PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF OCIMUM SANCTUM MEDICINAL PLANTS FOR THE TREATMENT OF HEPATOTOXICITY

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DOI: 10.47750/pnr.2022.13.S08.239

Abstract

Modern medicine does not have reliable liver protection drugs that prevent and treat liver injury caused by drugs. The leaves of sacred / holy basil (Ocimum sanctum) belong to the family of lamiaceae, which is traditionally used for its liver protection. We aimed to determine if Ocimum sanctum has hepatoprotective properties and, if so, whether or not they work synergistically with silymarin. Albino rats (150–200 g) were divided into five groups. Groups A and B are normal control groups and experimental control groups, respectively. Groups C, D and E received the alcoholic extract of Ocimum sanctum leaves (OSE) 200 mg / kg BW / day, silymarin 100 mg/kg BW/day and OSE 100 mg/kg BW/day + silymarin 50 mg/kg BW/day p.o., respectively, for 10 days. Hepatotoxicity was induced in Groups B, C, D and E on the eighth day with paracetamol 2 g/kg BW/day. The effect of hepatoprotection was evaluated through a study of serum proteins, albumin-globulin ratios, alkaline phosphatase, transaminase, and liver histology. The results of the study were presented as averages and standard deviations for each group (SEM). The study group was compared with the control group with a single-way ANOVA and Bonferoni test followed. A P-value of <0.01 was considered significant. In groups C, D and E, liver enzymes and albumin globulin ratio were significantly (P < 0.01) closer to normal than in group B. On histological inspection, groups C, D and E revealed reduced sinusoidal congestion, cloudy edoema, fatty abnormalities, and regenerating regions of the liver, but group B showed only liver necrosis. The extract of the alcoholic leaves of Ocimum sanctum has significant liver protection and synergy with Silimarin.

Keywords: Ocimum sanctum, Hepatotoxicity, Liver, Aqueous extract, Paracetamol.

INTRODUCTION

The liver controls a wide range of crucial metabolic processes, and any damage distorts these processes. According to estimates, liver problems cause roughly 20,000 fatalities annually in the United States. With more than 2,50,000 new cases reported each year, hepatocellular carcinoma is one of the 10 most prevalent cancers worldwide. [1] Herbal remedies that protect the liver include phenols, coumarins, lignans, essential oils, monoterpenes, carotenoids, glycosides, flavanoids, organic acids, lipids, and derivatives of xanthones. There have been reports that liver problems are cured by extracts from roughly 25 different plants. [2] Despite the enormous efforts made in the area of contemporary medicine, there are very few medications that promote liver function, protect the liver from harm, or aid in liver cell regeneration. [3] Throughout history, people have prevented and treated liver disease using plants and natural items. Numerous studies on the potential of these herbal components for hepatoprotection show that scientific data support the claims of their therapeutic usefulness. [5] There are more than 700 mono and polyherbal preparations from more than a hundred distinct plants that can be used. [6] Ocimum sanctum, often known as Green Tulsi or Sacred or Holy Basil, is a well-known medicinal herb that thrives in India’s wild as well as in homes and temples. It has long been believed to have revitalizing, toning, and vitalizing characteristics that promote longevity and a healthy existence. [7] Ocimum sanctum leaves have expectorant, diaphoretic, antiseptic, spasmolytic, stimulant, and anticatarrhal qualities. They are used to treat fever, pain, worm infestations, skin conditions, snakebites, and scorpion stings, in addition to colds and coughs. [9] Ocimum sanctum has been shown to have a significant hepatoprotective action against paracetamol, carbon tetrachloride, and anti-tubercular drug-induced hepatotoxicity in albino rats. [9-11] In order to expand on these results regarding the hepatotoxicity caused by paracetamol, we looked for a synergistic or additive effect when Ocimum sanctum was combined with a conventional hepatoprotectant.
Thus, the aim of our study was to:

1. Evaluate the hepatoprotective activity of Tulsi (Ocimum sanctum) leaves on paracetamol-induced hepatotoxicity in albino rats as compared with silymarin.

2. Evaluate whether the combination of Tulsi (Ocimum sanctum) and silymarin had synergistic or an additive hepatoprotective activity.

MATERIALS AND METHODS

Ethical Committee Clearance

The ethical clearance was obtained from the institute animal ethics committee (634/02/A/CPCSEA, dated 19/05/20022).

Experimental Animals

Healthy albino rats (Rattus norvegicus) of Wistar strain (both male and female), weighing 100–200 g each (obtained from animal house) received the standard diet with water ad libitum throughout the experiment as per the recommendation of the Committee for the Purpose of Animal Control and Supervision of Experiments (CPCSEA) for laboratory animal facilities. [12]

Drugs

All drug suspensions were prepared for the different groups with 3% (W/V) aqueous suspension of gum acacia as vehicle.

Test drug

alcoholic leaf extract of Ocimum sanctum (OSE). This was prepared as follows:

Fresh Ocimum sanctum leaves weighing one kilogram were gathered, properly cleaned with cold water, dried in the shade at room temperature, and then crushed in an electrical mixer-grinder. Ms. Belinda Lahon, PhD in Botany, University of North Bengal, recognised the leaves. A securely closed container was used to soak 100 grammes of this air-dried powdered leaf material in 90% ethyl alcohol for 15 minutes. After that, the soaking powder was transferred to a percolator, where it was tightly packed and macerated for 24 hours at room temperature before slowly percolating. Repeating the process with enough 90% alcohol over the course of the following 24 hours, until no further extraction was feasible. To obtain the dried leaf alcoholic extract of Ocimum sanctum, alcohol was evaporated to a soft extract and the remaining material was placed in a vacuum desiccator. [13] We obtained 5 g of a sticky and dark greenish-black (5% dry weight of powdered leaves). The OSE suspension was administered to the several groups at dosages of 200 mg/kg BW and 100 mg/kg BW in accordance with earlier research in other hepatotoxic model organisms. [9,10].

Standard hepatoprotective

Silymarin (SILY) powder (obtained from Micro Labs Ltd., Bangalore, India) was used to make the suspension in doses of 100 mg/kg BW and 50 mg/kg BW for the respective groups following the method of Mankani et al. and Mansour et al.[14,15]

Hepatotoxin

Paracetamol (PCM) powder (IPP) (obtained from Bharat Chemicals, Tarapur, Gujarat, India) was used to make the suspension in a dose of 2 g/kg BW for the respective groups.
Methods

This experiment was carried out for 10 days in 30 healthy albino rats. Before undergoing the experiment, the animals were allowed to acclimatize for one week to the laboratory environment.

Dosing and Administration of Drugs

Drug suspensions and vehicles were administered orally in a uniform volume of 5 ml/kg BW.

Induction of liver injury

On the eighth day of the experiment, a single dose of paracetamol was administered to B, C, D, and E. After overnight fasting, the animal’s diet was restricted 12 hours before consuming paracetamol. However, free water access has been allowed [16].

Laboratory Assessments

On the tenth day, animals were injected with light ether anaesthesia and blood was collected from their hearts. Blood was kept intact for 30 minutes and clots were distributed using glass bars. The samples were centrifuged for 15–20 min at 2000 rpm to separate the serum and then sent for liver function tests (LFT), namely total serum protein, albumin globulin ratio, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase [17-19]

Histopathological examination

Subsequently, the rats were sacrificed (the tenth day) under deep sea anesthesia, and the liver samples were excreted and washed with normal sodium. A record of each liver was made, with regard to size and shape, color, and presence or absence of any nodule. The liver was then immediately fixed with a 10% formalin solution. A paraffin embedding technique was carried out and sections were taken at 5-mm thickness, stained with hematoxylin and eosin and examined microscopically for histopathological changes.[20]

Statistical analysis

The results obtained by LFT are presented as averages and standard mean errors (SEM) in each group (average SEM). All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Bonferroni’s test to determine the intergroup variability. A comparison was made with the experimental control group (paracetamol) and with the standard (silymarin). The P-value is <0.01 (high significance), and the desired significance is

RESULTS

The LFT results are summarized in table 1 and expressed as mean SEM (n = 6). Histopathological examinations of liver group a showed that the hepatocytes were normally arranged with clear nuclei, central veins, and portal triads. We observed sinusoids congestion areas, cloudy swelling, and congestion of central vein congestion, centrilobular fatty change, and necrosis of hepatocytes in all animals of group B. Groups C, D, and E significantly reduced sinus congestion, cloud swelling, and fatty changes, and also regenerated areas.

DISCUSSION

When PCM was administered to animals, the overall protein and albumin globulin ratios in the serum declined significantly and the ALP, AST and ALT. In the C, D and E groups, paracetamol’s toxic effects of paracetamol were partially reversed in
animals. Compared to the PCM (experimental control) group, the C and E groups showed a significant increase in the albumin: globulin ratio and a significant decrease in serum ALP, AST and ALT levels. However, there was no significant difference in total protein concentrations between these groups. Group E compared to silymarin (standard) showed a significant decrease in serum AST and ALT alone. Thus, group C showed greater hepatoprotection than group E, considering the results of the LFT alone. The histology of the control group showed a normal hepatic architecture. The B group of animals showed areas of liver necrosis caused by paracetamol. Animals treated with PCM and OSE (group C), PCM and silymarin (group D) and PCM, OSE and silymarin (group E) revealed appreciable protection of PCM liver tissue. PCM, used to induce liver toxicity in experimental animals and leads to a covalent bond between the toxic metabolites N-acetyl P bezoquinoneimine and proteins’ sulfide groups. This leads to reduced liver glutathione exhaustion, cell necrosis, and lipid peroxidation. [21] Increased levels of transaminase and ALP show cell leakage and the loss of function integrity of liver cell membranes.[22–24] The administration of alcoholic extracts from Ocimum sanctum has demonstrated significant liver protection activity, as has been demonstrated in previous studies. [10] Synergistic hepatoprotection was observed in the OSE + SILY groups. The OSE group showed better hepatoprotection than the OSE + SILY group. But the OSE and OSE + SILY combination showed less efficacy than SILY alone. The components Eugenol, Flavonoid and Ursolic Acid present in the leaves of Ocimum sanctum have free radical removal and antilipoperoxidative effects. Therefore, the hepatoprotective effects of the Ocimum sanctum leaves are due to the antioxidant properties of their components. [25] The membrane stability properties of Ocimum sanctum are responsible for the hepatoprotective action of ocimium sanctum. [26] Furthermore, Ocimum sanctum fixed oil contains linoleic acid which is responsible for its anti-inflammatory activity. [27] Therefore, linoleic acid can also reverse inflammatory characteristics associated with liver injury and thus enhance the liver protection effect.

CONCLUSION

Therefore, Ocimum sanctum have a very significant hepatoprotective activity (P < 0.01). When administered simultaneously, the leaves of Ocimum sanctum and Silymarin have a very important synergistic hepatoprotective activity (P < 0.01). The group Ocimum sanctum showed better liver protection than the group combination Ocimum sanctum and Silymarin. However, in given doses, only Ocimum sanctum leaf extracts combined with silymarin show fewer hepatoprotective effects than silymarin alone. Silymarin is a known standard hepatoprotectant, while the presence of impurities in Ocimum sanctum can have a lower hepatoprotective effect. Furthermore, the combination group (Ocimum sanctum extract and silymarin) received lower doses of Ocimum sanctum (100 mg/kg) and conventional hepatoprotective silymarin (50 mg/kg) than the silymarin group alone.

REFERENCES