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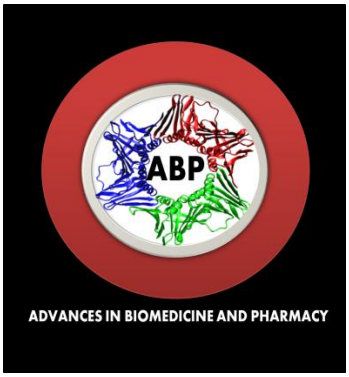

Original Article

Hepatoprotective and antioxidant properties of *Justicia gendarusa* against CCl_4 induced acute hepatic damage in rats.

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Abstract

To evaluate the effect of hepatoprotective activity of *Justicia gendarusa* on carbon tetrachloride (CCl_4) induced acute liver toxicity in rats, wistar albino rats were divided into 6 group each consisting of 6 animals: Group I served as control rats; Group II included CCl_4 treated rats; Group III and IV included rats treated with ethanolic extract (200 and 400 mg/kg respectively) of *Justicia gendarusa*` (EEJG); Group V included silymarin treated rats. Liver marker enzymes including serum glutamate oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, total protein, bilirubin were evaluated. Antioxidants parameters such as super oxide dismutase (SOD), lipid peroxidation (LPO), catalase (CAT) and reduced glutathione (GSH) were measured and histopathological evaluation of liver tissue was carried out. Our results indicated that treatment with EEJG significantly ($P<0.05$-$P<0.001$) reduced CCl_4 induced serum hepatic enzymes levels. Furthermore, EEJG significantly ($P<0.001$) reduced lipid peroxidation in the liver tissues and restored activities of normal levels antioxidant enzymes (GSH, SOD and CAT), which was further confirmed by the histopathological studies. The results of this study strongly indicates that hepatoprotective effect of EEJG is due to antioxidant properties.

Keywords: Hepatoprotective, *Justicia gendarusa*, Silymarin, Liver, Carbon tetracholride

Introduction

Liver is an important organ which plays a major role in metabolism and excretion of xenobiotics from the body. The activity of the liver in the metabolism of xenobiotics is mediated primarily by cytochrome P450 (CYP 450). Liver dysfunction is a major health problem which is a challenge not only for health care professionals but also for the pharmaceutical industry and drug regulatory agencies. The etiology of liver injuries and the damaging effects on the subject have been well discussed. These include viral infections, autoimmune disorders, ischemia, and several xenobiotics, such as drugs, alcohol, or toxins [1]. Liver cells are also damaged due to excessive alcohol consumption and microbial action [2].

CCl_4 is a well-known hepatotoxic industrial solvent and commonly used for free radical induced liver injury [3, 4]. Oxidative stress plays a crucial role in the development of carbon tetrachloride (CCl_4) - induced hepatotoxicity [5], and a connection between oxidative stress and lipid peroxidation has been reported. The available synthetic drugs to treat liver disorders are also causing further damage to the liver. On the other hand, Ayurveda, an indigenous system of medicine in India, has a long tradition of treating liver disorders with plant drugs [6].

Justicia gendarussa (Family-Acanthaceae) is a shade loving, quick growing; ever green pant which is mostly found in moist areas. It is considered to be a native of China and is distributed widely in India, Sri Lanka and Malaya. In traditional medicinal system, different parts of *Justicia gendarussa* have been mentioned to be useful in variety of diseases such as arthritis, jaundice, cephalgia, hemiplegia, eczema, etc [7, 8]. However, no studies have addressed the hepatoprotective effect of *Justicia gendarussa* on CCl_4 induced hepatotoxicity in rats.

In the present study, we aimed to investigate the potential effects of *Justicia gendarussa* in reducing damage and oxidative stress and in improving histopathological abnormalities in the liver of the rats treated with CCl_4 induced hepatotoxicity.

Materials and Methods

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu, India, and Carbon tetrachloride (SISCO Research Laboratory, Mumbai). Estimation of liver marker enzymes such as SGOT, SGPT, ALP, total bilirubin, total protein were performed by using kits provided by Agappe Diagnostics Pvt Ltd. All other chemicals were purchased from SD fine chemicals, Mumbai and were of analytical grade.

Plant materials

The fresh aerial parts of the *Justicia gendarussa* were collected from Kolappakam, Chennai, Tamilnadu were authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai and the voucher specimen was deposited in PARC (PARC/2016/3252). The aerial parts of the plants were allowed to dry in shade for three weeks. Dried leaves and stem were powdered separately.

Preparation of extracts

The shaded dried plant material of *Justicia gendarussa* was powdered separately in an electrical blender and stored at 5°C until further use. Coarsely powdered sample (500g) was defatted with petroleum ether (60-80º c) and then extracted with 1l of 95% v/v ethanol and water mixture by hot percolation method. The extract was concentrated under vacuum to solvent free residues (yield 17.06% w/w).

Phytochemical screening

The extract was screened for the presence of various phytochemical constituents employing standard screening test [9]. Conventional protocol for detecting the presence of starch, glycosides, saponins, tannins, phenolic compounds, terpenoids, steroids and flavonoids etc., was used.

Animals

Adult wistar rats (180- 220gm; 30 rats) were procured from KK College of Pharmacy, Chennai and divided into five groups of six animals each. The rats were housed in colony cages at an aberrant temperature of 25°C ± 2°C with a 12 h light/dark cycle. The animals had free access to standard pellet diet and drinking water. Behavioral studies were carried out in a quiet room between 9.00 am and 11.00 am to avoid circadian variation. The study was approved by Institutional Animal Ethical Committee, and work was carried out as per CPCSEA Guidelines, New Delhi.

Acute oral toxicity studies

Acute toxicity study was performed according to Organisation for Economic Co-operation and Development guideline No. 423 [10]. Adult wistar rats (180- 220gm) of either sex were divided into six groups and six in each. EEJG was administered orally as a single dose at different dose levels of 250, 500, 1000, 1500 and 2000 mg/kg. Animals were observed periodically for the symptoms of toxicity and death within 24 hr and then daily for 14 days.

Experimental Design

Animals were divided into five groups of six rats in each group. Normal group I: served as normal control, 4% w/v gum acacia 1.0 mL/kg/b.w. orally for 5 days with 1.0 mL liquid paraffin given subcutaneously on 2nd and 3rd day. Positive control group II: served as toxicant receiving 4% w/v gum acacia 1.0 mL/kg/b.w. orally for 5 days with 1:1 v/v, CCl4 in liquid paraffin,1.0 mL/kg/b.w. subcutaneously on 2nd and 3rd day. Test group III: received EEJG in (1 %, w/v, CMC) at 200 mg/kg/b.w. orally for 5 days with 1:1 v/v, CCl4 in liquid paraffin, 1.0 mL/kg/b.w. subcutaneously on 2nd and 3rd day. Test group IV: received EEJG in (1 %, w/v, CMC) at 400 mg/kg/b.w.orally for 5 days with 1:1 v/v, CCl4 in liquid paraffin,1.0 mL/kg/b.w. subcutaneously on 2nd and 3rd day. Test standard group V: served as standard receiving silymarin 100 mg/kg/b.w. orally for 5 days with 1:1v/v, CCl4 in liquid paraffin, 1.0 mL/kg/b.w. subcutaneously on 2nd and 3rd day.

Rats were anesthetized with ether on 6th day and blood was collected from retro orbital plexus and then sacrificed by cervical dislocation. The liver was isolated and preserved in 10% formalin solution and then subjected to hispathological studies [11].

Biochemical analysis

Serum alanine and aspartate aminotransferases (ALT & AST), alkaline phosphates (ALP) enzymes activity, total protein were estimated [12-15]. Serum total bilirubin was determined colorimetrically [16]. The liver homogenate was prepared and the clear supernatant was used for the estimation of the lipid peroxidation (LPO) [17], reduced glutathione (GSH) and antioxidant enzymes such as catalase (CAT) and super oxide dismutase (SOD) levels [18, 19].

Histopathological studies

The liver tissues were subjected to normal routine histological procedures, stained with hematoxylin-eosin and examined using the light microscope for any morphological changes [20].

Statistical analysis

The values were expressed as mean ± SEM. Analysis of variance (ANOVA) test followed by Tukey multiple comparison tests using Graph pad prism (Ver. 5.0) software for the determination of level of statistically significant.

Results

Phytochemical analysis

Preliminary phytochemical screening of the EEJG revealed the presence of starch, glycosides, saponins, tannins, phenolic compounds, terpenoids, steroids and flavonoids. Further separation of specific phytochemical is in progress.

Acute toxicity studies

EEJG produces no mortality at the doses upto 2000 mg/kg. Therefore, one-tenth of the maximum no mortality doses of extract were selected as therapeutic low dose (200 mg/kg) and just double as well as half dose of it as highest (400 mg/kg) respectively, in this study.

Effect of EEJG on SGOT, SGPT, ALP, Total bilirubin and Total protein

The effect of EEJG (200 and 400mg/kg) doses were studied on serum marker enzymes such as SGOT, SGPT, ALP, total bilirubin and total protein in CCl_4 induced hepatotoxicity in rats. CCl4 treated rats exhibited significant changes in marker enzyme such as SGOT by 170 %, SGPT by 250%, ALP by 86%, total bilirubin by 51% and total protein by 76% when compared to control rats. The percentage protected liver marker enzyme of EEJG (200 and 400 mg/kg) and silymarin (100 mg/kg) treated rats were; SGOT 50.16 ($P<0.05$), 62.38 ($P<0.01$) and 67.98 ($P<0.01$), SGPT 34.57 ($P<0.05$), 49.86 ($P<0.01$) and 52.54 ($P<0.01$), ALP 31.10 ($P<0.01$), 46.78 ($P<0.001$) and 48.98 ($P<0.001$), total bilirubin 46.67 ($P<0.05$), 59.09 ($P<0.01$), 65.67 ($P<0.001$), total protein 136.16 ($P<0.001$), 182.34 ($P<0.001$), 200.16 ($P<0.001$) as compared to CCl_4 treated rats (Table 1)

Effect of EEJG on LPO, GSH, SOD and CAT

The CCl_4 treated rats showed significant increase 189.13 ($P<0.001$) in the levels of LPO as compared to control rats. Treatment with EEJG (200 and 400 mg/kg) and silymarin (100 mg/kg) significantly prevented this heave in levels and the percentage protection in LPO were 38.56 ($P<0.01$), 51.13 ($P<0.001$) and 84.72 ($P<0.001$) respectively. The GSH, SOD and CAT levels had significantly increased in EEJG treated rats in a dose dependent manner as compared to CCl_4 rats. Moreover, CCl_4 intoxicated rats had shown significantly decrease in these parameters compared to control rats. The percent changes of GSH, SOD and CAT in CCl_4 intoxicated rats were as 25.64 ($P<0.001$), 32.67 ($P<0.001$) and 56.23 ($P<0.001$) respectively. The percent protection in GSH 19.02 ($P<0.001$), 34.89 ($P<0.001$) and 61.46 ($P<0.001$) and SOD 43.98 ($P<0.001$), 49.02 ($P<0.001$), 61.16 ($P<0.001$) while in CAT 53.68 ($P<0.01$), 80.15 ($P<0.01$), 107.16 ($P<0.001$) at the dose levels of EEJG 200 and 400 mg/kg and silymarin (100mg/kg) respectively. In higher dose levels of EEJG exhibited higher protection which were almost comparable to those of normal as well as silymarin treated rats (Table 2)

Histopathological observation

The Histopathological observation of the liver control group showed normal hepatocytes with portal traid. The liver section of CCl_4 treated rats showed severe centrilobular necrosis (N) with disappearance of nuclei and necrosis. This could be due to the formation of highly reactive free radicles as a result of oxidative stress induced by CCl_4. Treatment with EEJG (200 and 400 mg/kg) along with CCl_4 prevented the effects produced by CCl_4. In higher dose levels of EEJG showed a significant protection almost comparable to control and silymarin treated rats (Figure 1)

Table 1: Effect of EEJG and Silymarin on serum enzymes SGPT (U/L), SGOT (U/L) and SALP (U/L), Total bilirubin (mg/dL) and total protein on CCl_4 induced liver damage in rats

Groups	SGOT	SGPT	ALP	Total Bilirubin	Total Protein
Control	62.00±8.08	34.67±4.66	22.67±2.40	1.63±0.58	15.33±1.76
CCl4 rats	167.3±34.65##	119.3±14.89 ###	41.33±1.76 ###	10.03±1.20 ###	3.66±0.88##
EEJG 200mg/kg	83.33±10.73 *	78.00±8.71*	28.67±1.76 **	5.33±0.35*	8.66±1.20***
EEJG 400 mg/kg	62.67±6.36**	60.00±3.46 **	22.33±2.40 ***	4.06±0.75**	10.33±0.88***
Silymarin 100 mg/kg	54.67±4.05 **	56.67±4.66**	21.33±1.76 ***	3.46±0.40 ***	11.00±2.08 ***

Values are expressed as mean ± SEM of 6 animals in each group: $^{\#}$P<0.05, $^{\#\#}$ P< 0.01, $^{\#\#\#}$ P<0.001 is compared with respective control group; *P<0.05, **P< 0.01, ***P<0.001 is compared with CCl_4 treated groups

Table 2: Effect of EEJG and Silymarin on Liver (MDA nmole/min/mg of protein), GSH (nmole/ mg of protein), SOD (unit/mg of protein) and CAT (unit/mg of protein) on CCl_4 induced liver damage in rats.

Groups	LPO	GSH	SOD	CAT
Control	0.46± 2.08	34.24 ± 2.56	8.40± 2.16	60.14 ± 3.81
CCl4 rats	1.33± 3.92 ###	26.67± 3.58 $^{\#\#\#}$	5.77±0.04 ###	26. 39 ± 2.96 ###
EEJG 200mg/kg	0.96± 6.04 **	31.07± 2.30 ***	8.25±0.13 ***	40. 56 ± 3.48 **
EEJG 400 mg/kg	0.88± 1.04 ***	35.98 ± 9.16 ***	8.53±0.03 ***	47.98 ± 2.72 **
Silymarin 100 mg/kg	0.72± 1.12***	42.16 ± 1.02 ***	9.02 ± 1.25 ***	54. 54 ± 7.78 ***

Values are expressed as mean ± SEM of 6 animals in each group: $^{\#}$P<0.05, $^{\#\#}$ P< 0.01, $^{\#\#\#}$ P<0.001 is compared with respective control group; *P<0.05, **P< 0.01, ***P<0.001 is compared with CCl_4 treated groups.

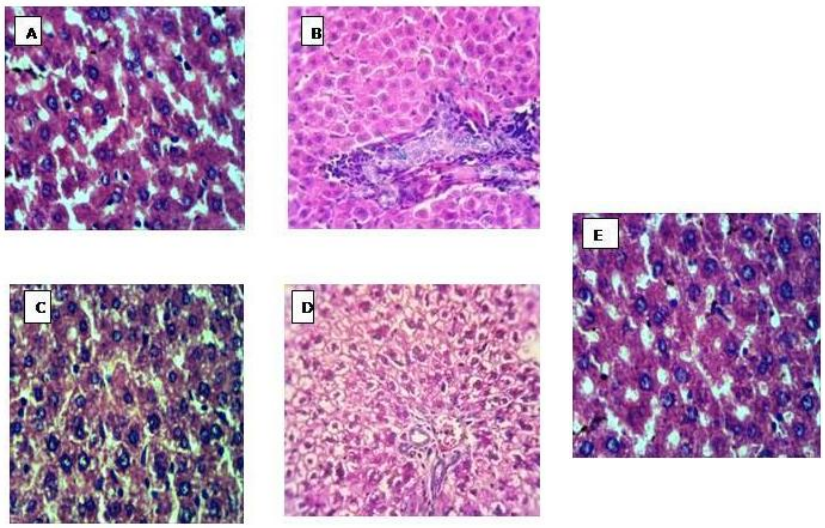

Figure 1: Liver sections stained with Haemotoxylin and eosin **(A)** Control group. **(B)** CCl4 treated rats. **(C)** 200 mg/kg of EEJG treated rats. **(D)** 400 mg/kg of EEJG treated rats. **(E)** Silymarin treated rats (100 mf/kg)

Discussion

Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical disease. There is a growing interest in the pharmacological evaluation of various plants used in Indian system of medicines [21]. In the present investigation, hepatoprotective activity of EEJG was evaluated against CCl4 induced hepatotoxicity in rats. The carbon tetrachloride (CCl_4) is the most commonly used hepatotoxin in the experimental study of liver disease [22]. Carbon tetrachloride is assumed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological changes in liver due to the production of free radicals. The hepatotoxic effect of CCl_4 is due to the conversion of CCl_4 to highly reactive CCl_3 molecules by cytochrome P450 enzyme present in the smooth endoplasmic reticulum of the liver and vital organs to the [23]. Highly reactive CCl_3 free radicals directly attack the endoplasmic reticulum and causes over production of SGOT, SGPT, ALP, bilirubin levels [24, 25]. These free radicals are responsible for cellular damage through lipid peroxidation [26]. The exposure of CCl_4 to the rats results in depletion of antioxidant activities [27].

In the present study, CCl_4 treated rats revealed a significant increase in the activities of serum hepatic enzymes levels such as SGOT, SGPT, ALP, bilirubin level. Treatment of EEJG (200 and 400 mg/kg) attenuated the increased level of serum enzyme level produced by CCl_4 and caused subsequent recovery towards normalization as compared to the control rats. Reduction in the levels of SGOT and SGPT towards the normal range indicates the regeneration of liver cells. Decreased level of total protein might be due to the functional failure of the functional cytochrome p450 complexes [28]. Our results are also consistent with previous results. Symonik-Lesiuk *et al*. (2003) reported that CCl_4 intoxication leads to changes in antioxidant enzymes and its intermediates involved in the bio-activation of CCl_4 that may truss those enzymes to prevent their inactivation. CCl_4 treated rats showed significant reduction in the levels of SOD and CAT but the treated 200 and 400 mg/kg of EEJG groups showed increase in the levels of these enzymes which indicates the antioxidant property of the *Justicia gendarusa*. Glutathione provides a first line of defense and scavenges free radical oxygen species (ROS). The decreased concentration of GSH in liver may be due to NADPH reduction or GSH utilization in the exclusion of peroxides [29]. GSH-dependent enzymes offer a second line of protection as they primarily detoxify noxious by-products generated by ROS and help to avert dissemination of free radicals [30]. In our present study CCl_4 treated rats showed marked reduction in the level of GSH compared to control animals which was reverted by the administration of EEJG (200 and 400 mg/kg) in a dose dependent manner . The increased level of MDA in CCl_4 treated rats is indicative of high level of lipid peroxidation which in turn is an indicator of membrane damage, alterations in structure and function of cellular membranes and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals [31]. Whereas CCl_4 plus EEJG treated rats showed lower levels of MDA than only CCl_4 treated rats, suggesting that EEJG reversed the elevation of lipid peroxidation. Hence, it is possible that the mechanism of hepatoprotection of EEJG free radical scavenging activity may be due to reduction of ferric ion in presence and absence of EDTA and as well as its hydrogen peroxide scavenging activity. The antioxidant activity of EEJG may be due to the presence of high phenolic and flavonoid content as determined by folin-ciocalteu's method and aluminium chloride calorimetric assay [32]. Therefore, the mechanism of hepatoprotection of *Justicia gendarusa* may be due its antioxidant potential.

The hepatoprotective effect of the EEJG was further accomplished by the histopathological examination. EEJG at different dose levels possess hepatoprotection but 400 mg/kg is more effective compared to CCl_4 treated groups. The liver section of CCl_4 rats showed centrilobular necrosis with disappearance of nuclei and necrosis. However liver sections from EEJG treated rats showed that the reduction in histopathological scores as well as cellular damage. On preliminary phytochemical investigation of EEJG revealed the flavonoids, alkaloids, carotenoids, phenolic compounds are the major chemical constituents. These antioxidants phytochemicals might contribute to the hepatoprotective and antioxidant activities of EEJG.

Conclusion

The EEJG has shown dose dependent hepatoprotective activity with activity at the dose level of 400 mg/kg comparable to CCl_4 and silymarin treated groups. Possibly the hepatoprotection of EEJG is due to antioxidant properties of major chemical constituents. However, further investigations on the isolation and characterization of components that may be responsible for hepatoprotective activity, are in progress.

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Conflict of interest

Authors declare that there is no conflict of interest to reveal.

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