HEPATOPROTECTIVE ACTIVITY OF LEAVES OF JATROPHA CURCAS LINN.

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Abstract

Jatropha curcas Linn. leaves are used in the traditional system of medicine for the treatment of hepatopathy, antidysentric, anthelmentic, depurative and purgative, chronic rheumatism, skin diseases, lactoguage, suppurative, styptic, toothache, gastropathy, bronchitis, chronic diarrhoehe present study describes the hepatoprotective activity of aqueous extract of leaves of Jatropha curcas against CCl_4- induced acute hepatic injury. Hepatic injury was achieved by injecting 2 ml/kg, s.c. of CCl_4 in 1:1 with olive oil. Aqueous extracts at the dose 200 mg /kg p.o. offered significant ($p < 0.01$) hepatoprotective action by reducing the serum marker enzymes like serum alkaline phosphatase (SALP) , serum glutamate oxaloacetate transaminase (SGOT), serum bilirubin, and serum glutamate pyruvate transaminase (SGPT). Histopathological studies further confirmed the hepatoprotective activity of aqueous extract of Jatropha curcas leaves when compared with the CCl_4 treated control groups. Reduced enzymic were restored to normal by administration of aqueous extracts of leaves of Jatropha curcas. The results obtained were compared with Liv.-52 (5 ml/kg, p.o.), the standard drug. In conclusion, aqueous extracts (200 mg/kg, p.o.) showed significant hepatoprotective activity similar to that of the standard drug, Liv-52 (5 ml/kg, p.o.).

Keywords: Jatropha curcas Linn.; Leaves; Hepatoprotective activity; Carbontetrachloride; Histopathological studies

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INTRODUCTION

In the absence of reliable liver protective drugs in modern medicine, there are a number of medicinal preparations in the traditional system of Indian medicine recommended for the treatment of liver disorders. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effect. Due to this reason many people in the world over turning towards traditional remedies or folk remedies.

Jatropha curcas Linn. commonly known as ratanjyot, mogalieranda, janglieranda, bhagerendra, physic nut (Family-Euphorbiaceae) is widely acclaimed in the old-age Ayurvedic drug with a wide range of biological activity. Fruits, seeds, leaves, flowers, latex, sap and root bark are mainly attributed with various medicinal properties like antidiarrhoeal, stomachic, antidysentric, anthelmentic, thirst, tridosha, urinary discharges, abdominal complaints, biliousness, anemia, fistula, diseases of heart(Ayurveda), purgatives, toothache, fever, wounds and refractory ulcers [1]. Methanolic extract of *Jatropha curcas* leaves was screen for brine shrimp toxicity [2]. In addition, the various plant parts from *Jatropha curcas* have been reported for antiviral [3], wound healing [4], anti malarial [5], anti-tumor and anti-HIV [6], anti- diarrhoeal [7], antimicrobial [8], anti- coagulant [9] and anti-inflammatory activities [10]. There is presence of vitexin, isovitexin [11] campesterol, β-sitosterol-3 β,7 β-diol and – tricontanol in leaves have been reported [12]. Other chemical constituents form this plants are Diterpenoids [13], protein, alkaloids [14]. *Jatropha curcas* leaves are used in folk remedies by Barbadians uses the leaf tea for marasmus, Panamanians for jaundice. Leaves are galactagogue, rubefacient, suppurative, insecticidal and are used in foul ulcers, tumors and scabies, given internally in jaundice. *Jatropha curcas* is used in herbal mixture to treat jaundice [15]. However, there is lack of scientific report regarding the hepatoprotective activities of *Jatropha curcas* leaves. The present investigations were carried out to evaluate the drug for its hepatoprotective activity.

MATERIALS AND METHOD

MATERIAL AND METHOD:

Animals used

Wistar albino rats (140±20 g) of either sex were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 22±2 ◦C. They were fed with standard pelleted feed and water ad labium. The study approved from the Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of the extract

The fresh leaves of *Jatropha curcas* were collected in the month of April-May from local area of Belgaum (Rakascope village), Karnataka. The leaves were authenticated by Prof. R. S. Goudar Botanist, R.L.S. College, Belgaum, Karnataka. The air-dried leaves were coarsely powdered and subjected to soxhlet continuous extraction using solvents petroleum ether (40-60°C), alcohol (95%) and water. The extracts were concentrated under reduced pressure and analyzed for the presence of phytochemicals by qualitative chemical analysis [16].

In vivo hepatoprotective activity studies.

The Animals were divided into six groups each group containing six animals. Group A (normal control) received single dose of 1 ml/kg i.p. (intraperitoneally) of sucrose solution for 4 days , 1 ml/kg s.c. of olive oil on second and third days. Group B (induction control) received 1 ml/kg i.p. aqueous sucrose solution for 4 days with 2 ml/kg of CCl_4 by subcutaneous route dissolved in an equal volume of olive oil on 2nd and 3rd days. Group C (standard control) received standard drug Liv-52 of 5 ml/kg of oral route for 4 days with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2nd and 3rd days. Group D, E and F received 200 mg/kg of each extracts by oral route for 4 days respectively, with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2nd and 3rd days for induction . On the 5th day, all the animals were sacrificed under ether anaeshesia, and blood and liver samples were collected. Blood was allowed to coagulate at 37^0C for 30 min and the serum was separated by centrifugation at1200 -1500 rpm for 15 to 20 minutes. For histopathological study, liver tissue was quickly removed after autopsy and fixed in 10% formosaline. [17].

Assessment of hepatoprotective activity.

Assessment of antihepatotoxic activity was done by collecting serum was analysed by determining the various biochemical parameters such as glutamyl pyruvate transaminase (GPT), glutamyl oxalacetic acid transaminase (GOT)[18], alkaline phosphatase (SALP)[19], serum bilirubin (SBLN) [20] were also carried out to assess the acute hepatic damage caused by CCl_4.

Table:1 Effect of Petroleum ether, Alcohol, Water extracts of *Jatropha curcas* leaves on serum GPT, GOT, ALP and Billirubin in Carbon tetrachloride intoxicated rats.

Treatment	SGOT (IU/L)	SGPT (IU/L)	SALP (KA units)	SBLN (Mg/DL)
Normal Control (Group A)	44.17±1.941	35.83±4.262	26.08±1.151	2.188±0.1254
CCL_4 Control (Group B)	152.2±4.792	142.8±5.529	90.35±7.821	12.5±1.547
Liv-52 (5ml/kg) (Group C)	59.33 ±8.09	42.83±6.65	44.13±2.24	5.232±1.36
Pet ether extracts (Group D)	96±6.986#	68.67±4.719#	80.5±6.892#	10.87±1.174#
Alcohol extract (Group E)	76.5±8.118*	61.33±4.32*	57.43±10.58*	5.853±0.8373*
Aqueous extract (Group F)	62.17±6.795*	48.67±5.785*	47.68±7.867*	5.84±1.056*

Values are mean ± S.D., $n = 6$ in each group.
*Represents statistical significance: $p < 0.01$.
#Represents statistical significance: $p < 0.05$.

Histopathological studies.

The antihepatotoxic activity was confirmed through histopathological studies on liver of rats while collecting the blood from heart for SGOT, SGPT, SALP and SBLN estimation. The liver tissue was collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene and embedded in paraffin. Sections (4–5 µm) were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observations, including cell necrosis, fatty change, hyaline degeneration, infiltration of kupfer cells and lymphocytes.

Histopathological Microphotograph of Rat liver tissue

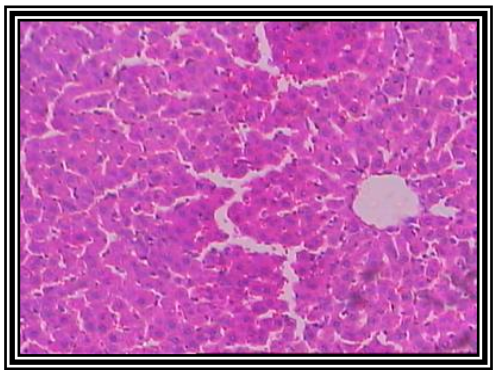

Fig-1Group A— Liver section of rats treated with normal shows central vein surrounded by hepatic cord of cells (normal architecture).

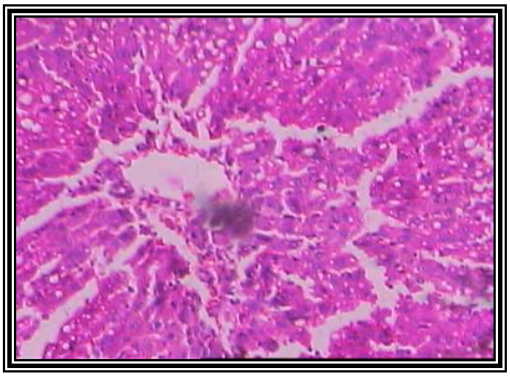

Fig-2 Group B— Liver section of rats treated with CCL_4 shows patches of liver cell necrosis with cytotoxic injury showing fibrotic changes.

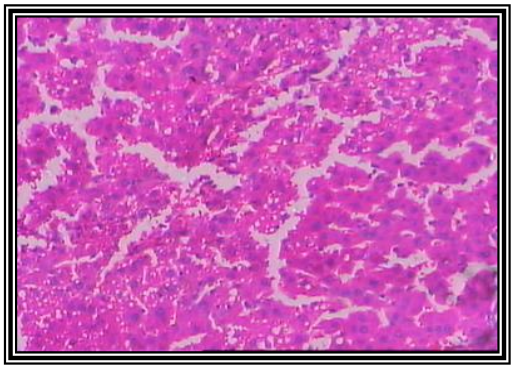

Fig. 3. Group C Liver section of rats treated with CCL_4 and Liv-52 (5ml/Kg) shows typical lobular arrangement. Hepatocytes show variable size, mild increase in fibrous connective tissues.

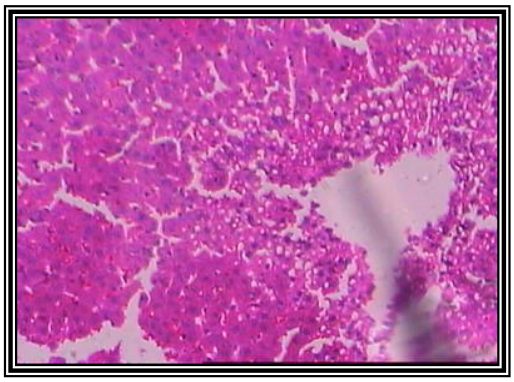

Fig. 4. Group D: Liver section of rats treated with CCL_4 with Petroleum ether extract shows inflammatory collections around central vein and focal necrosis with sinusoidal dilatation.

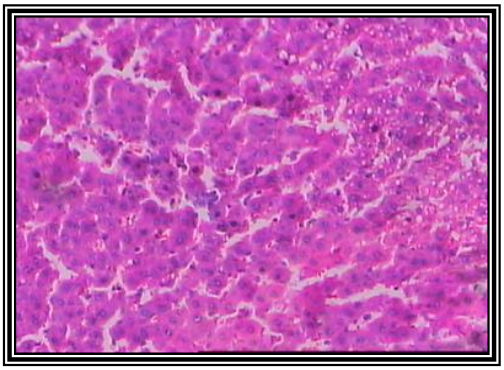

Fig. 5 Group E— Liver section of rats treated with CCL_4 and alcohol extracts show less inflammatory cells around central vein, absence of necrosis.

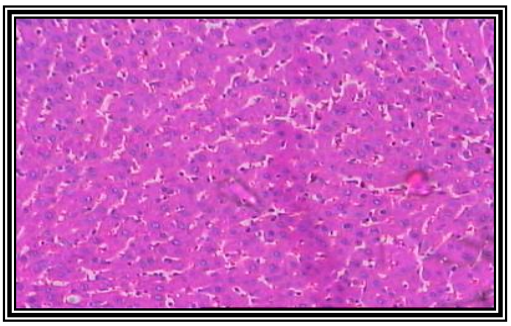

Fig. 6 Group F— Liver section of rats treated with CCL_4 and aqueous extracts shows minimal inflammatory cellular infiltration. Almost near normal liver architecture. Regeneration of hepatocytes around central vein.

Statistical analysis.
The results are expressed as mean ± S.D. *P*-values of 0.05 were considered statistically significant.

RESULT:
Preliminary phytochemical analysis
On preliminary phytochemical analysis, the petroleum ether extract of *Jatropha curcas* leave shows presence of steroid , alcoholic extract of *Jatropha curcas* leaves shows the presence of glycosides, saponins, carbohydrates, and alkaloids. Aqueous extract shown the presence of carbohydrates, flavonoids, steroids, saponins, and glycosides,

Antihepatotoxic activity.
The results of hepatoprotective effect of various extracts of *Jatropha curcas* leaves on CCl_4 intoxicated rats are shown in Table 1. In the CCL_4 treated group serum GOT, GPT, ALP and serum billirubin were increased 152.2 U/ I, 142.8 U/I, 90.35 KA , 12.5 IU/ml. while these value showed 44.17 IU/ I, 35.83 IU/I, 26.08 KA, 2.188 mg/dl in normal group, respectively. In contrast, the groups treated with alcoholic extract and aqueous extract decrease significantly ($P< 0.01$) the elevated levels of SGOT, SGPT, SALP, and serum billirubin. The effect of both alcoholic and aqueous extract (200 mg/kg) on serum marker enzymes and serum bilirubin in CCL_4 induced hepatic injury shown in Table 1. Hepatic injury induced by CCL_4 caused significant rise in marker enzymes SGOT, SGPT, SALP, and serum bilirubin. Administration of aqueous extract attenuated the increased levels of the serum enzymes, produced by CCL_4, and caused subsequent recovery towards normalization almost like that of Liv- 52 treatment (Table-1).

Histopathological studies
Histopathological observation supports the results obtained from serum enzymes assays. Hisopathological studies of the normal liver texture shows typical lobular arrangement. Lobules consist of hepatocytes arranged as cords radiating around centrally placed terminally hepatic vein. Hepatocytes seen are uniform in size, polyhedral in shape, with centrally located large nuclei. Portal tracts containing terminal branches of the hepatic portal vein and hepatic artery at the periphery in fibrous stroma are also seen (Fig. 1). The liver section of CCL_4 treated rats showed high tissue damage showing disturbs lobular arrangement, degenerative necrotic changes. Hepatocites shows ballooning degeneration and steatoic changes. Some amount of fibrosis seen in portal tract (Fig. 2). In the liver cells of Liv-52 and intoxicated CCl_4 treated rats shows typical lobular arrangement with variable size of hepatocytes. There is mild increase in fibrous connective tissues (Fig. 3). Histopathological architecture of liver sections of rats treated with petroleum ether, alcoholic extract and aqueous extract intoxicated with CCL_4 showed more or less distrurbance in lobular arrangement with degenerative early necrotic changes. Apoptotic cells and Mallory's bodies are seen with some amount of fibrosis (Fig. 4), ballooning, steatoic accumulation, fibrous tissues and inflammatory cells (Fig. 5), typical lobular arrangement with few steatoic accumulations (Fig. 6) respectively. The aqueous extract (200mg/kg) treated group showed minimal inflammatory condition with near normal and standard liver architecture possessing higher hepatoprotective activity.

DISCUSSION AND CONCLUSION:
It confirmed that, CCl_4 induces hepatotoxicity by metabolic activation. It selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($\bullet CCl_3$) that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum [21]. The ability of a hepatoprotective drug to the injurious effect or to preserve the normal hepatic physiological mechanisms, which have been affected by a hepatotoxin, is the index of its protective effects. To measure the degree of hepatotoxicity the various serum enzymes levels are the the indirect measure of hepatic injury, they show the status of liver. The decrease in enzyme level definite indication of hepatoprotective action of the drug. Protection of hepatic damage caused by CCL_4 administration was observed by recording SGPT,SGOT,SALP and serum bilirubin level in treated toxin control and normal group because serum transaminases, serum alkaline phosphatase, and serum bilirubin have been reported to be

sensitive indicator of the liver injury [22]. As a result of hepatic injury, it causes the leakage of enzymes from cells due to altered permeability of membrane which disturbs the transport function of hepatocytes [23]. This results in lowering of enzyme levels in the hepatic cells and raised level in serum.

The results presented in Table 1 explain that although both the treatments, alcoholic and aqueous extract offers hepatoprotection but aqueous extract is more effective than alcoholic extract. It is also notice that both the treatments are particularly sensitive to Serum bilirubin levels. These rapid decreases in serum bilirubin suggest that aqueous extract can be used in the acute conditions of jaundice. The histopathological studies are the direct evidence of efficacy of drug as protectant to liver injury. Simultaneous treatment of aqueous extract with CCL_4 exhibit less damage as compare to the hepatic cell as compare to the rats treated with CCL_4 alone. Intralobular veins though damage but to a lesser extent, liver shows typical lobular arrangement with few hepatocytes shows steatotic accumulation. The results of histopathological observation parameters also supports the results of biochemical parameters and explain the hepatoprotective activity of leaves of *Jatropha curcas* Linn and justifies the uses of decoction of the leaves of this plant in folk remedies for jaundice.

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