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Hepatoprotective Activity of *Justicia gendarusa* Linn. Leaves in Carbon Tetrachloride Induced Liver Injury in Mice

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Abstract

Aim of the present study is to screen phytochemical compounds present in Methanolic Extract of *Justicia gendarusa* (MEJG) leaves and determine the hepatoprotective activity using established Carbon Tetrachloride induced model. Mice were divided into five groups of six animals in each group. The hepatotoxicity induced by oral administration of CCl_4 dissolves in olive oil (1ml/kg b.w.), while vehicle control group given food and water only. Standard drug, silymarin (50 mg/kg) was used as the standard group. The MEJG at the doses of 200 mg/kg b.w. and 400 mg/kg b.w. received by treated group for seven consecutive days followed by a single oral dose of CCl_4 on 7 day. Blood samples and livers were collected for biochemical and histopathological analysis. The hepatoprotective activity of MEJG against the inducer exhibit significant ($p < 0.05$) results as indicated by the improvement in the liver function test. The histological findings of observation supported hepatoprotective activity of the plant. In conclusion, plant leaves of *Justicia gendarusa* possessed hepatoprotective activity, which requires further study in terms of its isolation of active phytochemicals present in the plant, which were responsible for the activity.

Keywords: Carbon tetrachloride, hepatoprotective, *Justicia gendarusa.*

1. Introduction

Liver having the largest organ of human body performs metabolism, detoxification of the exogenous xenobiotics, drugs, viral infections and chronic alcoholism [1]. To maintain a healthy liver is very crucial factor for health because liver diseases are some of the fatal disease in the world today and having serious challenge to International Public Health [2, 3]. By performing various role in body like metabolism, detoxifications liver goes under stress and liver failure and serious health problems arises [4]. Various synthetic medicines are recommended for liver diseases and most of them are immunosuppressive. To overcome this problem, herbal therapeutic strategies using plant-based treatment are in rigorous testing and several herbal hepatoprotective have been studied worldwide [5–8]. Thus, explaining plants for new sources of hepatoprotective agents has gained renewed interest amongst researchers. The plant *Justicia gendarusa*, Synonym: *Gendarussa vulgaris Nees* is consumed through folk Healers in Meghalaya [9] and in Assamese commonly known as *jatrashudhi*. The plant is distributed throughout the greater part of India and Andaman Islands. Traditionally, the leaves extract are used in body ache, pain and absecess [10]. The plant is used as febrifuge, diaphoretic, emetic, emmenagogue and anthelmentic [11]. Infusion of leaves, given internally in cephalalgia, hemiplegia and facial paralysis. Fresh leaves are used topically in oedema and rheumatism. Bark is used as emetic [12]. The leaves contain beta-sitosterol, lupeol, friedelin

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and aromatic amines. A few phytochemical and pharmacological studies of the plant species have been reported.

2. Materials and Methods

2.1 Plant material and Preparation extract

The plant, *Justicia gendarusa* Linn. (family-Acanthaceae) was collected in the month of June 2009, from Golaghat dist. Assam, India. The taxonomical identification and authentication of the plant was done at the Botanical Survey of India (BSI), Eastern circle, Shillong, Meghalaya. The voucher specimen (No. BSI/EC/Identification/2009/289, dated: 20/07/2009) is presented in our laboratory for further reference. The collected plant leaves were cleaned, shade dried, coarsely powdered (passes sieve no. 40) and stored in airtight container for further use. The leaves powder was defatted with petroleum ether then successively extracted in Soxhlet apparatus using ethyl acetate and methanol as solvent. The solvent was evaporated in a rotary vacuum evaporator. The vacuum dried extract was subjected to various phytochemical as well as pharmacological screening.

2.2 Phytochemical Screening

Phytochemical screening of Methanolic Extracts of *Justicia gendarusa* (MEJG) were subjected to qualitative detection of various primary and secondary phyto-constituents as per standard methods describe by Kokate [13].

2.3 Animal Used

Swiss Albino mice both sex with a weight between 20 and 25 g were used. The animals was kept under standard laboratory conditions (25±5^{0}C, 40-70% Relative humidity, 12 h light/dark cycle) and had access to standard diet and water *ad libitum*. The animals were acclimatized for a period of 14 days prior to performing the experiments. The experiments were carried out during the light period between 08.00-16.00 h. Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1283/c/09/CPCSEA) approved the experimental protocol. Protocol Approval Reference No. PBRI/IAEC/11/PN-163. Animal studies were performing as per regulations and in accordance to the guidelines of CPCSEA.

2.4 Pharmacologic Studies

2.4.1 Acute Toxicity Study

Acute toxicity study of the prepared methanolic leaves extract (MEJG) was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [14] the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, recommended starting dose is 300 mg/kg body weight. Acute toxicity was determined as per reported method.

2.4.2 Hepatoprotective Activity

In the hepatoprotective study, 200 and 400 mg/kg doses of MEJG were assayed against Carbon Tetrachloride (CCl_4) induced liver toxicity models with slightly modification [15]. Mice were divided into five groups of six animals in each group. The hepatotoxicity induced by oral administration of CCl_4 dissolves in olive oil (1ml/kg b.w.) toxic control group, while vehicle control group given food and water only, silymarin (50 mg/kg b.w.) was used seven consecutive days as standard drug for standard group. The MEJG at doses of 200 mg/kg b.w. and 400 mg/kg b.w. received orally by treated group for seven consecutive days followed by a single oral dose of CCl_4 in olive oil (1ml/kg b.w.) on 7 day. Animal were sacrificed using anaesthesia on 16th day after CCl_4 administration. Blood was collected from the retro orbital plexus and the liver was dissected out and processed immediately for biochemical assay and a small part of liver perfuse with saline and stored in 10% formaldehyde for histological examination.

2.5 Statistical Analysis

All the data are represent as mean ± standard error of mean and analyzed using the one-way ANalysis Of VAriance test (ANOVA) with the Bonferroni's Multiple Comparison Test $P<0.05$ t consider as significant level.

3. Results

3.1 Phytochemical Screening

Methanol Extract of *Justicia gendarusa* leaves (MEJG) showed the presence of phenolic compound, alkaloid, carbohydrate, glycosides, flavonoid and volatile oil, saponin, mucilage gum and lignin.

3.2 Acute Toxicity Study

From the acute toxicity study, it was concluded that no mortality of mice was observed up to the dose of 2000 mg/kg b.w. for the methanolic leaves extract. Hence, the extract was considered as non-toxic and 1/10th of dose 200mg/kg b.w. and a higher dose 400 mg/kg b.w. used in present study.

3.3 Hepatoprotective Activity

The Methanol Extract of *Justicia gendarusa* leaves (MEJG) exhibited significant ($p < 0.05$) hepatoprotective activity against the CCl_4 induced hepatotoxicity liver model shown in Table 1. The plant extract in two different doses improved liver function, as indicated by the reduction of elevated levels of the liver enzymes Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Serum ALkaline Phosphatase (SALP) compared with the control and toxic group. The various liver biochemical studies like Total Bilirubin (TB) and antioxidant enzyme such as Lipid Peroxidation (LPO) Superoxide Dismutase (SOD), Glutathione Reductase (GSH) and Catalase (CAT) showed significant ($P<0.05$) hepatoprotective activity against the vehicle and CCl_4-induced rats (Table 2). Liver histopathology of CCl_4 induced mice showed damage to the architecture of the liver, with severe necrosis of hepatocytes in the parenchymal region and hemorrhage detected (Fig. 1). These observations were improved in the MEJG and silymarin treated groups. The vehicle control group did not show any change in the level of liver enzymes and histology.

4. Discussion

The present work revealed the hepatoprotective potential of Methanol Extract of *Justicia gendarusa* leaves, MEJG in CCl_4 induced liver toxicity model. Liver damage induced by CCl_4 is commonly used model for the evaluation of hepatoprotective drugs. Administration of

Table 1: Effects of Methanol Extract of *Justicia gendarusa* leaves (MEJG) on various serum biochemical parameters in CCL_4 induced hepatotoxic mic.

Animal Groups	SGPT(IU/L)	SGOT(IU/L)	ALP(IU/L)	Total Bilirubin (mg/dL)
I-Vehicle Control	60.45 ± 4.31$^{\#}$	86.51 ± 4.72$^{\#}$	113.36 ± 4.05$^{\#}$	0.46 ± 0.240$^{\#}$
II-Standard Drug (Silymarin)+CCl_4	88.18 ± 4.99$^{*\#}$	108.45 ± 5.18$^{*\#}$	120.11 ± 3.24$^{*\#}$	0.91 ± 0.208$^{*\#}$
III-CCl_4 Control	148.15 ± 4.83*	188.98 ± 5.64*	179.28 ± 4.28*	4.26 ± 0.480*
IV- MEJG (200 mg/kg) + CCl_4	120.03 ± 4.94$^{*\#}$	143.90 ± 5.69$^{*\#}$	143.93 ± 6.72$^{*\#}$	2.21 ± 0.369$^{*\#}$
V- MEJG (400 mg/kg) + CCl_4	97.31 ± 4.23$^{*\#}$	117.80 ± 4.51$^{*\#}$	132.43 ± 3.47$^{*\#}$	1.68 ± 0.211$^{*\#}$

Values are expressed as mean ± SEM (n=6); $^{*}P<0.05$, vs Vehicle control group; $^{\#}P<0.05$, vs CCl_4 Control group.

Table 2: Effects of Methanol Extract of *Justicia gendarusa* leaves (MEJG) plant on various liver biochemical parameters in CCL_4 induced hepatotoxic mice

Animal group	LPO (nM/mg protein)	GSH (mM/gm tissue wt)	CATALASE (U/mg protein)	SOD (% inhibition of NBT)
I-Vehical Control	0.66 ± 0.113$^{\#}$	28.22 ± 1.54$^{\#}$	36.12 ± 1.52$^{\#}$	66.52 ± 2.03$^{\#}$
II-Standard Drug (Silymarin) +CCl_4	0.94 ± 0.082*#	23.34 ± 1.27$^{*\#}$	32.86 ± 1.32$^{*\#}$	64.87 ± 2.78$^{*\#}$
III-CCl_4 Control	5.21 ± 0.221*	10.54 ± 1.33*	16.34 ± 0.98*	25.76 ± 1.92*
IV- MEJG (200 mg/kg) +CCl_4	3.45 ± 0.224$^{*\#}$	18.85 ± 0.91$^{*\#}$	22.34 ± 1.32$^{*\#}$	31.30 ± 2.03$^{*\#}$
V- MEJG (400 mg/kg)+CCl_4	1.68 ± 0.087$^{*\#}$	20.45 ± 1.11$^{*\#}$	29.66 ± 1.45$^{*\#}$	49.23 ± 2.29$^{*\#}$

Values are expressed as mean ± SEM (n=6); $^{*}P<0.05$, vs Vehicle control group; $^{\#}P<0.05$, vs CCl_4 Control group.

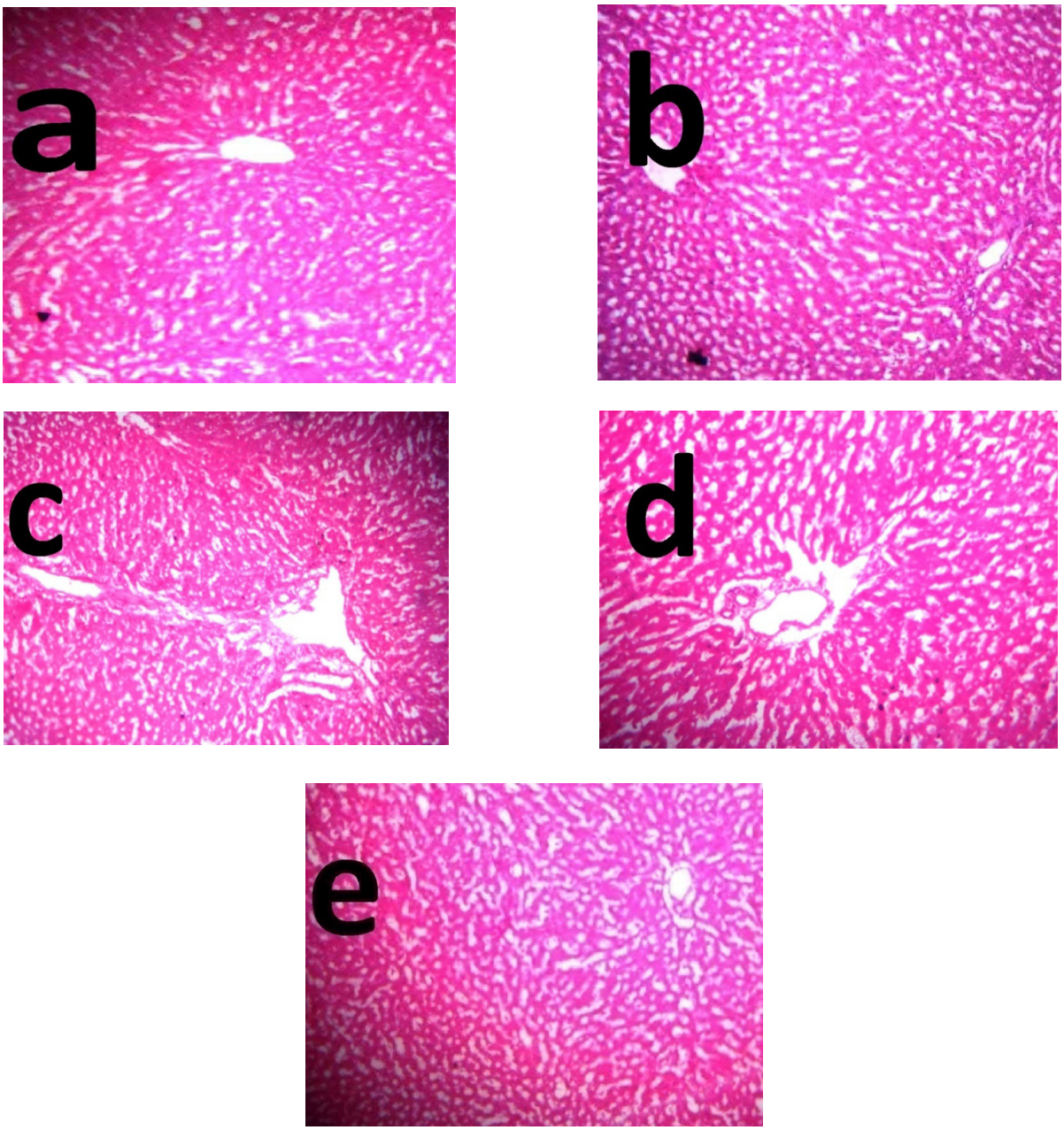

Fig. 1. Liver photomicrographs. (a) Vehicle control group showing normal liver architecture. (b) Hepatotoxic liver after treatment with CCl_4 (c) CCl_4-induced hepatotoxic liver after treatment with 50mg/kg silymarin, showing normal architecture of the hepatocytes. (d & e) Showing mild necrosis and normal architecture of the hepatocytes, mild steatosis and mild infiltration by lymphocytes in CCl_4-induced hepatotoxic liver after treatment with MEJG 200 mg/kg and 400mg/kg b. w. dose respectively.

CCl_4 causes acute liver damage, which produces changes in liver function that ultimately leads to destruction of hepatocellular membrane. The CCl_4 treatment forms various free radicals, which are activated by the cytochrome P-450 and involved in the pathogenesis of liver damage in chain reaction [16]. Thus, it could be said that the free radicals and oxidative processes play an important role in hepatotoxicity. Photochemical study of the plant extract has demonstrated that MEJG contains a high total phenolic content. The total phenolic content

has been known to contribute to the antioxidant activity of extracts [17] and antioxidant activity has also been linked to the hepatoprotective effect [18]. Therefore, these findings in MEJG exert a hepatoprotective activity. In Phytochemical screening the MEJG contains contain flavonoid, alkaloid, carbohydrate, glycosides, and lignin, which also reported to exert hepatoprotective activity [19–21]. The Methanolic leaf Extract of the plant *Justicia gendarusa* (MEJG) contributes to hepatoprotective activity which are supported by serum biochemical results and histopathological finding as stated above.

5. Conclusion

It can be concluded that the Methanolic Extract of *Justicia gendarusa* leaves (MEJG) possesses hepatoprotective activity against CCl_4 induced liver toxicity by regulating various biochemical parameters such SGPT, SGOT, ALP and total bilirubin and liver metabolites. However, further study is required to identify the chemical constituents (at molecular level) present in the extract of the herb responsible for the biological activity.

6. Conflicts of interest

All authors have none to declare

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