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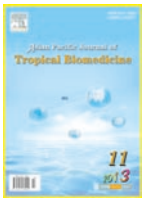

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Hepatoprotective effect of leaves of *Erythroxylum monogynum* Roxb. on paracetamol induced toxicity

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PEER REVIEW

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Comments

The present research work provides valuable information. Seening the extent of damage to the society done by liver related this can be helpful. Authors have justified the work by using a suitable model and doing both hiatopathologiacal and biochemical work. It is suggested that this work should be carried out further to investigate constituents responsible. Synergy part must be kept in mind while doing any further research work.
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ABSTRACT

Objective: To investigate the hepatoprotective activity of methanolic extract of leaves of *Erythroxylum monogynum* (*E. monogynum*) on paracetamol induced toxicity.
Methods: Methanolic extract of leaves of *E. monogynum* was given in doses of 100 mg/kg, 200 mg/kg and 400 mg/kg for 7 d and toxicity was induced by paracetamol (2 mg/kg) on Day 8. Silymarin (50 mg/kg) was used as reference standard. After 24 h of toxicity induction blood samples were collected from retro-orbital plexsus and analyzed for serum parameters like serum glutamic pyruvic transaminase, serum glutamic oxaloacetate transaminse, alkaline phosphatase and total bilirubin. Livers isolated were studied for histopathological changes.
Results: Phytochemical analysis of methanolic extract of *E. monogynum* leaves showed the presence of carbohydrates, flavonoids, phenols and saponins. Prior administration of this extract restored the elevated levels serum markers as compared to toxic group which is also confirmed by the histopathological changes observed.
Conclusions: The present study showed that methanolic extract of leaves of *E. monogynum* possess hepatoprotective action against paracetamol induced hepatotoxicity.

KEYWORDS
Erythroxylum monogynum, Hepatoprotection, Paracetamol, Silymarin

1. Introduction

Liver is the most important organ of the human body involved in metabolism, detoxification and excretion of various endogenous and exogenous substances. Such physiological activity of the liver results in the production of highly reactive species known as free radicals. Free radicals combine with the membrane lipids by covalent bond altering the membrane permeability of the cells leading to tissue damage[1]. Approximately 10% of world population is affected with liver diseases. This includes chronic hepatitis, alcoholic steatosis, fibrosis, cirrhosis and hepatocellular carcinoma[2]. Morbidity and mortality resulting from liver diseases is a major public health problem worldwide, especially in developing countries.

The management of liver disease is still a challenge to modern medicine. The only drugs available for the treatment of liver disorders are corticosteroids and immunosuppressive agents. However, these suffer with several adverse effects. This has led to increased dependence on complementary and alternative medicine, especially herbal therapy. Plant drugs are known to play

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a vital role in the management of liver diseases[3]. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but for minimal side effects and easy availability in nature[4].

Erythroxylum monogynum Roxb. (Erythroxylaceae) (*E. monogynum*) is a well known plant in traditional medicine which is found widely in southern parts of India. Several parts of the plant are used in Indian traditional medicine for various therapeutic effects. In the southern parts of Andhra Pradesh, extract of the leaves mixed with yoghurt is administered to kill intestinal worms[5], and the leaf juice is used for the treatment of jaundice[5,6]. The infusion of bark and wood is used as stomachic, diaphoretic, stimulant, diuretic and also in mild cases of dyspepsia and continued fever[7]. *E. monogynum* has been reported for its antibacterial activity[8].

Acetaminophen (N-acetyl-p-aminophenol, Paracetamol) induced toxicity in rats is one of the widely used experimental model to evaluate the hepatoprotective activity of herbal extracts[9]. At therapeutic doses, paracetamol is considered a safe drug. However, it can cause hepatic necrosis, nephrotoxicity, extra hepatic lesions, and even death in humans and experimental animals when taken in overdose[10]. Literature review reports that no scientific validation has been done on the leaves of *E. monogynum* as a hepatoprotective agent. So the present work aims at evaluating hepatoprotective activity of leaves of *E. monogynum* against paracetamol induced toxicity in the male Wistar albino rats.

2. Materials and methods

2.1. Experimental animals

Male Wistar albino rats, weighing between 180-200 g were used to determine the hepatoprotective activity. The animals were housed in clean polypropylene cages which consists of sterile paddy husk acting as a bedding agent and maintained under standard conditions of temperature (24±2) °C under 12 h light/dark cycle. They were fed with standard pellet diet and water *ad libitum*. All the animals were acclimatized to laboratory conditions before commencement of the experiment. The procedure followed in this study was done in accordance with Animals Ethics Committee guidelines.

2.2. Reagents and Instruments

Paracetamol was obtained as a gift sample from Cipla Ltd., India. Silymarin was purchased from Yucca Enterprises, Mumbai. Assay kits for analysis of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and total bilirubin (TB) were purchased from Accurex Biomedical PVT. Ltd., Mumbai. All other chemicals were high analytical grade which were used for performing the hepatoprotective activity.

2.3. Collection and preparation of methanolic extract of E. monogynum.

Plants of *E. monogynum* were collected from Khammam District of Andhra Pradesh, India. A specimen of the plant was submitted to Botanical Survey of India (BSI), Hyderabad and authenticated by the same. The leaves of the plant material were dried in shade and then powdered, which was later extracted by cold maceration technique using methanol as a solvent for 7 d with intermittent shaking. The last trace of the solvent was removed by Rota Evaporator and finally dried in vaccum. The percentage yield of leaves of methanolic extract of *E. monogynum* was found to be 12.9%.

2.4. Preliminary phytochemical screening

The preliminary phytochemical screening was done by following standard qualitative chemical methods[11]. The methanolic extract of *E. monogynum* was screened for the presence of carbohydrates, alkaloids, triterpenoids, saponins, phenols, sterols and flavonoids.

2.5. Acute toxicity study

The acute oral toxicity was carried out according to OECD-425 guidelines. Five male Wistar albino rats were selected and administered a dose of 3 g/kg. The respective dose was well tolerated by all the animals without showing any signs of toxicity and mortality. So we assumed that LD_{50} was beyond 3 g/kg. Three different graded doses of 100 mg/kg, 200 mg/kg and 400 mg/kg were selected to determine the hepatoprotective activity.

2.6. Assessment of hepatoprotective activity

In the paracetamol induced liver toxicity model, male Wistar rats weighing between 180-200 g were selected and divided into six groups containing six animals in each group[12]. Group I treated as normal given 2 mL/kg *p.o.* of gum acacia (2%) for 8 d. Group II received 2% gum acacia *p.o.* for 7 d and single dose of paracetamol (2 mg/kg) *p.o.* on Day 8. Group III administered with silymarin (50 mg/kg) *p.o.* for 7 d. Groups IV-VI received plant doses 100 mg/kg, 200 mg/kg and 400 mg/kg for 7 d. Silymarin, paracetamol and plant extract were dissolved in 2% gum acacia.

On Day 8, all the groups (III-VI) received paracetmol (2 mg/kg) except Group I, after 24 h of induction of toxicity by paracetamol, blood samples were collected from the

retrorbital plexsus. The collected blood was centrifuged at 2 500 r/min for 15 min to separate serum which is used for analysis of biochemical parameters such as SGPT, SGOT, ALP and TB.

2.7. Histopathological studies

One animal from each group was sacrificed by cervical dislocation, and the liver was removed. The liver specimen isolated from treated and control groups were fixed in 10% buffered formalin for 24 h and then stained with haematoxylin-eosin for photomicroscopic observation of the liver histopathological architecture.

2.8. Statistical analysis

All the results were expressed as mean±SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test using Graph pad Prism-5 software. $P<0.05$ was considered as significant.

3. Results

3.1. Preliminary phytochemical analysis

Preliminary phytochemical analysis showed the presence of phytoconstituents such as carbohydrates flavonoids, triterpenoids and saponins.

3.2. Acute toxicity study

No adverse effects and no mortality of the animals were observed during the period of study, 14 d up to the dose of 3 000 mg/kg body weight *p.o.* for the methanolic extract of leaves of *E. monogynum*. Hence, three doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight *p.o.* of the extract were selected for the hepatoprotective activity against paracetamol induced toxicity.

3.3. Hepatoprotective assessment

The results obtained from the hepatoprotective study of methanolic extract of *E. monogynum* are summarized in Table 1.

Table 1

Effects of drug treatment on biochemical parameters in paracetamol intoxicated rats.

Groups	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	TB (mg/dL)
Control	29.37±2.61	30.37±1.14	184.00±6.60	0.30±0.06
Toxic	21.30±2.27*	150.90±2.93*	511.10±9.51*	2.15±0.09*
Silymarin	34.70±1.69**	36.40±1.15**	281.50±3.04**	0.51±0.04**
100 mg/kg *E. monogynum* group	41.95±0.94**	43.08±1.06**	305.90±9.30**	1.38±0.07**
200 mg/kg *E. monogynum* group	40.88±0.95**	41.43±1.00**	293.90±5.11**	0.91±0.06**
400 mg/kg *E. monogynum* group	38.10±2.48**	39.32±0.87**	288.00±2.01**	0.60±0.06**

Data are expressed as mean±SEM (n=6), *$P<0.05$ compared with control, **$P<0.05$ compared with paracetamol.

Data showed that Wistar rats treated with paracetamol (2 mg/kg *p.o.*) alone developed significant hepatocellular damage as evident from a significant increase in serum

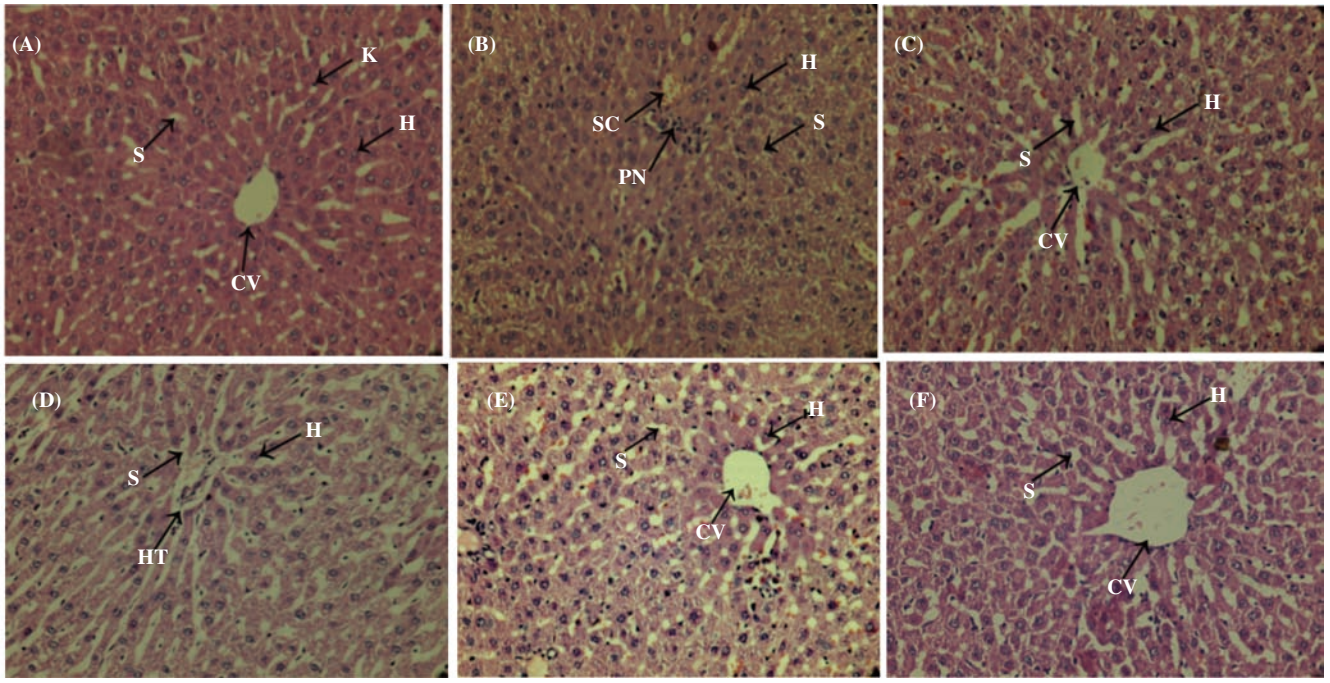

Figure 1. Histopathological changes occurred in rats during paracetamol intoxication and prevention by the treatment with methanolic extract of leaves of *E. monogynum*.

A: Normal control, B: Toxic control, C: Silymarin (50 mg/kg+paracetamol), D: *E. monogynum* (100 mg/kg+paracetamol), E: *E. monogynum* (200 mg/kg+paracetamol), F: *E.monogynum* (400 mg/kg+paracetamol). CV: Central vein, H: Hepatocytes, HT: Hepatic triad, K: Kupffer cells, PC: Piecemeal necrosis, SC: Sinusoidal congestion, S: Sinusoids.

biomarkers SGPT, SGOT, ALP and total bilirubin as compare to control group. Pretreatment of the rats with methanolic extract of leaves of *E. monogynum* at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg, prior to paracetamol administration caused a significant reduction in the values of SGPT, SGOT, ALP and TB almost comparable to that of silymarin.

3.4. Histopathological observations

Histological observation of the liver (Figure 1) supported the results obtained from serum enzyme assays and showed heaptoprotective activity of methanolic extract of leaves of *E. monogynum*. Figure 1a shows the nomal control. Paracetamol alone treated rats showed piecemeal necrosis in the liver (Figure 1b), including sinusoidal congestion. Pretreated methanolic extract groups IV-VI showed less severe necrosis and fatty changes of hepatocytes with well defined hepatic architecture comparable to standard group (Figures 1c-f).

4. Discussion

Paracetamol (acetaminophen) is widely used as a non narcotic analgesic and antipyretic drug[13]. When taken in toxic doses, it becomes a potent hepatoxin, generating fulminated hepatic and renal tubular necrosis which is lethal in humans and many species of animals like rodents[14]. The laboratory features of hepatotoxicity induced by acetaminophen resemble other kinds of acute necroinflammatory liver disease with prominent increases of serum SGPT and SGOT levels. The histopathological appearances of the liver biopsy or autopsy revealed a variably extensive centrizonal necrosis without steatosis and with a relatively light inflammatory infiltration[15].

Paracetamol is primarily metabolized by sulphation and glucuronidation to unreactive metabolites, and then activated by the cytochrome P-450 system to result in liver injury[16]. The characteristic zone 3 necrosis of acetaminophen appears to be produced by an electrophilic metabolite of the drug (N-acetyl-p-benzoquinonimine, NAPQI). NAPQI is initially detoxified by conjugation with reduced glutathione to form mercapturic acid[17]. However, when the rate of NAPQI formation exceeds the rate of detoxication by glutathione, NAPQI will oxidize tissue macromolecules, such as lipids or protein thiols, and alter the homeostasis of calcium after depleting glutathione leading to cell death.

Lipid peroxidation has been postulated to be the destructive process in liver injury due to acetaminophen administration[18]. The coincidence of antioxidant activity and liver tissue protective effects after acetaminophen administration suggest that both free radical generation and lipid peroxidation may be involved in this kind of drug injury process.

Strengthening the above mechanisms involved in the generation of toxicity by paracetamol, there are significant increased levels of biochemical parameters in the toxic group of the present study, which is also evident from the histopathological profile. Thus, it clearly indicates that toxicity is either due to depletion of glutathione or lipid peroxidation.

Silymarin treated group maintained the normal architecture and restored the levels of the serum markers and bilirubin levels compared to toxic group, but ameliorated activity than the methanolic extract of *E. monogynum*.

The methanolic extract of *E. monogynum* showed the dose dependent activity which is evident from the decreased level of serum enzymes and total bilirubin at dose of 400 mg/kg as compared to 100 mg/kg and 200 mg/kg. Further from the histopathological studies, it is revealed that methanolic extract of *E. monogynum* at the dose of 400 mg/kg is comparable to that of silymarin.

The preliminary phytochemical analysis of methanolic extract of *E. monogynum* revealed the presence of triterpenes, flavanoids and saponins. These phytochemicals are well documented for their hepatoprotective action[19,20]. Hence, from this study, it can be concluded that methanolic extract of leaves of *E. monogynum* possesses hepatoprotective activity against paracetamol induced toxicity in rats.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Liver is the biochemical factory. Almost 80% of the liver can be out of function without major indications. Liver disease is the fifth big killer in England and Wales, being the fourth major cause of death in the USA. Till date, a lot is required to be done to treat diseases like cirrhosis which can only be managed to prevent them from getting worse.

Any work on this global menace is more than welcome.

Research frontiers

The present research studied the hepatoprotective effect of leaves of *E. monogynum* on paracetamol induced toxicity. Paracetamol is common toxic to cause liver injury. The study on biochemical parameters is well supported by histopathology evidence.

Related reports

Paracetamol is common toxic to cause liver injury worldwide. Cytochromes P450 2E1 and 3A4 in liver convert paracetamol to a highly-reactive metabolite, *viz.* NAPQI. NAPQI reacts with cellular membrane molecules, resulting in widespread hepatocyte damage and death, leading to acute hepatic necrosis. Sylimarin is a promoters of ribosomal RNA synthesis, stimulating liver regeneration and as inhibitors of the transformation of stellate hepatocytes into myofibroblasts, the process responsible for the deposition of collagen fibres leading to cirrhosis.

Innovations and breakthroughs

E. monogynum is a well known plant in traditional medicine used for jaundice, which is usually caused by viral infection. In India, wood of plant is used as an adultrant to sandalwood. The present study supports the use of plant in jaundice and can be a good supportive therapy for traetmnet of liver disease.

Applications

With help of some clinical data, the present research can be converted into economically viable project.

Peer review

The present research work provides valuable information. Authors have justified the work by using a suitable model and doing both histopathological and biochemical work. It is suggested that this work should be carried out further to investigate constituents responsible. Synergy part must be kept in mind while doing any further research work.

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