocetate and L-glutamate. The rate of this response is monitored by an indicator reaction coupled with malatedehydrogenase (MDL) in which the oxaloacetate formed is converted to malate ion in the presence of NADH (nicotinamide adenine dinucleotide). The rust of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGOT action.

L- Aspartate+2-oxoglutarate —AST→ oxaloacetate + L glutamate

Pyruvate +NADH —MDH→ L-Malate + NAD

Sample+NADH —LDH→ L- Lactate + NAD

Where:

AST: Aspartate amino transferase

MDH:Malate dehydragenase

LDH:Lactate dehydragenase

Procedure:

Pipette	Sample(µl)
Working reagent	1000
Sample	100

Mix well and aspirate.

Estimation of Serum Alkaline phosphatase (ALP):

Principle:

Estimation of Serum alkaline phosphatase hydrolyses p-nitro phenyl phosphate in the presence of oxidizing agent Mg^{+2}. This reaction is measured as absorbance is proportional to the ALP activity.

Pnitrophenylphosphate +H2O —ALP, Mg^{+2}→ p-nitrophenol+Phosphate

ALP: Alkalinephosphatase

Procedure:

Pipette	Sample(µl)
Working reagent	1000
Sample	20

Mix well and separate

Estimation of Serum bilirubin:

Principle:

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form a pink colored azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly counter in acidic medium. But, indirect and unconjugated bilirubin is solubilised using a surfactant and then it is reacts similar to direct bilirubin.

Procedure:

	Blank(µl)	Standard	Test
Working reagent	500	500	500
Distilled water	25	-----	-----
Standard	-----	25	------
Sample	-----	-----	25

Mix well. Incubate for 5 minutes at 37ºC temperature for Total bilirubin and direct bilirubin. Read absorbance at 546/630 nm against Mixture blank.

Estimation of Serum cholesterol:

Principle:

In the presence of cholesterol esterase, fat esters are dissociated in to cholesterol and fatty acids, cholesterol oxidase then changes the cholesterol in to hydrogen peroxide and cholesterone. In the presence of peroxidase, hydrogen- bleach reacts with 4-aminoantipyrineand phenol to form a quinoneimine dye.

The estimation of cholesterol involves the following enzymatic reaction.

Cholesterolesters —cholesterolesterase→ cholesterol + fattyacid

Cholesterol+O2 —cholesterol oxidase→ choles-4-en-3-one+ H2O2

H2O2 + 4-Aminoantipyrine+phenol —POD→ Quinonimine + H2O2

Where:

POD: peroxidase

The absorbance of quinoneimine measured spectrometrically at 505nm was proportional to cholesterol concentration in the example.

Procedure:

	Blank (µl)	Standard (µl)	Sample (µl)
Working regent	1000	1000	1000
Distilled water	20	----	----
Standard	----	20	----
Sample	----	----	20

Mix well and incubateat37^0c for 10 min. Aspirate blank followed by standard and tests then measure the absorbance of the sample and standard against total at 510/630nm.

Estimation of Serum Triglycerides (Enzymatic method):

Principle:

Triglycerides+H20 ⟶ Glycerol+Free fatty acids

Glycerol+ATP ⟶ Glycerol-3-phosphate+ATP

Glycerol-3-phosphate+O2 ⟶ DHAP +H2O2

2H2O2+4 AAP ⟶ Quinonimine + 4H2O

Where:

GK: Glycerolkinase

GPO: Glycerol-3-phosphateoxidase

DHAP: Dihydroxyhydrogenacetone phosphate

ATP:Adenosinetriphosphate

AAP:Amino antipyrine

LPL :Lipoprotein lipase

The intensity of chromogen quinoneimine formed disproportional to the triglyceride focusing the sample when measured at 510nm.

Procedure:

	Blank (µ)	**Standard (µl)**	**Sample (µl)**
Working agent	1000	1000	1000
Distilled water	20	----	----
Standard	----	20	----
Sample	----	----	20

Mix well, incubate at 37^0c for 10minutes. Measure absorbance of standard and sample against blank within one hour.

Estimation of Serum Total Proteins:

Principle:

The peptide bond of proteins reacts with CU+2 ions in alkaline solution to from a blue violet complex (Biuret reaction), each copper ion complexing with 5 or 6 peptide bonds. Tarterate is added as additive while iodine is used to prevent auto reduction of alkaline copper complex. The color formed is proportional to the protein attention and is measured at 546 nm.

Procedure:

	Blank (µl)	**Standard (µl)**	**Sample (µl)**
Working regent	1000	1000	1000
Distilled water	20	----	---
Standard	---	20	---
Sample	----	----	20

Incubate for 10 min. at 37 0c. Read absorbance of standard and each sample at 546 nm against reagent blank.

Livers of the animals were regulated with ice-chilled10%Kcl soln and centrifuge at 2000 rpm to 10 minutes. Then gather the supernatant liquid and estimate the antioxidant parameters like Catalase, Super oxidase and Lipid peroxidation.

Antioxidant parameters

Catalase:

Principle:

In U.V. range H2O2shows a repeated increase in absorption with decreasing wavelength. The decomposition of H2O2 can be followed straight by the decrease in absorbance at 240nm. The change in absorbance per unit is a measure of catalase activity.

Procedure:

The liver homogenates containing 5µg total protein was varied separately with 700µl,5mM hydrogen peroxide and incubatedat37°C.The disappearance of peroxide was experimental at 240nm for 15min. One unit of catalase activity is that which lessens 1µmolofhydrogen peroxide per minute.

Observation:

Check absorbance at time interval of (0sec, 15sec, 30sec, 45sec, 60sec, 75sec, 90sec, 105sec, 120sec).

Superoxide dismutase:

Principle:

The enzyme is essential for survival in all oxygen absorbing cells. It is found in the cytosol and inter membrane space of mitochondria of eukaryotic cells. It contains copper and zinc. In normal cells, this essential alone is the precursor of hydrogen peroxide.

Superoxide dismutase scavenges the super oxide (O^-2) and thus provides a first line defense against free radical injury. SOD'S are a enzyme that catalyze the dismutation of superoxide anion (O2)

to hydrogen peroxide and molecular oxygen in the following manner.

$2H2O2+ 2O^{-} \rightarrow 2H2O+ O2$

In the erythrocytes, the super oxide anion (O^{-2}) networks with peroxides to form hydroxyl radicals (OH), which causes heamolyses in the absence of SOD activity. SOD measurement was carried out on the ability of SOD to inhibit natural oxidation of epinephrine to adreno chrome.

Procedure:

2.8 ml of sodium carbonate buffer (0.05 mM) and 0.1 ml of tissue homogenate or sucrose (Blank) was incubated at 30^{0}C for 45 minutes. Then, the absorbance was adjusted to 0 to sample. Thereafter, the reaction was started by adding 10µl of adrenaline solution (9mM). The change in absorbance was recorded at 480nm for 8-12 minutes. Throughout the assay, the temperature was preserved at 30^{0}C. Similarly, SOD calibration curve was prepared by taking 10units/ml as standard solution. 1units of SOD produce approximately 50% of inhibition of auto-oxidation of adrenaline. The results are spoken as unit (U) of SOD activity per mg of tissue.

Lipid Peroxidation:

Procedure:

Thiobarbituric acid sensitive substances (TBARS), the last product in lipid peroxidation pathway, were measured using the modified method of Ester Bauer and Cheese man, 1990.Liver tissue (200mg) was standardized in 10 volumes of ice-cold 50mM Phosphate buffer(pH 7.4) and the homogenates were centrifuged at 12,000rpm for 15min at 4°C. The supernatant was used for the assay. Protein attention so different homogenates were measured according to the method of Bradford. Protein (1mg) was incubated at 37°Cfor1h and then 1ml 20% TCA and 2ml 0.67% TBA was added and animated for 30min at100°C. Precipitate was removed by centrifugation at 1000g for 10min. The absorbance of the samples was stately at 535nm against a blank that contains all the reagents except the sample. TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA which is 1.56×105mmol−1cm−1as 99% of TBARS is MDA.

Histopathological Studies:

Processing of isolated liver:

The animals were forwent and the liver of each animal was isolated and was cut into small pieces, preserved and fixed in 10% formalin for two days. Then the liver piecewaserodedinrunningwaterforabout12hoursto removetheformalin and was followed by dehydration with isopropyl alcohol of swelling strength(70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with about three changes for 12 hours each.

Dehydration was performed to remove all traces of water. Further alcohol was removed by using chloroform and chloroform removed by paraffin access. The clearing was done by using chloroform with two vagaries for 15 to 20 minutes each. After paraffin in filtration the liver bits were subjected to automatic tissue processing unit.

Embedding in paraffin vacuum: Hard paraffin in was melted and the hot paraffin was poured into L-shaped blocks. The liver pieces were then released into the molten paraffin quickly and allow cooling.

Sectioning: The blocks were cut using microtome to get sections of thickness of 5. The sections were taken on a micro slide on which egg albumin i.e., sticking substance was applied. The units were allowed to continue in an oven at 600C for 1 hour. Paraffin melts and egg albumin denatures, there by fixing tissue to slide.

Staining: Eosin is an acid stain, hence it stains all the cell constituents pink which are basicin nature i.e., cytoplasm. Haematoxylin, a basic stain which stains the entire acid cell components blue. i.e. DNA in the nucleus.

RESULTS AND DISCUSSION

Toxicity studies:

In the present study the Methanolic extract of Ocimum sanctum trees was exposed for toxicity studies. For the LD50 dose determination, Methanolic extract was administered upto dose 2gm/kg body weight and extract did not food any mortality, thus 1/10th, 1/20th of maximum dose tested were particular for the present study. LD50 of extracts of Ocimum sanctum was intended and found to be as follows: Methanolic extract– 1800 mg/kg.

Hepatoprotective activity:

Halothane induced toxicity:

Physical parameters

Wet liver weight and Wet liver volume:

Halothane treatment in rats caused in enlargement of liver which was plain by increase in the wet liver weight and volume. The groups were treated with Silymarin and Methanolic extract of *Ocimum sanctum* leaves showed important restoration of wet liver weight and wet liver volume faster to normal.

Table 4: Effect of Methanolic extract of *Ocimum sanctum*on Wet. Liver Weight & Wet liver volumes in Halothane induced hepato toxic rats

Group	Treatment	Dose	Wet Liver weight(gm/100gm) (Mean±SEM)	Liver volumes(ml/100gm) (Mean±SEM)
A	Normal control	10ml/kgp.o	2.875 ± 0.375	2.75±0.10
B	Toxicant Control	Halothane-600mg/kg,p.o.	5.35± 0.150	4.4±0.15
C	Standard	100mg/kg,p.o +Halothane	3.55 ± 0.05**	3.0±0.05**
D	OSME	100mg/kg,p.o +Halothane	4.15± 0.10*	3.665±0.015*
E	OSME	200mg/kg,p.o +Halothane	3.675 ± 0.125**	3.40±0.150**

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where, *represents significant at p<0.05, **signifies highly significant at p<0.01, ***represents very significant at p<0.001.

Biochemical Parameters

Effect of methanolic extract of *Ocimum sanctum* leaves on biochemical Parameters in Halothane induced hepatotoxic rats.

Effect of serum marker enzymes:

Rats treated with Halothane advanced a significant hepatic damage observed as elevated serum levels of hepato specific enzymes like SGPT, SGOT and SALP when compared to usual control. Pretreatment with Silymarin, methanolic extract presented good protection against Halothane induced toxicity to liver. Tukey-Kramer's test indicates a important reduction in elevated serum enzymes levels with extract frozen animals compared to toxicant change animals.

Table 5: Effect of methanolic extract of *Ocimum sanctum* leaves on SGPT, SGOT, ALP level sin Halothane induced hepato toxic rats

Group	Treatment	Dose	SGPT levels(U/) (Mean±SEM)	SGOT levels/) (Mean±SEM)	ALP levels(d) (Mean±SE)
A	Normal Control	10ml/kgp.o	28.175±0.325	34.05±4.5	33.0±0.50
B	Toxicant Control	Halothane-600mg/kg,p.o.	108±2.50	188.05 ±2.50	92.95±0.550
C	Standard	100mg/kg,p.o +CCl4	37.5±1.0***	51.5±1.0**	40.05±0.55**
D	OSME	100mg/kg, p.o + CCl4	87.5±2.0*	143.75±8.750*	81.2±0.30*
E	MPME	200mg/kg, p.o + CCl4	53.9±1.30**	88.5±3.0**	51.75±0.250**

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test.

Where, *represents significant at p<0.05,**represents very significant at p<0.01,and***represents very important at p<0.001.

Serum direct bilirubin and total bilirubin:

Raise of direct and total bilirubin level safter direction of Halothane indicate its hepatotoxicity. Pretreatment with Silymarin, Methanolic extract significantly summary levels of direct and total bilirubin levels when compared to toxic control group indicating Hepatoprotective effect of Methanolic cutting of *Ocimum sanctum* leaves.

Table 6: Effect of Methanolic extract of *Ocimum sanctum* leaves on direct bilirubin, directbilirubin levels in Halothane induced hepatotoxicity rats.

Group	Treatment	Dose	Direct bilirubin levels (mg/dl) (Mean±SEM)	Total bilirubin Levels (mg/dl) (Men±SEM)
A	Normal Control	10ml/kgp.o	0.173±0.0120	0.293±0.029
B	Toxicant Control	Halothane-600mg/kg,p.o.	1.679±0.099	1.8±0.005
C	Standard	100mg/kg, p.o +CCl4	0.256±0.012***	0.463±0.049***
D	MPME	100mg/kg, p.o + CCl4	1.26±0.032**	1.33±0.059**
E	MPME	200mg/kg, p.o + CCl4	0.50±0.040**	0.736±0.1105**

Values are mean ±SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, *represents significant at p<0.05, **signifies highly significant at p<0.01, and ***signifies very significant at p<0.001.

Serum total protein levels:

Halothane action considerably reduced serum total protein levels. Pretreatment with Silymarin and methanolic extract of *Ocimum sanctum* leaves showed a significant surge in total protein levels as likened with toxicant control group.

Serum cholesterol and Triglycerides:

From the results it was found that rats treated with Halothane showed a marked increase in lipid and triglycerides levels when compared to normal control group. In rats pretreated with Silymarin and Methanolic extract of *Ocimum sanctum* leaves these rum fat and triglycerides levels had knowingly reduced when compared to toxic and control group.

Table 7: Effect of Methanolic extract of *Ocimum sanctum* leaves on Serum total protein, Total cholesterol & Triglyceride levels in Halothane induced hepatotoxic rats.

Group	Treatment	Dose	Total protein levels (gm/dl) (Mean±SEM)	Total Cholesterol levels (mg/dl) (Mean±SEM)	Triglyceride levels (mg/dl) (Mean±SEM)
A	Normal Control	10ml/kgp.o	5.45±0.07	135.6±4.59	0.515±0.0126
B	Toxicant Control	Halothane-600mg/kg, p.o.	2.89±0.25	324.05±10.5	2.38±0.0879
C	Standard	100mg/kg, p.o +CCl4	4.86±0.06***	165±3.46***	0.890±0.0382***
D	MPME	100mg/kg, p.o + CCl4	3.52±0.78**	284.5±4.53**	2.015±0.0182**
E	MPME	200mg/kg, p.o + CCl4	4.45±0.12***	214±5.50***	1.08±0.0694***

Values are mean±SEM (n=6) oneway ANOVA followedby Tukey-Kramer's test. Where, *represents significant at p<0.05, **represents highly significant at p<0.01, ***represents very significant at p<0.001.

Antioxidant Parameters:

Table 8: Effect of Methanolic extract of *Ocimum sanctum* leaves on catalase, Superoxide dismutase and Lipid peroxidation in Halothane induced hepatotoxicity

Group	Treatment	Dose	CAT (Mean±SEM)	SOD (Mean±SEM)	LPO (Mean±SEM)
A	Normal Control	10ml/kgp.o	91.8±3.412	14.5±0.5774	3.83±0.60
B	Toxicant Control	Halothane-600mg/kg,p.o.	22.3±0.8819	3.23±0.088	89.26±0.1856
C	Standard	100mg/kg,p.o+CCl4	83.05±0.622***	11.0±0.352***	6.53±0.202***
D	OSME	100mg/kg,p.o+CCl4	36.4±0.585**	5.8±0.152**	7.86±0.03**
E	OSME	200mg/kg,p.o+CCl4	51.03±1.093***	7.7±0.2517***	7.26±0.0667**

a=n mole of MDA/mg of protein. b= Units/mg of protein
c=µmoleofH2O2consumed/min/mg of protein, d= µg/mg of protein
Values are mean ± SEM (n=6) one way ANOVA trailed by Tukey-Kramer's test. Where, *signifies significant at p<0.05, ** represents highly significant at p< 0.01, *** signifies very significant at p<0.001

Functional parameters:

Thiopentone induced sleeping time

From the results, it was found that rats treated with Halothane have showed a marked reduction in onset of sleep and increase in duration of a sleep time when compared against normal control group. Onset of sleep had significantly increased in the rats pretreated with Silymarin and Methanolic extract of Ocimum sanctum leaves while the length of sleeping time had significantly decreased when compared to toxicant group.

Table 9: Effect of Methanolic extract of Ocimum sanctum leaves on Onset of sleep & Duration of sleep in Halothane induced hepatotoxicity rats

Group	Treatment	Dose	Onset of time(sec) (Mean±SEM)	Duration of sleeping (min) (Mean±SEM)
A	Normal Control	10ml/kgp.o	177.5±2.5	96.75±3.775
B	Toxicant Control	Halothane-600mg/kg,p.o.	65±10.0	225.6±7.68
C	Standard	100mg/kg,p.o+CCl4	157.5±7.5***	115±2.74***
D	OSME	100mg/kg,p.o+CCl4	101.5±3.5*	192.83±3.712**
E	OSME	200mg/kg,p.o+CCl4	130±5.0**	152.33±3.84***

Values are mean ± SEM(n=6) oneway ANOVA followed by Tukey-Kramer's test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, ***and represents very significant at p<0.001.

Histo pathological studies of the liver in Halothane induced hepatotoxicity

Normal control group: Section studied shows liver parenchyma with intact architecture. Some of the hepatocytes show mild nuclear pleomorphism with prominent nucleoli. There are seen scattered mononuclear inflammatory infiltrations within all the zones.

Halothane treated group: Section calculated shows liver parenchyma with effaced architecture. All the zones show areas of hemorrhage, necrosis, micro vesicular steatosis, macro vesicular steatosis, degenerative hepatocytes. There are seen total sotf mixed inflammatory infiltration within all the zones.

Silymarin + Halothane treated group: Section willful shows liver parenchyma with focal eroded architecture. Few of the perivenular hepatocytes and focal midzonal hepatocytes show macrosteatos is and microsteatosis. Some of the vital veins and sinusoids show dilatation with focal congestion. Also seen are mild stromal inflammatory infiltration comprising of lymphocytes and macrophages.

Methanolic extract (100mg/kg) + Halothane: Section studied displays liver parenchyma with partially destroyed architecture. Some of the perivenular hepatocytes and focalmidzonal hepatocytes show necrosis. While some of the hepatocytes show macrosteatosis and microsteatosis. There are seen moderate mixed fiery infiltration comprising of neutrophils and lymphocytes.

Methanolic extract (200mg/kg) +CCl4: Section studied displays liver parenchyma with partial effaced building. Few of the perivenular hepatocytes and focalmidzonal hepatocytes show macrosteatosis and microsteatosis. There are seen modest mono nuclear inflammatory penetrations within the perivenular hepatocytes and also inmidzonal hepatocytes.

Plate 1: Histopathology of Liver:

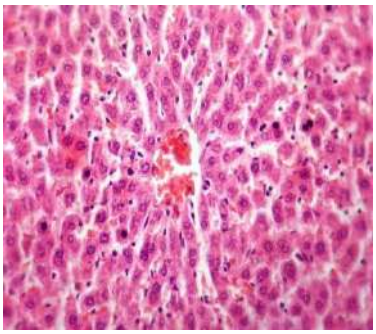

Fig1: Control group

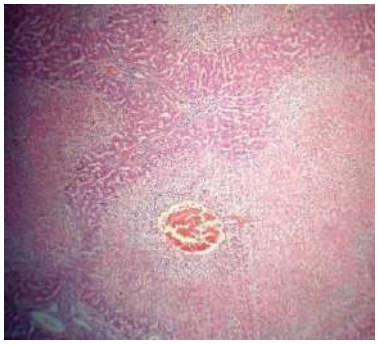

Fig2: Halothane treated group

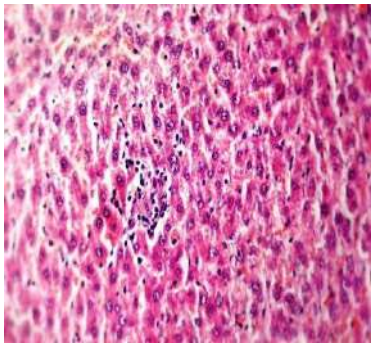

Fig 3: Standard group

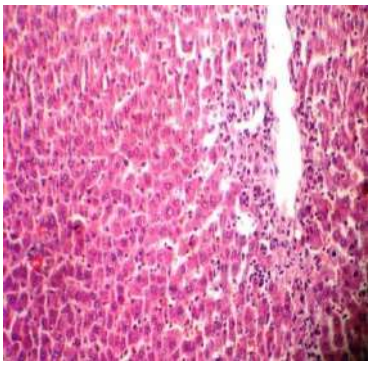

Fig 4: Test group-I

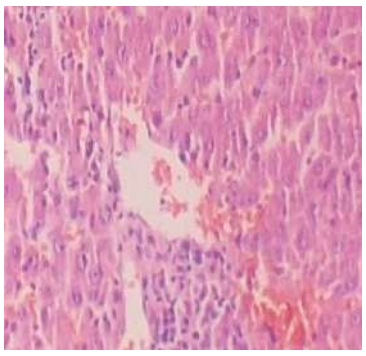

Fig 5: Test group-II

Hepatoprotective activity:

Liver participates in a change of metabolic activities possibly by virtue of presence of number of enzymes and thus may self expose too many toxicants, elements and drugs which could injure it. In our hepatoprotective study, Halothane is used as hepatotoxicant to induce liver injury, since this agent is used/ agreed frequently to the human lives for either medicinal or non-medical purposes.

Mechanism of Halothane-induced hepatotoxicity:

The Halothane is absorbed by CYP 450 enzyme system to tri chloro methyl radical (CCl3•). This in turn reacts with molecular oxygen and gets converted to tri chloro methyl peroxy radical. This essential forms `covalent bonds with sulfydryl group of several membrane molecules like GSH leading to their reduction and causes lipid peroxidation. Thelipid peroxidation initiates a cascade of responses leading to tissue necrosis.

Physical parameters:

Wet Liver Weight and Wet Liver Volume:

In case of toxic liver, Wet liver weight and Wet liver volumes are enlarged. Toxicants induced hepato toxicity crop fatty changes and also it is observed that there is a fall in serum lipids in another series of experiments. In this case water is retained in the cytoplasm of hepatocytes leading to enlargements of liver cells, resultant in increased total liver mass and volume.[73] It is reported that liver mass and volume are important parameters in ascertaining the hepatoprotective effect of the medications. Action with Methanolic extract of the leaves of *Ocimum sanctum* significantly reduced the wet liver weight and wet liver volumes of creatures and hence it possesses statistically important hepato protective activity.

Biochemical Parameters:

Estimation of Serum Marker Enzymes:

Hepato toxin gets converted into activists in liver by action of enzymes & these attacks the unsaturated fatty acids of skins in presence of oxygen to give lipid peroxides. The functional integrity of hepatic mitochondria is different, leading to liver damage.

During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resultant in increased concentrations. Halothane running for 25 days significantly amplified all these serum enzymes.

SGT is a cytosolic enzyme mainly present in the liver. The level of SGPT in serum increases due to escape of this cellular enzyme in to plasma by toxicants induced hepatic injury. Serum levels of SGPT can increase due to injury of the tissues producing acute hepatic necrosis, such as virus-related hepatitis and acute cholestasis. Alcoholic liver damage and cirrhosis also can associate with mild to moderate elevation of transaminases. In the current study treatment of rats with methanolic extract of the leaves of *Ocimum sanctum* significantly reduced the levels of SGPT in serum which is an indication of hepatoprotective activity.

SGOT is a mitochondrial enzyme unconfined from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as plain viral hepatitis & acute cholestasis. Alcoholic liver injury and cirrhosis can also associate with mild to moderate elevation of transaminases. In the current study treatment of animals with methanolic cutting of *Ocimum sanctum* leaves significantly diminished the levels of SGOT in serum which is an indicative of hepatoprotective activity.

In case of toxic liver, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by amplified production of ALP by hepatic parenchymal or duct cells. In the current study treatment of animals with Methanolic extract of *Ocimum sanctum* leaves significantly lessened the levels of ALP in serum as a sign of hepatoprotective activity.

Direct and Total Serum bilirubin:

In case of deadly liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin. Such as that can occurin generalized liver cell injury. Certain drugs (e.g.,rifampin and probenecid) interfere with the net acceptance of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia.[74] Bilirubin level rises in viruses of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease.[78] In the present study treatment of animals with methanolic extract of *Ocimum sanctum* leaves significantly reduction the levels of bilirubin (direct and total) in serum which is an indication of hepatoprotective activity.

Cholesterol and Triglycerides:

Toxicant induces hypercholesteremia and hypertriglyceridemia, may be due to the activation of enzyme HMG CoA reductase, the rate-limiting step in fat biosynthesis.[79] The increased serum

triglyceride level in Halothane-treated rats may be due to the reduced activity of lipo protein lipase, which is complex in the uptake of triglyceride-rich lipoprotein by the extra hepatic skins.[79] Pretreatment with Methanolic extract of *Ocimum sanctum* leaves reduced the elevated fat and triglyceride levels, suggesting.

That the extracts prevented ethanol-induced hyperlipidemia may be due to their hepatoprotective activity. Treatment with OSME significantly reduced the levels of fat and triglycerides in Halothane induced hepato toxic animals, signifying the hepato protection.

Total Protein:

Liver toxicity reductions the Total Protein level in serum due to the damage to the tissues. Since the methanolic extract of *Ocimum sanctum* leaves show increase in Total Protein level in serum of animals it owns statistically important hepatoprotective activity.

Functional Parameters:

Thiopentone induced sleeping time:

Toxicant also alters the metabolic action of hepatocytes, thereby inducing hepatic damage. Barbiturates are a class of xenobiotics that are extensively absorbed in the liver. Deranged liver function leads to delay in the clearance of barbiturates, resulting in a longer duration of hypnotic effect. In the present study, management of thiopentone sodium to rats pretreated chronically with a toxicant caused in an increased duration of thiopentone sleeping time. Pre-treatment with Methanolic extract of *Ocimum sanctum* leaves decreased thiopentone-induced slumber time, an indirect sign of their hepato protective effect.

Antioxidant parameters:

Superoxide dismutase and Catalase:

Our study further revealed that lingering exposure to ethanol decreased the activities of the ROS scavenging enzymes, viz. SOD and CAT. This is in line with assumption suggested earlier by Sandhir and Gill, that decrease in the action of antioxidant enzymes SOD and CAT next Halothane exposure may be due to the damaging effects of free radicals, or alternatively could be due to a direct effect to acetaldehyde, formed from oxidation of, Halothane on these enzymes. In our studies, it discloses that OSME could restore the activity of both these antioxidant enzymes and possibly could reduce group of free radicals and hepatocellular injury.

Lipid Peroxidation:

Formation of ROS, oxidative pressure and hepatocellular injury has been implicated to alcoholic liver disease. It has been documented that Kupffer cells are the major sources of ROS during chronic Halothane ingesting, and these are primed and triggered for enhanced creation of pro-inflammatory factors. Additionally, alcohol-induced liver injury has been associated with augmented amount of lipid peroxidation. Indeed, OSME supplementation in our study was potentially effective in dampening lipid peroxidation, suggesting that OSME possibly has antioxidant stuff to reduce Halothane-induced membrane lipid peroxidation.

The hepato toxicity of Halothane results from its metabolic conversion to free radical product CCl3 by CytP-450. Once CCl3 has been fashioned it reacts very rapidly with O2 to produce $CCl3OO^{-}$, a much more reactive radical than CCl3. These free radicals attack microsomal lipids chief to its peroxidation and also covalently bind to microsomal lipid sand proteins. This results in the generation of reactive oxygen species (ROS), which includes the super-oxide anion O2, H2O2 and the hydroxyl radical. Although various enzymatic and non-enzymatic system shave stood developed by cell to copeup with the ROS and other free radicals, when a disorder of oxidative stress establishes, the defense capacities against ROS becomes insufficient.[85] ROS also affects the antioxidant defense devices, reduces the intra

cellular concentration of GSH, and decreases the activity of SOD and CAT. It has also known to decrease the detoxification system produced by GST. Increasing evidence indicates that oxidative stress causes liver injury, cirrhosis growth and carcinogens. In our studies, it exposes that OSME could restore the movement of both these antioxidant enzymes.

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