ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 11 | November - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

HEPATOPROTECTIVE EFFECT OF OCIMUM SANCTUM LEAF EXTRACT IN ALCOHOL-INDUCED LIVER DAMAGE

Pharmacology

Fardan Qadeer Associate Professor, Department of Pharmacology, Era's Lucknow Medical College, Lucknow

Fariha Fatima* Associate Professor, Department of Pharmacology, Era's Lucknow Medical College, Lucknow *Corresponding Author

Abeer Zubair Khan Associate Professor, Department of Anatomy, Hind Institute of Medical Sciences, Barabanki

Maaz Syed Intern, Era's Lucknow Medical College, Lucknow

ABSTRACT

Ocimum sanctum, often known as green tulsi, has been utilized for its hepatoprotective properties since ancient times. In this work, we assessed Ocimum sanctum's hepatoprotective effects in alcohol-induced hepatitis and its synergistic hepatoprotection with silymarin. **Materials and Methods:** Background Albino rats (150–200 g) were divided into four groups. Groups I and II were control and test, respectively. Test group received the alcoholic extract of Ocimum Sanctum leaves (OSE) 200 mg/kg BW/day and group III received silymarin 100 mg/kg BW/day and group IV OSE 100 mg/kg BW/day + silymarin 50 mg/kg BW/day respectively, for a period of 4 weeks. The hepatoprotective effect was evaluated by performing liver function test to assess the serum proteins, albumin globulin ratio, alkaline phosphatase, transaminases and liver histopathology. The results were presented as mean and standard error of mean (SEM) for each group. The study group was compared with the control group by one-way ANOVA. A P-value of <0.05 was considered significant. **Results:** In groups II, III and IV, liver enzymes and albumin globulin ratio were significantly ($P < 0.05$) improved compared to group I. Alcohol induced hepatotoxicity produces endotoxins which activates Kupffer cells and results in peri central lobar hypoxia. Reduction in lobular inflammation, zone 3 injury, and degenerative areas of the liver were observed on histopathological examination in groups II, III and IV. **CONCLUSION:** In cases of alcohol-induced hepatotoxicity, the alcoholic leaf extract of Ocimum sanctum exhibits considerable hepatoprotective action and synergism with silymarin.

KEYWORDS

Hepatoprotective, alcohol, hepatotoxicity, Ocimum sanctum, silymarin

INTRODUCTION

In India, drinking alcohol is a significant contributing factor to morbidity and mortality. According to estimates, alcohol intake was a contributing factor in around 3.3 million fatalities in India in only 2010 [1]. The most frequent cause of liver cirrhosis is alcoholism [2], which not only worsens health but also increases the financial burden on a community and a nation.

Tulsi or Ocimum Sanctum, is a member of the Lamiaceae family. It is a tropical plant that is both planted and grows as a weed. Ocimum sanctum is traditionally consumed in a variety of ways, such as fresh leaf, dried powder, or herbal tea. [3]

The medicinal potential of Ocimum sanctum leaves has received more attention in recent years. It has been shown that these leaves have expectorant, diaphoretic, antiseptic, spasmolytic, stimulant, and anticatarrhal effects and they are used to treat fever, pain, worm infestations, skin illnesses, snakebite, and scorpion stings in addition to treating colds and coughs.[4]

Ocimum sanctum has been shown to have significant hepatoprotective action against paracetamol, carbon tetrachloride, and anti-tubercular drug-induced hepatotoxicity in albino rats. [5-7] Therefore, the purpose of this study was to assess the hepatoprotective effects of Ocimum sanctum on alcohol-induced hepatotoxicity and to look for a synergistic or additive impact when Ocimum sanctum was combined with a conventional hepatoprotectant.

MATERIALS AND METHODS

Study Design: Experimental
Study Period: 9 months
Animals: Swiss Albino Mice (20-25gm).

The animals were maintained in polycarbonate cages, under the room temperature of 25 ± 2°Cand 45-55% relative humidity, with a 12-hour light/dark cycle. They were allowed food and water ad libitum. Animals were given a standard pellet diet obtained from Unilever Limited.

Animals were obtained from CPCEA-approved animal houses (IITR, Lucknow).

All experiments were performed after approval from the Institutional Animal Ethics Committee of Eras' Lucknow Medical College (Approval Number: IAEC/ELMCH/1/19/7)

All experiments were performed as per the guidelines of animal care by CPCEA

Test Drug

Ocimum sanctum alcoholic leaf extract (OSE) was prepared by the validated method

The OSE extract was used in a dose of 100 mg/kg BW and 200 mg/kg BW for the respective groups.

Standard Drugs

Silymarin (SILY) was obtained from Charak Pharmaceuticals Ltd. It was powdered to make a suspension in the doses of 100 mg/kg BW and 50 mg/kg BW for the respective groups following previous studies.

Sample Size Calculation

The sample size was calculated as 24 animals divided into 4 groups of 6 animals each.

$$n = \frac{(\sigma_1^2 + \sigma_2^2)(Z_\alpha + Z_\beta)^2}{d^2}$$

Type I error = 5%	Type II error = 20%	σ_1=2.36	σ_2= 2.48
Power of study=80%	Loss of data=10%	d= 13	

n= 6 each group

Induction Of Alcoholic Hepatitis[15]

Laboratory-grade Ethanol was obtained from Sigma Aldrich Laboratories Ltd. Ethanol in a dose of 5 mg/kg orally per day for a period of 4 weeks was given for the induction of Alcoholic hepatitis. This was given in all the animals along with the drugs for a period of 4 weeks

The animals were divided into the following groups:

Group	Intervention
II: Alcoholic hepatitis	Vehicle: 5 ml/kg BW per day
III: Test	OSE 200 mg in vehicle 5 ml/kg BW per day
III: Standard	SILY 100 mg in vehicle 5 ml/kg BW per day
IV: Test+ Standard	100 mg OSE+ 50 mg SILY in vehicle 5 ml/kg BW per day

Dosing and Administration of Drugs

The drug suspensions and the vehicle were administered per orally by an intragastric feeding tube at a uniform volume of 5 ml/kg BW

Laboratory Assessments

Blood was be collected from the hearts of the animals under pentobarbitone anaesthesia. The serum was separated and was sent for liver function tests (LFT), namely total serum protein, albumin globulin ratio, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). [6]

Histopathological Examination

The animals were be sacrificed at the end of 4 weeks under deep pentobarbitone anaesthesia (200 mg/kg) and the liver samples was excised and washed with normal saline. The gross specimen was be observed for the size and shape, colour and presence or absence of any nodule. Then, the liver was fixed immediately in 10% formalin solution. A paraffin embedding technique was carried out and sections were taken at 5 mm thickness, stained with haematoxylin and eosin and was examined microscopically for histopathological changes.

Statistical Analysis

All data were analyzed by Analysis Of Variance Test [ANOVA] using GRAPH Pad Prism 16.0 Version. P value <0.05 was considered significant

RESULTS

The LFT results are encapsulated [Table 1] and expressed as mean ± SEM (n = 6). In group III, Total protein, A/G ratio and liver enzymes were significantly improved to near normal range after treating with silymarin. (P <0.05). There were significant improvement of LFT parameters in animals which was treated with test drug i.e.

Ocimum sanctum, compared to Group I. (p<0.05). Liver enzymes and albumin globulin ratio were significantly (P < 0.01) improved compared to group I in all the other groups, however, Group III had improvement to near normal range compared to Group II and IV. (p<0.05)

Table 1: Effects of the alcoholic leaf extract of Ocimum sanctum on total protein, albumin globulin ratio, serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in alcohol induced hepatitis in albino rats

GROUP	Total protein (g/dl)	Albumin globulin ratio	Serum ALP (KA units)	Serum AST (IU/L)	Serum ALT (IU/L)
I: Alcoholic hepatitis	4.85 ± 0.57	0.17 ± 0.08	28.5 ± 1.05	750 ± 34.64	351.6 ± 20.41
II: Test	6.26 ± 0.27	1 ± 0.14	16.5 ±1.76	265 ± 12.24	73.33 ± 12.11
III: Standard	7.56 ± 0.29	1.38 ± 0.22	11.66 ± 1.5	103 ± 23.3	49.16 ± 7.35
IV: Test+ Standard	6.88 ± 0.24	1.08 ± 0.21	15.16 ± 1.6	170 ± 12.64	64.16 ± 7.35
P -value	0.0001	0.0001	0.0001	0.0001	0.0001

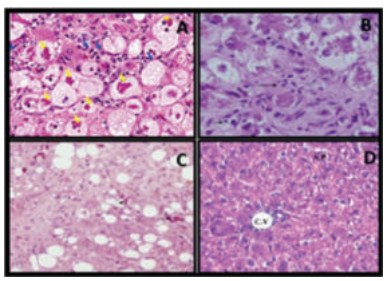

Figure 1: A. Macrovesicular steatosis in alcoholic hepatitis B. Neutrophilic inflammation(arrow) and ballooning of hepatocyte C. Prominent Mallory hyaline body(arrow) D. evidence of regeneration.

We observed steatosis, zone 3 injury, lobular inflammatory infiltrates especially rich in neutrophils, ballooning degeneration, Mallory- Denk bodies and megamitochondria in all animals with alcohol hepatitis (group I) neutrophilic inflammation, ballooning of hepatocytes and prominent Mallory hyaline bodies. we observed macrovesicular steatosis,[Figure 1A-C]. In groups II, III and IV, there was marked reduction in lobular inflammatory changes, steatosis, Degeneration and zone 3 injury with areas of regeneration as well. [Figure 1D].

DISCUSSION

Ocimum sanctum Linn also known as Tulsi, is a medicinal herb that is widely used in conventional medicine. According to studies, Tulsi can be used to treat bronchitis, cancer, hypertension, and diabetes. Both anti-inflammatory and anti-microbial activities are present in this plant. In research on animals, it was shown that Ocimum sanctum essential oils and extracts might reduce oxidative stress by boosting glutathione peroxidase, and catalase that leads to hepatoprotective effects. Protection against stomach ulceration is provided by the anti-ulcer and anti-secretory action. Tulsi has biochemically active ingredients such as Eugenol, carvacrol, ursolic acid, -caryophyllene, and rosmarinic acid that have medical properties. [8,9]

Hepatotoxicity brought on by alcohol is complex. Endotoxins cause the Kupffer cells to become activated, which causes the hepatic parenchymal cells to enter a hypermetabolic state and begin to cause liver damage. The pericentral lobular hypoxia that results from this hypermetabolic state causes the generation of damaging free radicals and consequent cell death [10]. Alcohol has been shown to change how well Kupffer cells operate, including their ability to phagocytose, produce cytokines and engage in bactericidal action. [4] Alcoholics have higher levels of TNF alpha which is triggered by the monocyte-macrophage system. The Kupffer cells are the main cell type of this lineage. It is also clear that long-term drinking opens calcium channels as well.

The generation of free radicals has long been thought to contribute to ethanol-induced hepatotoxicity. Early events in alcoholic liver disease are likely affected by the development of free radicals often known as oxidative stress. The transcription factor Nf kappa B is activated by oxidative stress which also increases the production of adhesion molecules. [5]

Alcohol lowers catalase and superoxide dismutase function, inhibits GSH peroxidase, and raises GSH levels in the liver. Superoxide dismutase and GSH peroxidase activity is thought to diminish as a result of the harmful effects of free radicals created after exposure to ethanol or alternatively, as a direct result of acetaldehyde which is produced when ethanol is oxidized [6].

In this study, we evaluated the hepatotoxicity caused by alcohol and looked for a synergistic or additive impact when Ocimum sanctum and a conventional hepatoprotectant were combined.

We discovered that alcoholic hepatitis in albino rats led to a statistically significant elevation in serum ALP, AST, and ALT levels while decreasing levels of total blood proteins and albumin globulin ratio. Alcohol had the opposite effects in groups II, III, and IV. The blood levels of ALP, AST, and ALT were much lower in the III and IV groups compared to the alcohol group, and the albumin:globulin ratio increased significantly. However, there was no discernible distinction between these groups' total protein levels.

The blood AST and ALT levels in Group IV were significantly lower than those in Silymarin. When comparing the findings of the LFT alone, group III demonstrated higher hepatoprotection than group IV. Steatosis, lobular inflammatory infiltrates, Mallory-Denk bodies, megamitochondria, and zone 3 damage were all seen in the alcohol group's histology. The rats administered OSE (group II), silymarin (group III), or both OSE and silymarin (group IV) showed notable protection of hepatic tissue against alcohol.

Similar to the results of prior investigations, administration of the alcoholic extract of Ocimum sanctum leaves shown considerable hepatoprotective action.[10] With the OSE + SILY group, synergistic hepatoprotective effect was seen. OSE demonstrated superior hepatoprotection compared to OSE + SILY. However, OSE and the

OSE + SILY combo were less effective than SILY by itself.

Ocimum sanctum leaves contain eugenol, flavonoids, and ursolic acid components that have anti-lipoperoxidative and free radical scavenging properties. Therefore, the antioxidant activities of Ocimum sanctum's components may be the cause of the leaves' hepatoprotective effects. [10] Ocimum sanctum's ability to stabilize membranes is what gives it its hepatoprotective effects. [9]

Additionally, linoleic acid, which is responsible for the anti-inflammatory properties of Ocimum sanctum may also be responsible for correcting the inflammatory aspects linked to hepatic damage.

CONCLUSION

Ocimum sanctum has considerable hepatoprotective effect ($P<0.05$). Ocimum sanctum leaves have a synergistic action with silymarin which is statistically significant ($P < 0.05$). Compared to the Ocimum sanctum and silymarin combination group, the Ocimum sanctum group demonstrated greater hepatoprotection. The Ocimum sanctum leaf extract alone and in combination with silymarin had a weaker hepatoprotective effect than silymarin alone in the dosages tested. The Ocimum sanctum extract may have resulted in a lesser hepatoprotective effect compared to silymarin which is a well-known standard hepatoprotective.

Acknowkedgement: Nil

REFERENCES

1. Chattopadhyay RR, Sarkar SK, Ganguly S, Medda C, Basu TK. Hepatoprotective activity of Ocimum sanctum leaf extract against paracetamol included hepatic damage in rats.
2. Lahon K, Das S. Hepatoprotective activity of Ocimum sanctum alcoholic leaf extract against paracetamol-induced liver damage in Albino rats. Pharmacognosy research. 2011 Jan;3(1):13.
3. Mahajan N, Rawal S, Verma M, Poddar M, Alok S. A phytopharmacological overview on Ocimum species with special emphasis on Ocimum sanctum. Biomedicine & Preventive Nutrition. 2013 Apr 1;3(2):185-92.
4. Eshraghian A. Anti-Inflammatory, gastrointestinal and hepatoprotective effects of Ocimum sanctum Linn: an ancient remedy with new application. Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy) (Discontinued). 2013 Dec 1;12(6):378-84.
5. Pandey G, Madhuri S. Pharmacological activities of Ocimum sanctum (tulsi): a review. Int J Pharm Sci Rev Res. 2010 Nov;5(1):61-6.
6. Bano N, Ahmed A, Tanveer M, Khan GM, Ansari MT. Pharmacological evaluation of Ocimum sanctum. J Bioequiv Availab. 2017;9(3):387-92.
7. Almatroodi SA, Alsahli MA, Almatroudi A, Rahmani AH. Ocimum sanctum: role in diseases management through modulating various biological activity. Pharmacognosy Journal. 2020;12(5).
8. Shukla ST, Kulkarni VH, Habbu PV, Jagadeesh KS, Patil BS, Smita DM. Hepatoprotective and antioxidant activities of crude fractions of endophytic fungi of Ocimum sanctum Linn. in rats. Oriental Pharmacy and Experimental Medicine. 2012 Jun;12:81-91.
9. Mondal S, Mirdha BR, Mahapatra SC. The science behind sacredness of Tulsi (Ocimum sanctum Linn.). Indian J Physiol Pharmacol. 2009 Oct 1;53(4):291-306.
10. Maurya AK, Bisht LS, Dobhal M, Parveen N, Rawat T. PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF OCIMUM SANCTUM MEDICINAL PLANTS FOR THE TREATMENT OF HEPATOTOXICITY. Journal of Pharmaceutical Negative Results. 2022 Nov 10:1963-6.