The Hepatoprotective Effects of Jatropha integerrima Leaves Extracts on Carbon Tetrachloride (CCl4) Induced Liver Damage in Rats

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Research Article

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Abstract

Liver illnesses represent a pressing worldwide health challenge, characterized by notable rates of both death and morbidity. The gravity of the problem is exacerbated by the restricted range of treatment alternatives that are frequently accompanied by unfavorable side effects. There is a growing interest among researchers in the exploration of plant-based hepatotherapeutic medicines, mostly driven by their abundant accessibility and comparatively reduced incidence of adverse effects. In the present context, Jatropha integerrima, a plant indigenous to the region, has attracted considerable attention due to its wide range of bioactive chemicals that are recognized for their antioxidative and anti-inflammatory characteristics. The present work aims to systematically investigate the Hepatoprotective properties of an ethanolic extract obtained from the leaves of Jatropha integerrima in a rat model of liver injury produced by carbon tetrachloride (CCl4).A cohort of 40 rats was carefully partitioned into eight groups, with each group consisting of eight rats. The study design incorporated several treatments and control groups in order to thoroughly evaluate the protective properties of Jatropha integerrima leaf extract. In this study, Group I was designated as the control group, serving as the standard reference without any intervention. Intraperitoneal injections of carbon tetrachloride (CCl4) were delivered to experimental groups consisting of elements from Groups II, III, IV, and V, resulting in the successful induction of liver injury. In the context of Group III, a commonly used hepatoprotective medication known as silymarin was provided as a reference substance. Different dosages of the Jatropha integerrima leaves extract were administered to Groups IV and V, with doses of 200 and 400 mg/kg, respectively. The results of the study demonstrated a significant decrease in the blood levels of liver enzymes and bilirubin in the groups administered with the plant extract, compared to the positive control. This reduction was shown to be dependent on the dosage, as indicated by the statistical significance of the findings ($p<0.05$). Furthermore, it was observed that both groups treated with plant extracts showed a statistically significant reduction ($p<0.05$) in overall oxidative stress levels. This reduction was followed by a notable rise ($p<0.05$) in total antioxidant levels when compared to the positive control group. The hepatoprotective activity of the plant extract was further supported by histopathological investigation of the liver tissue, which demonstrated the protection of hepatocytes against cellular damage caused by CCl4.In summary; the results of this investigation emphasize the hepatoprotective properties exhibited by extracts derived from Jatropha integerrima leaves in mitigating liver damage generated by CCl4 in rats. The findings presented in this study provide significant insights into the therapeutic capabilities of this natural treatment and emphasize its potential as a promising Hepatoprotective agent. These research pursuits have the potential to make important contributions to the advancement of medicines for liver illnesses, therefore addressing a major worldwide health challenge through the utilization of plant-based remedies.

Introduction

Liver illnesses pose a significant global health burden, resulting in substantial morbidity and mortality [1]. The present concern arises from a variety of underlying reasons, with specific attention given to the danger presented by Hepatotoxic agents, particularly carbon tetrachloride (CCl4) [2]. CCl4 is widely

recognized as a prominent inducer of hepatic injury owing to its ability to stimulate the generation of reactive oxygen species and elicit oxidative stress inside the liver. The subsequent oxidative attack leads to damage to hepatocytes and the creation of a pro-inflammatory environment, hence creating favorable conditions for the emergence and advancement of liver disorders [3]. Given this context, scholars have increased their investigation into natural substances with hepatoprotective properties. The growing interest in this field has led to an increase in research on the medicinal properties of plant extracts, focusing specifically on Jatropha integerrima [4]. Through a comprehensive examination of the protective attributes exhibited by these extracts in relation to liver damage generated by CCl4, researchers want to identify new strategies for alleviating the severe consequences of liver illnesses. This endeavor holds the potential to provide a glimmer of hope for those affected by this pervasive global health threat [5]. Jatropha integerrima, commonly referred to as "spicy Jatropha" or "physic nut," is a botanical species that is native to tropical and subtropical areas. Throughout the annals of history, traditional folk medicine has accorded it a significant position, utilizing its therapeutic properties to address a diverse range of health ailments [6]. Prior scientific research on this particular plant has revealed a plethora of pharmacological possibilities, such as significant antioxidant activities, anti-inflammatory benefits, and analgesic qualities [7]. The aforementioned discoveries have generated a keen interest in investigating the potential therapeutic uses of Jatropha integerrima across different settings. Nevertheless, despite the increasing corpus of scholarly investigations pertaining to this botanical specimen, there exists a notable dearth of knowledge concerning its capacity to safeguard the hepatic organ against deleterious effects induced by hazardous agents such as carbon tetrachloride (CCl4) [8]. The liver, being an essential organ, is very vulnerable to damage caused by toxins. Therefore, it is of utmost importance to investigate if Jatropha integerrima has the ability to protect the liver from damage created by CCl4. This knowledge is critical in fully comprehending the therapeutic capabilities of Jatropha integerrima [9]. The primary objective of this study is to address the existing gap in knowledge by conducting a thorough investigation of the hepatoprotective properties of Jatropha integerrima extracts. This research intends to provide insights into the possible therapeutic function of these extracts in mitigating liver disorders resulting from toxic assaults.

The main objective of this study is to investigate the possible hepatoprotective effects of Jatropha integerrima leaf extracts against carbon tetrachloride (CCl4)-induced liver damage in a rat model. In order to accomplish this goal, the research utilizes a diverse methodology to thoroughly evaluate the effects of various substances on hepatic well-being. The primary objective of this study is to assess the biochemical parameters. This involves the analysis of particular biomarkers present in the bloodstream, which offer valuable information on the functioning of the liver. The indicators included alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin concentrations [10]. Deviant amounts of these markers may suggest impairment or malfunctioning of the liver. The objective of this study is to assess the potential of Jatropha integerrima leaf extracts in ameliorating the adverse impacts of CCl4 on hepatic function through the measurement of relevant parameters. Additionally, the research included a histological evaluation. This involves the examination of liver tissue thin slices under a microscope following the application of hematoxylin and eosin (H&E)

staining [11]. Histopathology enables researchers to observe and analyze morphological modifications in the liver, including necrosis, inflammation, and other CCl4-induced abnormalities. The objective of this investigation is to determine if the extracts have the ability to mitigate the structural damage induced by the toxic chemical, CCl4, by conducting a comparative analysis of the liver tissue of rats treated with the extracts and those exposed just to CCl4.

Finally, the study examines the antioxidant properties. Liver damage is a significant consequence of oxidative stress, which is typified by an inequilibrium between free radicals and antioxidants within the physiological system [12]. Antioxidant enzymes, including as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), play a crucial role in mitigating oxidative stress by effectively neutralizing free radicals [13]. In contrast, the measurement of Malondialdehyde (MDA) levels serves as an indicator of the magnitude of lipid peroxidation, a direct outcome of oxidative injury [14]. The present study evaluates several antioxidant parameters in order to investigate the potential of Jatropha integerrima leaf extracts in mitigating oxidative stress and augmenting the liver's protective systems against damage generated by CCl4.

The primary objective of this study is to conduct a complete assessment of the hepato protective properties of leaf extracts from Jatropha integerrima. This will be achieved by the examination of several biochemical, histological, and antioxidant parameters. Through this approach, the aim is to offer a comprehensive comprehension of how these extracts might potentially protect the liver against the detrimental impacts of CCl4, hence illuminating their therapeutic capabilities within the realm of liver disorders.

Methodology

Plant Material Collection and Identification

Jatropha integerrima plants were systematically collected during their seasonal availability (June to September) from the University of Punjab, Lahore. Subsequently, the plants were processed into herbarium sheets by undergoing a meticulous procedure involving pressing, drying, and mounting. To ensure accurate identification, the renowned botanist Dr. Iram Mujahid Iqbal from the Scientific Institute of Punjab University validated the specimen. The authenticated specimen was cataloged and deposited at the Punjab. Herbarium under the reference number Lah# 2011.

Preparation of Plant Material

The harvested plant material, specifically the study portions, underwent a stringent cleansing process using distilled water and was subsequently air-dried under shaded room temperature conditions. The desiccated leaves were then meticulously ground into a fine powder, and the resulting powdered plant material was weighed precisely. To preserve its integrity and prevent moisture or contaminants from compromising its quality, the powdered plant material was securely sealed in polythene bags equipped with zippers.

Extraction Procedure

The extraction process adhered to a well-established protocol in accordance with Franchina method for preparing metabolic extracts from medicinal plant samples. To commence, 100 grams of the dried powdered plant material were placed in a 1-liter (1000 mL) round bottom flask, where analytical-grade ethanol was added in a 1:3 ratio. This mixture was left to soak for duration of 72 hours at room temperature. Vigilant stirring of the plant suspension was carried out regularly to ensure consistent homogenization and complete dissolution. Subsequently, the suspension was filtered using filter papers. The extract was then concentrated using a rotary evaporator under reduced pressure, maintaining a temperature of 36 °C. The resultant plant extract was accurately weighed and stored in an airtight glass jar, preserving its integrity for subsequent in vivo trials [15].

Experimental Animals

Forty Sprague Dawley (SD) rats, characterized by uniform weights ranging from 150 grams to 200 grams, were procured from the animal house. These rats were housed in stainless steel cages within the experimental room, maintaining a constant temperature of 25 °C and adhering to a 12-hour light/dark cycle. To facilitate acclimatization, the rats received standard feed and access to water during this period.

Chemicals

The chemicals utilized in this study included analytical-grade ethanolic, distilled water, silymarin, and carbon tetrachloride (CCl4).

Treatment Procedure

1. Group I: This control group received only water and standard food for a period of 21 days.
2. Group II: The positive control group was administered intraperitoneal injections of carbon tetrachloride (0.5 mL/kg) dissolved in olive leaf extract for 21 days.
3. Group III (SC group): Rats in this group were given silymarin at a dosage of 100 mg/kg/day and 0.5 mg/kg/day of CCl4 via oral gavage for 21 days.
4. Group IV: This group received Jatropha integerrima ethanolic extract at a dosage of 200 mg/kg/day for 24 hours, followed by a dose of 0.5 mg/kg/day of CCl_4 via oral gavage for 21 days.
5. Group V: Rats in this group were administered a ethanolic extract of Jatropha integerrima at a dosage of 400 mg/kg/day, followed by a dose of 0.5 mg/kg/day of CCl4 via oral gavage for 21 days.

Rat Biochemical and Histopathological Studies

On the 22nd day, after a 12-hour fasting period, the rats were euthanized, and various analyses were conducted:

Blood Collection: Blood was collected via cardiac puncture, allowed to clot in plain glass tubes for approximately 40-45 minutes, and then centrifuged at 3000 rpm for 15 minutes. The resulting serum was stored at 4°C for subsequent biochemical analyses.

Tissue Sampling: A fresh liver slice was excised, rinsed thoroughly with distilled water, and immersed in 10% buffered formalin. The liver slice was fixed in formalin for 24 hours, enabling its subsequent histopathological assessment.

Result

The value of Bilirubin, Alanine transaminase, Aspartate transaminase and alkaline phosphatase in the positive control group 1.8±0.05 mg/dl,213.6667±3.28^{b},255.67±7.75^{c} and 417±7.76^{b} respectively is significantly increased as compared with the negative control group 0.77±0.033 mg/dl, 119.67±21.06^{a}, 122±8.71^{b} and 215.33±31.95^{a} that show liver toxicity in the positive control group rats. A significant decrease in AST levels was observed in plant treated groups LI-1 and LI-2 (84±11.54^{ab} and 68.3333±2.33^{a} respectively) as compare with positive control group (275.6667±7.75^{c}). Similarly, the serum levels of bilirubin, ALT, and ALKP were also decreased in the plant extract and *silymarin* treated groups (LI_1, LI_ 2 and SC), as compared to the positive control group (Figure 1.1).

Figure 1.2 shows the histopathology of a normal rat, a rat treated with CCL4, and a rat treated with extracts, all of which showed healthy cells with normal morphology, including round nuclei with centrally located plus homogenous cytoplasm and flat endothelial cells surrounding the central vein and sinusoid (NC). CCL4 rat liver, on the other hand, displayed portal inflammation with no ballooning and necrosis, less injury of endothelial cells around the central vein, and fewer fat vacuoles in hepatocytes; this was in contrast to the mild portal inflammation observed in the reference silymarin treated group (Figure 1.2 (PC)) (SC). Slight hepatitis, modest ballooning, and mild dilatation of the sinusoids were seen in the 200 mg/kg extract-treated group (Figure 1(CCl4+ LI-1)), but these changes were less pronounced in the 400 mg/kg extract-treated group (Figure 1(CCl4+ LI-2)).

Discussion

Toxic metabolites formed by CYP-450 metabolism are responsible for the toxicity and cell death caused by carbon tetrachloride in the liver. Through irreversible conjugation with the sulfhydral groups of glutathione, it is transformed into N-acetyl Pbenzoquinineimine, an oxidant that in turn depletes glycogen and glutathione. This research looked at how well Jatropha integerrima ethanol extract protected against CCl4-induced liver damage in rats.

Liver tissue necrosis and increased levels of liver marker enzymes followed the injection of a lethal dosage of CCL4. Enzyme (ALT, AST, and ALP) and bilirubin levels were reduced (p0.05) in the treatment group after rats were given plant extract at 200 and 400 mg/kg/day for 21 days. When compared to the standard (silymarin), these biochemical parameter shifts were statistically significant. Histopathology of

the liver tissue confirmed the remarkable recovery seen in the extract-treated animals (p0.05). Both the hepatotoxin-treated and extract-treated groups had morphological alterations and tissue necrosis. These crucial flavonoids may account for the hepatoprotective effects of Jatropha integerrima.

Conclusion

CCl4 causes hepatotoxicity in rats, as shown in the present research. Hepatotoxic rats had their liver structure buffered from CCl4 toxicity thanks to a Jatropha integerrima ethanolic extract. The results indicate that the elevated CCl4-induced biochemical parameters may be mitigated by treatment with ethanolic extract. This data demonstrates that the ethanolic extract effectively enhanced hepatocyte activity. This research suggests that the hepatoprotective properties of the flavonoids and phenolic compounds in Jatropha integerrima leaves might be used therapeutically. The outcomes of using jatropha integerrima extract to treat liver damage were superior to those of using the gold standard medication silymarin.

Declarations

I confirm that the animal care committee approved and oversaw the study I confirm that the authors declare no competing interests

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Figures

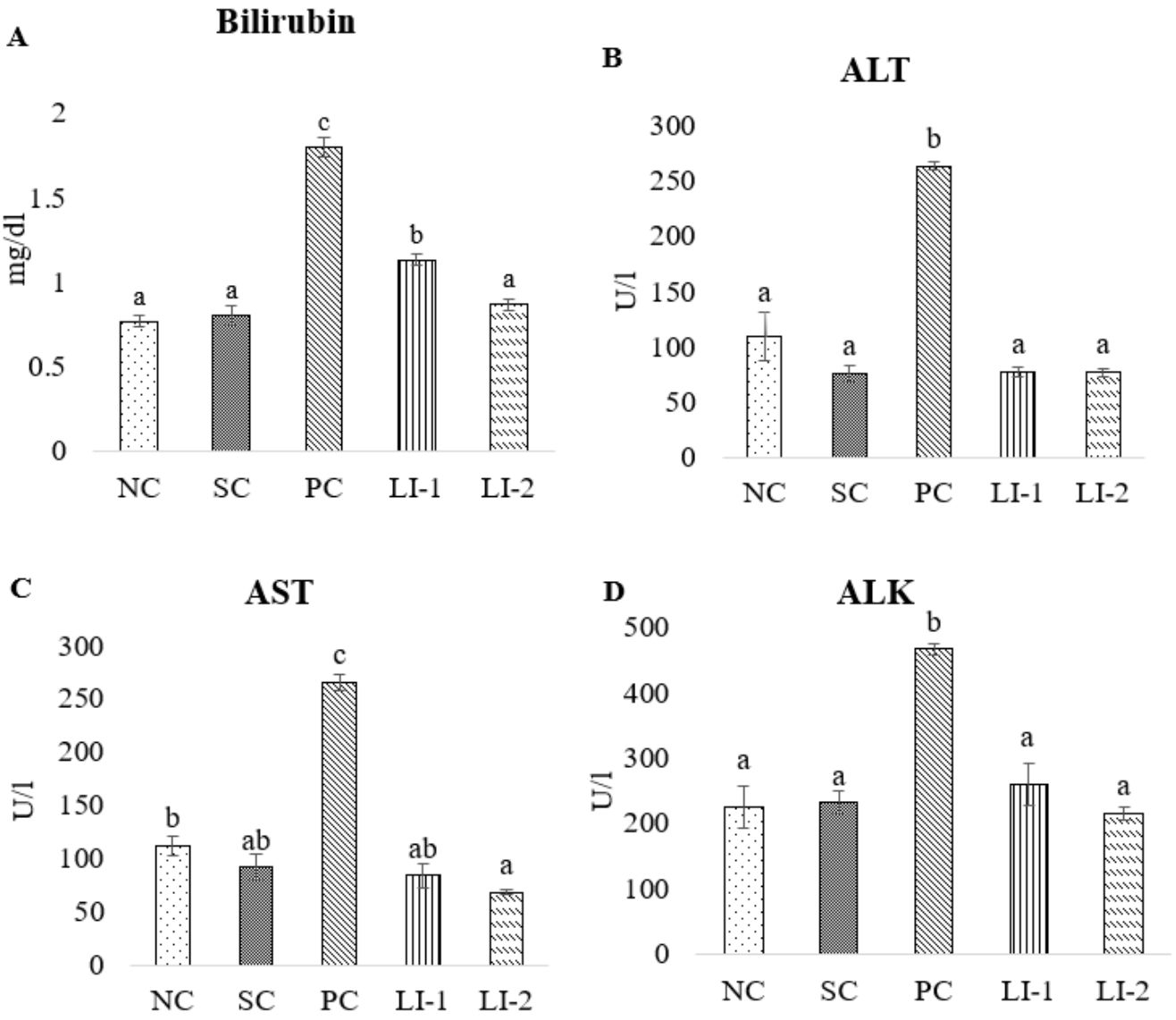

Figure 1

Figure 1.1 Liver enzyme and bilirubin levels in rats with CCl4-induced liver damage and the action of the LI plant. NC Positive Control (LI-1); Jatropha integerrima (200mg dosage); Standard Control (PC); Negative Control (SC); and Negative Control (LI-2) (400mg dose).

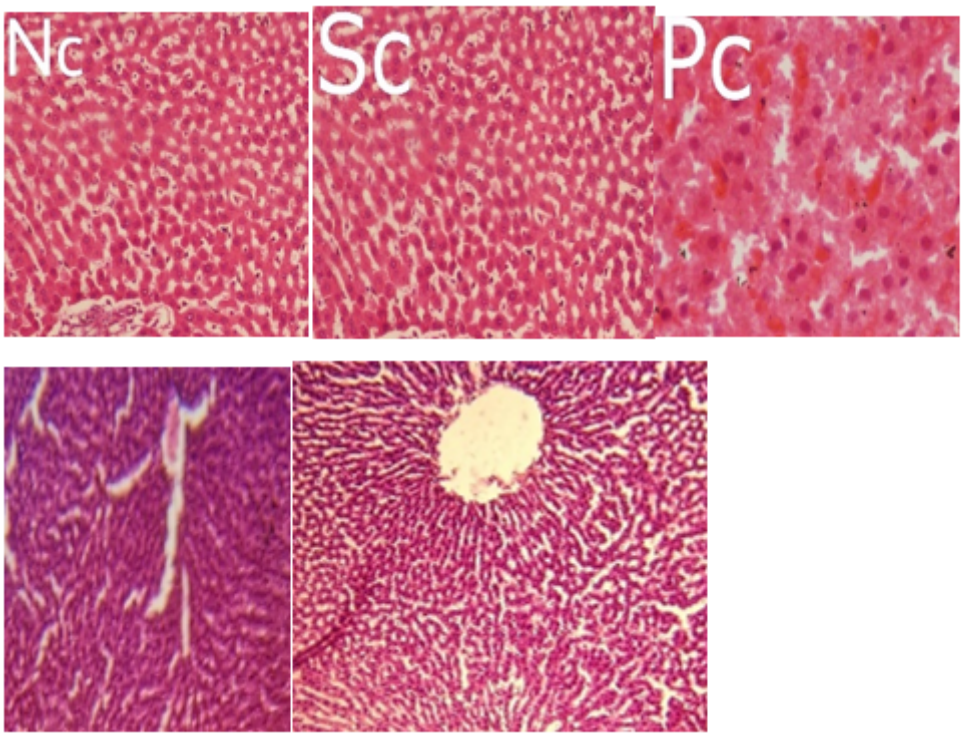

Figure 2

Figure 1.2 shows the histopathology of a normal rat, a rat treated with CCL4, and a rat treated with extracts.