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Original Article

Ethanolic Extract of Citrus Sinensis Peel Markedly Mitigate Paracetamol-Induced Hepatotoxicity in Rats

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ABSTRACT

Background: Herbal medicines have historically been crucial for enhancing human health. Previous studies have highlighted the antioxidant properties of various parts of Citrus sinensis. In light of widespread reliance on plant-based remedies, this study focuses on a particular herbal medicine, Citrus sinensis, known for its rich content of vitamin C, pectins, phenolic compounds, and flavonoids.

Objective: This research aims to investigate the hepatoprotective potential of the ethanolic extract of Citrus sinensis peel in vivo, particularly focusing on its antioxidant capabilities.

Methods: The study utilized biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, and total proteins to assess the hepatoprotective action of the Citrus sinensis peel extract in serum of Wistar albino rats. The rats were divided into five groups, with varying treatments including a control group, a paracetamol-induced hepatotoxicity group, and groups receiving different doses of the Citrus sinensis extract.

Results: Treatment with the ethanolic extract of Citrus sinensis peel at doses of 200 mg/kg and 400 mg/kg significantly mitigated the paracetamol-induced elevation of serum levels of AST, ALT, ALP, bilirubin, and total proteins. These improvements were comparable to those observed with the standard drug, silymarin.

Conclusion: The study confirms the hepatoprotective effects of Citrus sinensis peel extract against paracetamol-induced liver damage. This protection was evident both biochemically and histopathologically, suggesting the potential therapeutic utility of Citrus sinensis in liver disorders.

Keywords: Alanine aminotransferase, Antioxidant, Aspartate aminotransferase, Bilirubin, Citrus sinensis, Hepatoprotective, Liver markers, Paracetamol, Silymarin, Total proteins.

INTRODUCTION

The use of herbal remedies has significantly improved human health. Eighty percent of people worldwide rely on traditional therapies, primarily plant-based remedies (1). Herbal medicines have a long history of use in treating liver disorders and are the most widely used strategy in many Western polls (2). However, only 5–15% of the estimated 250,000 species of higher plants recorded have been examined for their potential therapeutic value (3). These practices, replacing enduring beliefs, have been transmitted from generation to generation through oral history and/or restricted literature. Standardization remains a challenge, and clinical trials are lacking to confirm efficacy. Some plant extracts used for digestive or biliary disorders may be harmful due to potent, liver-toxic alkaloids. Molecules like phyllanthin, glycyrrhizin, picroside, silibinin, and baicalein—derived from Phyllanthus amarus, licorice root, Picrorhiza kurroa, milk thistle, and sho-saiko-to—have proven antifibrotic, antioxidative, anticarcinogenic, and/or antiviral properties (4). They hold the potential to be foundational molecules for developing novel hepatoprotective drugs (5). Although herbal treatments are effective for many disorders, their misuse is frequent. Therefore, thorough research of these natural medicines is required in light of current knowledge (6).

Vitamin C, pectins, phenolic compounds, and flavonoids are abundant in Citrus sinensis. The main flavonoids in citrus fruits are narirutin, hesperidin, eriocitrin, and naringin (7). One orange provides 116% of the recommended daily intake of vitamin C, a major

water-soluble antioxidant. Vitamin C inhibits the production of free radicals, thereby preventing tissue damage in aqueous environments (8). Consumption of orange juice without added salt and sugar has been linked to reduced severity of inflammatory diseases such as rheumatoid arthritis, osteoarthritis, and asthma (9, 10). Beta-cryptoxanthin, an orange-red carotenoid most abundant in Citrus sinensis, is highly effective in reducing lung cancer risk (11, 12). Citrus sinensis also possesses antifungal, anti-inflammatory, and anxiolytic properties (13, 14), while its peel extracts exhibit hypolipidemic, anti-inflammatory, and antioxidant properties, attributable to bioflavonoids such as PMFs (polymethoxylated flavones) (15, 16). Researchers have demonstrated that plant flavonoids are primarily responsible for antioxidant activity (17, 18). The present study investigates the in vivo hepatoprotective effects of Citrus sinensis against paracetamol-induced liver injury.

MATERIAL AND METHODS

Preparation of Crude Ethanolic Extract

Citrus sinensis peels were dried in a shaded area for 5 to 7 days. The peels were then diced and ground into a fine powder. This powder was placed in a sealed bottle, and ethanol was added until the material was completely immersed. The mixture was shaken twice daily for 7 to 10 days. Subsequently, ethanol was evaporated using a rotary evaporator, yielding the crude extract as a semisolid solution.

Chemicals and Drugs

Paracetamol was procured from M/S English Pharmaceuticals, Lahore. Silymarin (Siliver®) was obtained from a local pharmacy. Ethanol was sourced from Labscan Asia Co., Ltd., Bangkok, Thailand. Serum diagnostic kits for biochemical assays were acquired from DiaSys Diagnostic System GmbH, Holzheim, Germany. All solvents used in this study were of analytical grade.

Experimental Animals

Wistar rats of either sex, young and healthy, were acquired from the Islamia University of Bahawalpur (IUB). The animals were housed in specially designed plastic cages with sipper bottles at the Department of Pharmacology, Faculty of Pharmacy, University of Sargodha. They received humane care and were maintained at a constant room temperature (25-10°C) as per the guidelines of the National Research Council (NRC, 1996). The rats were allowed a week to acclimate to their new environment and had unlimited access to a standard laboratory diet and water. The Animal Ethics Committee of the University of Sargodha approved the study. Additionally, the study protocol received approval from the Institutional Animal Ethics Committee of the Faculty of Pharmacy, University of Sargodha.

Acute Toxicity Studies

The acute toxicity of the ethanolic extract of Citrus sinensis was investigated in Wistar albino rats. The animals were fasted overnight prior to the test. The CPCSEA (Annexure-2d) methodology and the fixed dose OECD guideline No. 420 were employed for the study. Each test dose was administered to a group of six rats, with the screening dose determined based on the LD50 value. The animals were observed continuously for 24 hours.

Hepatoprotective Potential Evaluations

Assessment of the Hepatoprotective Effects in Paracetamol-induced Liver Damage

Procedure

Wistar albino rats were selected and divided into five groups, each consisting of six animals. Group I served as the vehicle control group and received 1 ml/kg p.o. (per os, orally) of the vehicle. Group II was administered 640 mg/kg p.o. of paracetamol in 1% methyl cellulose. Group III received a standard dose of 25 mg/kg of silymarin, while Group IV was given 200 mg/kg of the ethanolic extract of Citrus sinensis dissolved in 1% Tween 80. Group V received 400 mg/kg p.o. of the ethanolic extract of Citrus sinensis suspended in 1% Tween 80. Each group, except for Group I, received 640 mg/kg p.o. of paracetamol (in 1% methyl cellulose) at 0 hours. Silymarin at a dose of 25 mg/kg was administered orally to Group III at intervals of 6, 12, and 18 hours. The ethanolic extract of Citrus sinensis was administered to Groups IV and V at doses of 200 mg/kg and 400 mg/kg, respectively.

Twenty-four hours after the final dose of treatment, the animals were sacrificed. Blood (5 mL) was drawn from the brachial artery under light sedation with ether. The serum was then separated from the blood by centrifugation for fifteen minutes at 3000 rpm. This serum was used to determine the levels of ATP, AST, ALP, total bilirubin, and total protein using a biolyzer and diagnostic tools. The liver was then excised for histopathological examination and placed in a 10% formalin solution. The data were presented as mean ± standard deviation. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Dunnett's test for post-hoc analysis. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

Biochemical parameters:

The hepatoprotective properties of silymarin (25 mg/kg) and the ethanolic fraction of Citrus sinensis (EFCS) (at doses of 200 mg/kg and 400 mg/kg) were evident through their impact on total bilirubin, total proteins, as well as on AST, ALT, and ALP. Compared to the vehicle-treated control group, the group treated with paracetamol exhibited significantly higher blood levels of ALT, AST, ALP, and total bilirubin, along with significantly lower levels of total proteins and the albumin/globulin ratio (Table 1). Table 1 also summarizes the effects of silymarin (25 mg/kg) and EFCS at doses of 200 mg/kg and 400 mg/kg on the paracetamol-induced increase in these parameters. Notably, EFCS at doses of 200 mg/kg and 400 mg/kg significantly reduced the levels of serum enzymes and serum bilirubin while significantly increasing the levels of serum total proteins and the albumin/globulin ratio, compared to the standard medication, silymarin. These results demonstrate the hepatoprotective efficacy of the ethanolic extract of Citrus sinensis.

Table 1: Hepatoprotective activity of Citrus sinensis peel ethanol extract against paracetamol-induced hepatotoxicity: Serum Enzymes, Bilirubin Total, and Total Proteins

Group Title	ALT (U/L)	ASP (U/L)	ALP (U/L)	Bilirubin total (mg/dl)	Total Proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio (%)
Vehicle Control	38** ± 16.2	41** ± 6.18	131** ± 12.3	0.300** ± 0.010	7.300** ± 0.082	5.000** ± 0.093	2.30** ± 0.082	2.170** ± 0.082
Paracetamol Control	302 ± 26.9	440 ± 22.0	380 ± 23.1	5.000 ± 0.08	4.100 ± 0.165	1.100 ± 0.129	3.00 ± 0.051	0.360 ± 0.017
Silymarin Standard	60** ± 37.7	107** ± 9.1	139** ± 13.5	0.583** ± 0.021	7.000** ± 0.073	4.800** ± 0.052	2.60** ± 0.052	1.817** ± 0.049
EFCS 200 mg/kg	99** ± 45.5	200** ±50.3	280* ± 28.6	0.613** ± 0.019	7.300** ± 0.144	4.600** ± 0.144	2.700 ± 0.144	1.700** ± 0.144
EFCS 400 mg /kg	90** ± 15.7	139** ± 26.2	268* ± 32.2	0.500** ± 0.010	7.000** ± 0.103	4.600** ± 0.103	2.80** ± 0.030	1.640** ± 0.103

Histopathological parameters

Histopathological examinations of the negative control mice revealed normal liver cells with a distinct nucleolus and nucleus, well-conserved cytoplasm, and a healthy central vein (Fig A). Microscopic analysis of the paracetamol-treated animals revealed significant necrosis, fatty alterations, degeneration, and ballooning. (Fig B). Liver slices from silymarin-treated animals revealed liver architecture that was similar to that of untreated animals. (Fig C). According to Fig. D, animals given Citrus sinensis' ethanolic fraction demonstrated a significant amount of hepatic protection against toxic substances.

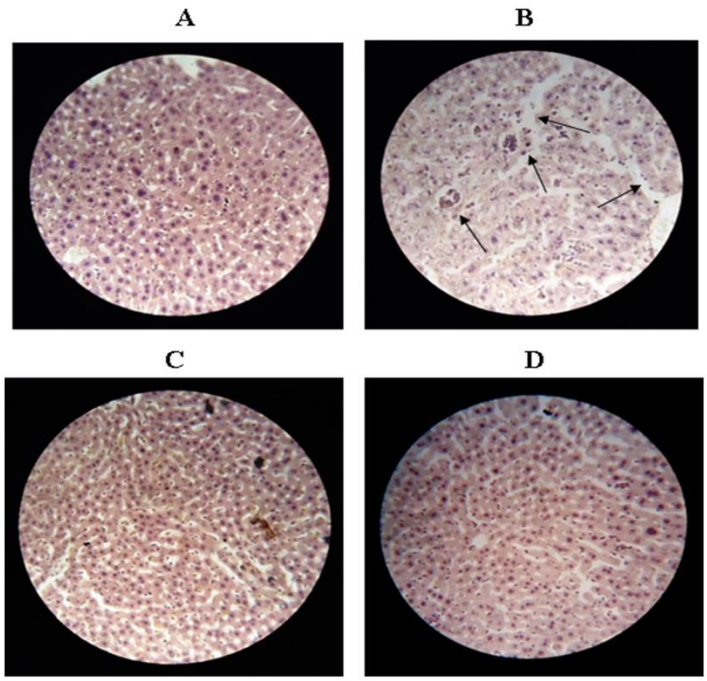

Fig. 1: A typical liver histogram showed untreated rat liver cells with prominent nuclei, well-conserved cytoplasm, and a healthy central vein. B: The liver of a paracetamol-impaired rat showed significant necrosis, fatty alterations, degeneration, and ballooning. C: Rat liver histograms treated with silymarin showed normal liver cells with distinct nuclei and nucleoli, well-conserved cytoplasm, and a healthy central vasculature. D: Rat liver histogram treated with an ethanolic extract of citrus sinensis peel that was similar to normal.

DISCUSSION

In the current research, paracetamol served as a toxic agent to evaluate the protective action of Citrus sinensis against hepatic injury. The extent of toxicity was assessed using histopathological analyses and biochemical enzyme markers, including ATP, AST, ALP, serum bilirubin, and protein levels. Adherence to OECD guidelines facilitated the determination of acute oral toxicity (19). Citrus sinensis was deemed safe at doses up to 2000 mg/kg, as determined by the LD50 method. However, a dose of 400 mg/kg was selected for its hepatoprotective action. Paracetamol at a dosage of 650 mg/kg body weight induced hepatotoxicity. The liver's metabolism of paracetamol via the cytochrome p450 enzyme system, leading to the production of the minor but potent alkylating metabolite NAPQI, was central to this process. NAPQI initiates its harmful effects by covalently bonding to cellular proteins and depleting glutathione, disrupting calcium homeostasis.

Supporting these observations, biochemical parameters in the current study showed a significant increase in the paracetamol control groups. Histopathological profiles corroborated the presence of noticeable damage in the same group. This indicates that injury is caused by mechanisms such as lipid peroxidation, depletion of glutathione reserves, or generation of free radicals. Notably, the administration of 400 mg/kg of Citrus sinensis peel ethanolic extract significantly reduced serum enzyme and bilirubin levels. Furthermore, histopathological studies confirmed the restoration of normal liver histology, thus supporting the organ's protection.

This study's findings align with those of Lagha-Benamrouche & Madani (2013) (20), reinforcing the hepatoprotective potential of Citrus sinensis. The strengths of this research include its comprehensive approach in assessing both biochemical and histopathological changes. However, limitations exist in the scope of the study, such as the need for further research to isolate and identify the specific active constituents responsible for the hepatoprotective effect. While this study provides valuable insights, it also highlights the necessity for continued research to fully understand the mechanisms underlying the hepatoprotective properties of Citrus sinensis.

CONCLUSION

The findings of this study demonstrate that an ethanolic extract of Citrus sinensis peel significantly reduced serum levels of ALT, AST, ALP, and bilirubin. Furthermore, histopathological examination indicated that Citrus sinensis peel might possess the capacity to mitigate liver damage, restoring liver function towards a near-normal state. These hepatoprotective effects are likely attributed to the plant's reputed antioxidant properties. Future research should focus on isolating, characterizing, and purifying more active ingredients from Citrus sinensis. Additionally, further experimental studies are warranted to elucidate the precise mechanisms through which this plant exerts its hepatoprotective action.

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