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ORIGINAL ARTICLE

Hepatoprotective activity of *Osmium sanctum* leaf extract against paracetamol induced hepatic damage in mice

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ABSTRACT

Introduction:
Hepatotoxicity is associated with diseases that cause increased deaths worldwide. Medicines, chemicals, and dietary disturbance cause liver damage. However, for hepatoprotection medicinal plants are safer, effective, and pharmaceutical alternatives for liver damage. This study aimed to evaluate the effect of *Ocimum Sanctum* leaf extract (OSLE) on paracetamol- induced hepatic damage in mice.

Methods:
Mice were divided into four groups. Group A normal control, Group B 250mg/kg of paracetamol, Group C 250mg/kg of paracetamol and OSLE, and Group D 500mg/kg of OSLE and 250mg/kg were given respectively for 7 days. The hepatoprotective effect was measured by assaying Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and the histology of the liver. The study group was compared with the normal control group by one-way Anova, followed by Dunnet C.

Results:
Paracetamol induced elevation in AST by 155% ($p<0.05$), and ALT by 334% ($p<0.05$) compared to control mice. In addition, treatment with a high dose of OSLE (500mg/kg) demonstrated a better hepatoprotective activity compared to the low dose (250mg/kg). The liver biomarker for 250mg/kg were AST (65%) and ALT (27%) while for 500mg/kg were AST (95%) and ALT (77%). Histologically, livers from these mice revealed liver cell disarrangement, inflammation, and vacuoles hepatocytes. In comparison, livers for the treatment group (OSLE) showed inflammation and vacuole hepatocytes reduction.

Conclusion:
Taken together, OSLE provides hepatoprotective effects against paracetamol-induced liver injury in mice with no toxicity even at a higher dose.

Keywords
Hepatotoxicity, Hepatoprotective activity, Histopathology, medicinal plants, Tulsi

Introduction

The liver is a multifunctional, vital organ in the human body that regulates homeostasis. [1] The liver controls the metabolic functions, secretion, storage, and actively takes part in the process of detoxification. [2] Therefore, liver diseases are responsible for higher mortality rates worldwide.

According to the World Health Ranking (2018), Malaysia ranked 125, globally for liver disease. In the year 2010, more than 2 million deaths were associated with liver pathology namely hepatitis, cirrhosis, and liver carcinoma, contributing 4% of all deaths worldwide. [3] Liver diseases can be classified as non-inflammatory, inflammatory, and degenerative. Chronic liver disease induces cirrhosis in 633,000 patients per year with a prevalence of 4.5 to 9% worldwide. [4]

Hepatotoxins cause hepatotoxicity which is derived from the chemicals, dietary supplements, pharmaceutical drugs, and medicinal plants. Hepatotoxicity is also defined as a condition of being destructive to the liver and the associated risk factors encompass alcoholism, environmental pollution, viral induced hepatitis, hepatotoxic drugs such as high doses of acetaminophen, and more. [1] Hepatotoxicity is an injury or liver damage caused by exposure to drugs or other non-pharmacological agents. [5]

Paracetamol is also known as acetaminophen (IUPAC name). It is commonly used for antipyretic and pain relievers since the year 1955 and has been available over-the-counter as a single formulation or in combination with other substances used in almost all ages. [6] Acute liver failure, centrilobular hepatic necrosis, renal tubular necrosis, and hypoglycaemic coma are indications of paracetamol toxicity. [7] N-acetyl-p-benzoquinone imine (NAPQI) is primarily related to paracetamol hepatotoxicity. Cellular damages caused by NAPQI are directly related to the dose of paracetamol consumed. [8] If paracetamol is ingested at hepatotoxic doses, most drugs are metabolized by the CYP2E1 pathway resulting in Glutathione (GSH) depletion, by activation of GST-S-transferase, and with the build-up of NAPQI at toxic concentration. [9]

Medicinal plants are rich in biologically active chemical components known as phytochemicals which give rise to pharmacological properties such as antioxidant, hepatoprotective, anti-inflammatory, antiviral, and more. [10, 2] *O. sanctum,* is an aromatic plant in the Lamiaceae family, which is originated in north-central India and spread throughout the eastern tropical forests. It is naturally growing, and it will germinate in wet soil areas. This shrub has been utilized as traditional medicine typically to treat cough, anxiety, asthma, diarrhea, eye disease, arthritis, skin disease, back pain, indigestion, vomiting, hiccups, fever, snake bite, scorpion bite, malaria, cardiac and genitourinary disorder. [11] Tulsi protects against toxic chemical-induced injury boosting antioxidant like glutathione and increasing anti-oxidant enzyme activities such as superoxide dismutase and catalase. These enzymes protect cellular organelles and membranes by scavenging the free radicals generated by oxygen lack and other toxoids. [12]

The present study was undertaken to investigate the effect of *O. sanctum* leaf extract (OSLE) on paracetamol-induced hepatic damage in mice.

Methods

The study was conducted from Dec. 1, 2020, to March 31, 2021, at QIU, Perak, Malaysia.

Preparation of *O. sanctum* leaf extracts (OSLE)

The leaves of the plant were washed in cold water, dry under shade at room temperature for one week, and grounded to powder with the help of an electric grinder. Next, powdered leaves were soaked in 90% of ethyl alcohol, where it was firmly packed and allowed to macerate for 24 hours at room temperature. Afterward, the solution was filtered through Whatman's filter paper, and the filtrate was evaporated with the help of a rotary evaporator at 55-60˚C. Finally, the residue was kept for two days in the freeze dryer until it dried completely, and a dark green sticky-like paste was obtained and stored in the freezer at -20˚C. [13]

Experimental Animals

Male Swiss albino mice (weight 22-35g) were used for this experiment, and all were kept at the animal holding house of the Faculty of Medicine, Quest International University. We maintained standard laboratory conditions for the caged animals. The temperature was maintained at 25 ± 2˚C with an acclimatization period of ten days. We provided the animals' standardized pellet diet and water ad libitum.

Treatment of mice with OSLE

Mice were randomly assigned into four groups of four mice each and treated orally. Group I was normal control, receiving normal pellet food and water for seven days. Group II served as paracetamol control, receiving 250mg/kg of paracetamol dissolved in 10 mL of distilled water for seven days. Group III received OSLE at a dose of 250mg/kg dissolved in 10 mL of distilled water for 7 days and received paracetamol 250mg/kg 3 hours after extract treatment. Group IV received OSLE at a dose of 500mg/kg dissolved in 10mL of distilled water for seven days and received paracetamol 250mg/kg 3 hours after extract treatment dose. All the animals were fasted for 12 hours and anesthetized with carbon dioxide. Blood was collected from the mice by cardiac puncture.

Biochemical Investigation

Collected blood, after clotting was centrifuged to collect the serum at the rate of 2000 rpm for 15-20 minutes and stored at -80˚C. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using commercial kits (Elabscience, USA). [14]

Histopathological Studies

The liver from the mice was separated and placed in 10% buffered formalin for 24 hours. Then, paraffin embedding techniques were carried out, and the section was taken at 5 mm thickness, stained with haematoxylin & eosin for 5 minutes, and viewed under microscopy at 10X lens for histological changes. [15]

Data management and statistical analysis

The results of the study were expressed as the mean ± SEM. Statistical analysis of the data was performed with One-way Anova. All the calculations were performed using Statistical Package for the Social Sciences (SPSS) statistical software. Significant differences were set at p values lower than 0.05 ($p < 0.05$).

Ethical committee approval

All animals were handled as per the international animal care and welfare and the Institutional rules and regulations for the experimentation on laboratory animals. Ethical clearance was also obtained from the Joint Research Ethics Committee of Quest International University (QIU).

Results

Effects of paracetamol treatment on ALT and AST Levels

Serum biochemical parameters in the control and various experimental group are delineated in Table 1. Administration of hepatotoxic agent, paracetamol (PCM), to mice by oral route caused significant liver damage as indicated by an increase in the level of hepatocellular injury biomarkers, ALT, and AST. Paracetamol induced elevation in AST by 155% ($p<0.05$), and ALT by 334% ($p<0.05$) when compared to the control group. Elevated levels of these enzymes are indicative of cellular damage in the liver.

Table 1: Effect of OSLE on alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Group	Dose (mg/kg)	ALT Mean ± SEM	AST Mean ± SEM	P value
A	Control	7.50±1.89	65.63±4.60	
B	PCM (250)	25.08±1.27	101.88±1.38	<0.001*
C	OSLE (250) + PCM (250)	6.02±0.79	66.41±4.30	
D	OSLE (500) + PCM (250)	19.33±2.98	96.64±2.43	

*P<0.05 experimental groups compared with paracetamol group

Data were expressed with one way ANOVA using SPSS, as mean ± SEM (n= 4). A= Normal control; B= Paracetamol treated control; C= Paracetamol + 250mg/kg of OSLE treated group and D= Paracetamol + 500mg/kg of OSLE treated group.

Effects of OSLE on ALT and AST Levels

Mice were given OSLE at 250mg/kg and 500mg/kg doses significantly decrease the levels of ALT and AST ($p<0.05$) when compared to Paracetamol (PCM) administered controls. As compared to the high dose (500mg/kg), low dose (250mg/kg) demonstrated a better hepatoprotective activity. The decline in liver biomarkers in descending orders was AST (65%) >ALT (27%) for 250mg/kg. For 500mg/kg, AST decreased by (95%) whereas ALT decreased by (77%).

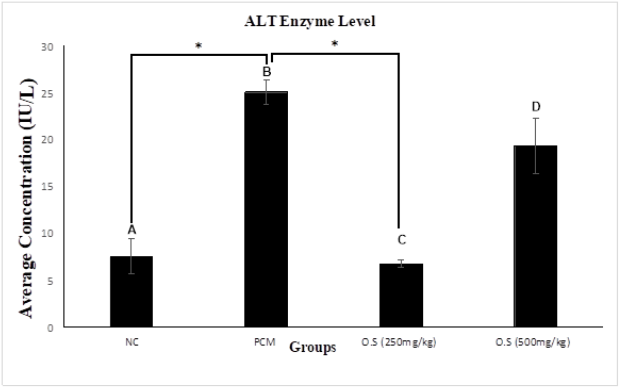

Figure 1: Effect of OSLE on liver (ALT Enzyme Level are expressed as IU/L).

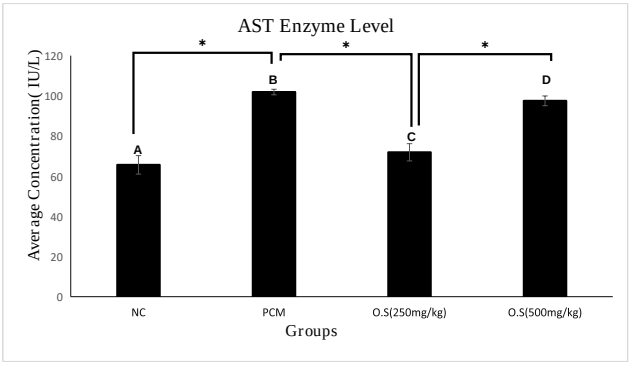

Figure 2: The effects of OSLE on liver (AST enzyme level is expressed as IU/L)

Effects of OSLE on liver histology

The hepatoprotective effect of OSLE and Paracetamol-induced liver damage was confirmed by histopathological examinations. As shown in the figure, no histological abnormality was recognized in normal control animals. Meanwhile, liver cell disarrangement, dilated hepatic sinusoids, infiltration, inflammation, and vacuoles were observed in paracetamol (PCM) administered mice. However, treatment with OSLE showed signs of protection in the doses of 250mg/kg and 500mg/kg, which showed a reduction or absence of inflammatory cells, vascular congestion, cellular degeneration, and vacuoles. As compared to the high dose (500mg/kg), the low dose (250mg/kg) demonstrated a better hepatoprotective activity.

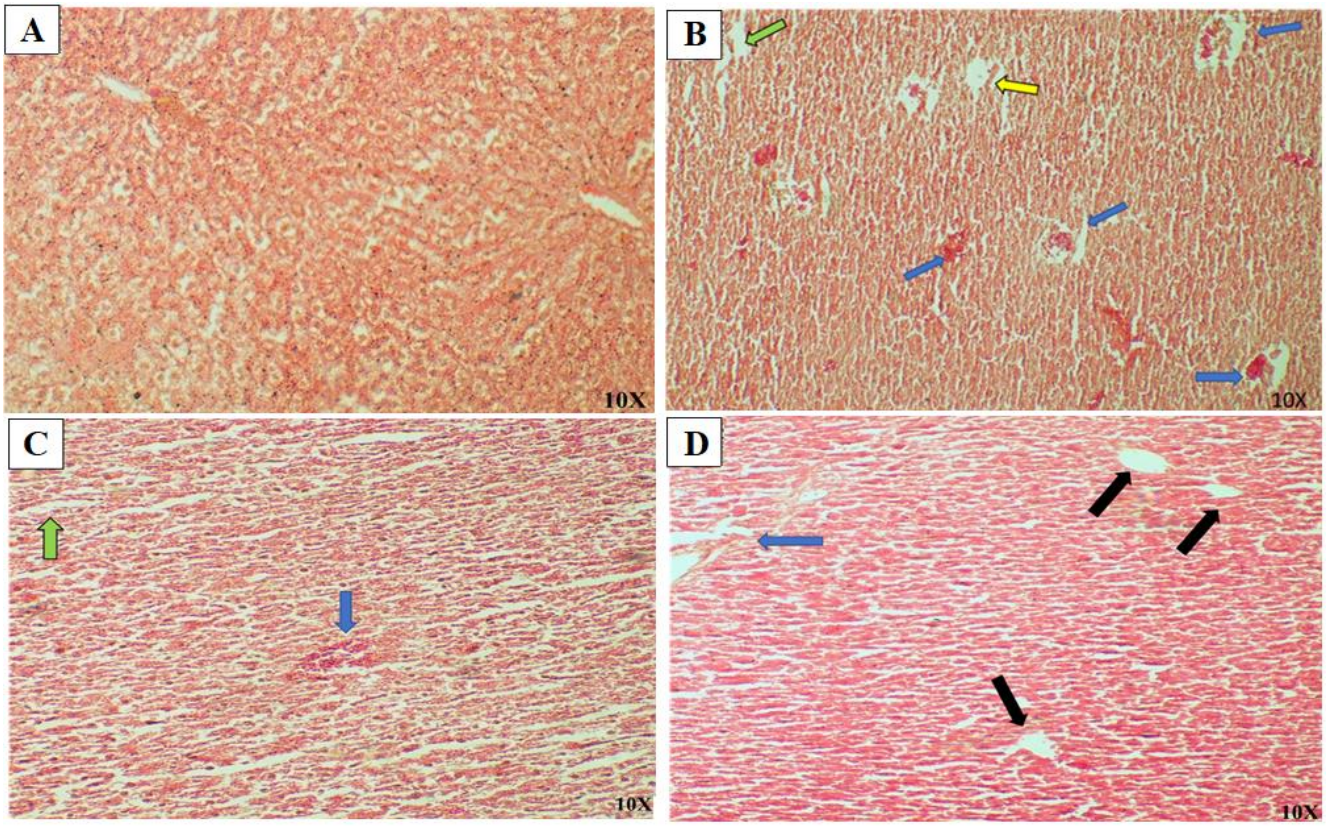

Figure 3: The histological structure of the mice liver. (A) Normal control (original magnification, 10x); (B) Paracetamol treated mice; (C) Paracetamol and 250mg/kg of O.S in the liver of treated mice; (D) Paracetamol and 500mg/kg of O.S in the liver of treated mice. The arrow displayed the status of cell in different conditions; blue arrow (Inflammatory cell infiltration), a yellow arrow (Vacuole formation), a green arrow (Dilated hepatic sinusoids), a black arrow (steatosis)

Discussion

Metabolic homeostasis is a crucial role of the liver which actively performs the biotransformation, detoxification, and excretion of harmful substances from the body. [16] Several studies demonstrated overdose of paracetamol lead to hepatocellular damage or necrosis in experimental animals and humans. Paracetamol works by covalent binding of n-acetyl-p-benzoquinone-amine to the sulfhydryl group of protein, which leads to the necrosis of the cell. [17] In the present study, the leaf extract of *O. sanctum* was evaluated for hepatoprotective activity against paracetamol-induced hepatic damage in mice. Liver function tests and histological studies were done to assess the hepatoprotective properties of this plant.

It was observed that the values of serum enzyme were significantly reduced in animals receiving OSLE of 250mg/kg and 500mg/kg. In the present study, OSLE treatment compared to PCM treatment showed a significant decrease in serum AST and ALT which may be due to the hepatoprotective effect of *O. sanctum* leaves antioxidant properties. [18] Hepatotoxicity affects the transport system leading to leakage of the AST and ALT reflected as increased titer in the serum. [19] Raised level of these enzymes in acetaminophen induced hepatic damage suggests leakage from cell and membrane damage. [20] *O. sanctum* has membrane-stabilizing properties responsible for its hepatoprotective action. [21] OSLE showed significant hepatoprotective activity, supported a previous study [22] because *O. sanctum* has eugenol and a small amount of eugenol can prevent toxin-induced damage in the liver. However, too much of eugenol can cause liver damage. Thus, 250 mg/kg of OSLE showed more significant hepatoprotective activity than 500mg/kg. Earlier research on the alcoholic leaf extract of *O. sanctum* on albino rats showed beneficial effects on the liver. The administration of alcoholic extract of *O. sanctum* leaves showed significant hepatoprotective activity. [13] *O. sanctum* increases the level of the antioxidant glutathione and increases superoxide dismutase and catalase activity. [12] Liver sections in histopathological examination in this study also support the biochemical investigation, as shown in Figure 3. OSLE reduced inflammation, vascular congestion. and degeneration of the cells, when compared with the PCM group, which may be due to the presence of linoleic acid contributing to the anti-inflammatory activity. [23] These results demonstrate that pre-treatment of OSLE

protects mice from the significant liver damage of paracetamol.

Conclusion

Taken together, the alcoholic leaf extract of *O. sanctum* demonstrated high hepatoprotective activity with no significant toxicity to the mice's liver. The recommended dose for the extract to confer the best hepatoprotection via pre-treatment and post-treatment was 250mg/kg as this dose contributed the highest reduction in ALT and AST levels when compared to Paracetamol (PCM) administered controls. The present research contributes a novel insight into the hepatoprotective activity of alcoholic leaf extract of *O. sanctum* against paracetamol-induced hepatic damage in mice.

Limitation and future scope

The bioactive components of *O. Sanctum* need to be separated in future studies and the potency to prevent the damage of other organ systems needs to be investigated. More animal trials should be conducted before human studies.

Abbreviations

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), International Unit per Litre (IU/L), International Union of Pure and Applied Chemistry (IUPAC), N-acetyl-p-benzoquinone imine (NAPQI), *Ocimum sanctum* leaf extract (OSLE), Paracetamol (PCM), Standard error of the mean (SEM), World Health Organization (WHO).

Relevance of the study

Herbal medicine is the backbone of traditional medicine in many countries and plays an important role in curing the disease. The present study provides additional pharmacological properties for *O. sanctum*. The paracetamol-induced hepatic damage causes forms toxic metabolites by the cytochrome P450. From this study, it has been proven that *O. Sanctum* has a hepatoprotective effect mediated by the increased levels of GSH.

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Authors' contribution

a. Study planning: KD

b. Data collection: KD, OK

c. Data analysis/ interpretation: KD, OK

d. Manuscript writing: KD, OK

e. Manuscript revision: KD, OK

f. Final approval: KD, OK

g. Agreement to be accountable for all aspects of the work: KD, OK

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Availability of data and materials

All data underlying the results is available as part of the article, and no additional source data is required.

Competing interests

We declare no competing interests.

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