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Protective effect of leaf extracts of *Ximenia Americana* Linn. on Acetaminophen induced hepatotoxicity in rats

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ABSTRACT

The extracts of Ximenia americana Linn.were studied to evaluate the Hepatoprotective activity in acetaminophen induced hepatotoxicity in rats. The plant extracts (200 and 400 mg/kg, p.o) showed a remarkable Hepatoprotective activity against acetaminophen induced hepatotoxicity as judged from the serum marker enzymes and Liver tissues. Acetaminophen induced significant rise in Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP), Total Bilirubin, and Gama glutamate transpeptidase(GGTP), with reduction in total protein content. Treatment of rats with different doses of plant extracts (200 and 400 mg/kg, p.o) significantly (P<0.05) altered serum marker enzymes levels to normal against acetaminophen treated rats. Histopathological liver samples were compared with standard (Silymarin treated). The results of this study indicates that Ximenia americana Linn. leaves have potent Hepatoprotective action against acetaminophen induced hepatotoxicity in rats.

Keywords :*Ximenia americana* Linn., Acetaminophen, Biochemical parameters, histopathology.

INTRODUCTION

Therapeutic potentials of herbal drugs ranges from parts of plants through, simple extracts to isolated active constituents. There have been resurgence of interest on plants and plant derived products as a source of medicine in last few decades. About 80% of world population relies on folklore medicine for curing ailments related to liver. Liver is a vital organ of the body and plays a major role in metabolism, performing a number of functions including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and detoxification of Xenobiotics, environmental pollutants and chemotherapeutic agents. A small number of these medicinal plants as well as formulations used are scientifically evaluated for their activity. In the context of our ongoing search for new natural substances possessing

Hepatoprotective efficacy, the present investigation was undertaken by utilizing the plant *Ximenia americana* Linn.of Olacaceae family.

The plant is known as Chiru- illantai in Tamil and Kondanakkera in Telugu. It is a straggling variable shrub or small tree, up to 4.5 cm in height, with spiny branches. Bark reddish brown, rough with deep fissures, leaves are ovate to broadly elliptic oblong or round. Flowers are white or yellowish white fragrant auxiliary racemes. The ripe fruits are aromatic and acidic sweet. There are few reports on phytochemical composition like presence of Oleanene palmitate[1], Oleonolic acid saponin[2]. Pharmacological activities pertaining to wound healing,[3] antimicrobial,[4] antioxidant [5] and antitrypanosomal [6] activities have been reported.

MATERIALS AND METHODS

The leaves of *Ximenia americana* Linn. were collected in December 2010, in the regions around the Maredimilli, East Godavari District, Andhrapradesh, India and authenticated by Prof. R. Jayaraman, Plant Anatomy Research Centre, Tamilnadu. A voucher specimen (pcp -2011/102) has been kept in our laboratory for future reference. The leaves were dried in shade and pulverized. The powder treated with petroleum ether for dewaxing as well as to remove chlorophyll. After that powder dried and subjected to hot extraction method (decoction), the aqueous extract separated from marc by filtration, decoction was concentrated at 45^{o}c. Two different concentrations of aqueous extracts were used for present investigation.

Chemicals

Acetaminophen was purchased from Lupin Ltd., Mumbai, India. Silymarin (Micro Labs, Hosur, India),1-chloro2,4-dinitrobenzoic acid(CDNB), 5,5-dithio –bis- 2-nitrobenzoicacid (DTNB) and reduced glutathione (GSH) were supplied by Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Acute toxicity studies

The institutional Animal Ethical Committee (Sanction No. 265/CPCSEA/ October 2000) approved the pharmacological protocols, according to prescribed guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Acute toxicity of the leaf extracts of *Ximenia americana* Linn.was evaluated by using 6 mice (One Group) by using the up and down procedures as per OECD 2001 guidelines. 1/ 20th and 1/10th of maximum tolerated dose 200 and 400mg /kg body weights were chosen for study respectively.

Animals used

Male albino rats (Wistar strain) weighing 150-200g were used for the experiments. The selected animals were maintained under standard laboratory conditions (temperature 27±2^{0}c relative humidity 55±10% and 12 h light and dark cycles and fed standard diet and water *ad libitum*. The animals were adapted to laboratory conditions for 7 days prior to the experiments**.**

Hepatoprotective activity [7]

At the end of experimental period, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analysed for various biochemical parameters.

Table 1: Design of experiment (Rats are divided into five groups, each group consisting of six animals)

S.No	Group	Treatment and Dose
1	*Group I*	*Control received the normal Saline (2ml/kg)*
2	*Group II*	*Received Acetaminophen (750 mg/kg p.o) at every 72 h for 10 days*
3	*Group III*	*Received Silymarin (50mg/kg p.o) for 10 days and simultaneously administered Acetaminophen 750 mg/kg body wt. every 72 h.*
4	*Group IV*	*Received aqueous extract of Ximenia americana Linn. (200 mg/kg p.o) for 10 days and simultaneously administered Acetaminophen 750 mg/kg body wt. every 72h.*
5	*Group V*	*Received aqueous extract of Ximenia americana Linn. 400 mg/kg p.o for 10 days and simultaneously administered Acetaminophen 750 mg/kg body wt. every 72h.*

Assessment of liver functions

Biochemical parameters i.e. aspartate aminotransferase (AST) [8], alkaline phoshatase (ALT) alkaline phospatase (ALP), [9] α - glutamate transpeptidase (GGTP), [12] total bilirubin [11] and total protein [12] were analysed according to the standard procedures.

Histopathological studies

After the collection of blood samples, liver samples were collected, washed with normal saline and were fixed in 10% buffered neutral formalin for 48 h and then washed with water to remove fixative. The tissues were fixed in Bouin's solution for 6 h and processed for microtome sections and examined under light microscope. [13]

Statistical Analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way ANOVA followed by Dunnett' s multiple comparison test using Graph pad 5. $P < 0.05$ was considered as statistically significant.

RESULTS

The effect of *Ximenia americana* Linn.on serum marker enzymes are presented in table 2. The levels of serum AST, ALT, ALP, total Bilirubin and GGTP were markedly elevated and that of protein decreased in acetaminophen treated animals, indicating liver damage. Administration of *X. americana* Linn. at the dose of 200 and 400 mg/kg remarkably prevented the acetaminophen-induced hepatotoxicity in a dose dependent manner.

Table 2: Effect of *Ximenia americana* Linn. on biochemical parameters in Acetaminophen induced hepatotoxicity in rats

Groups	Bilirubin (mg/dl)	Total Protein (g/dl)	AST/SGOT (U/L)	ALT/SGPT (U/L)	ALP (U/L)	GGTP (U/L)
Group -I	0.101±04	7.2±0.06	261±1.93	52.6±1.2	187.3±1.76	25.82±0.255
Group - II	0.313±0.01	6.16±0.042	410.2±6.36	141.2±2.3	576.2±2.91	61.92±0.23
Group - III	0.106±03*	6.32±0.06*	310.4±7.92*	63±0.89*	224.9±1.20*	35.08±0.23*
Group - IV	0.210±0.0.1*	6.14±0.042*	357.4±1.45*	117±2.10*	411.2±1.46*	40.84±0.19*
Group - V	0.2±0.15*	6.26±0.043*	331.61±1.62*	82±1.13*	280.2±4.6*	37.08±0.238*

*Values are Mean± S.E.M; n=6. Statistical analysis was performed by one way analysis of one way ANOVA followed by Dunnett's multiple comparison test *P < 0.05 vs control.*

Fig 1. Section of liver tissues of control rats showing normal histology with mild peri portal inflammation

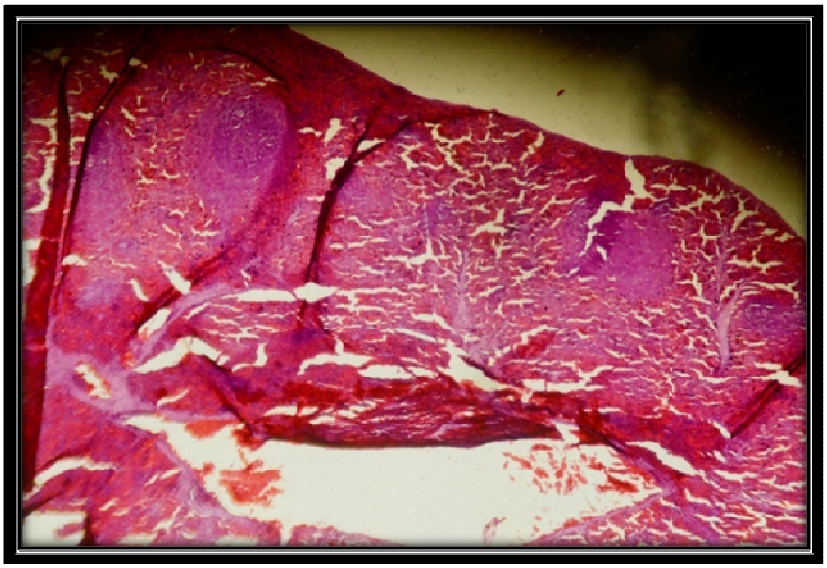
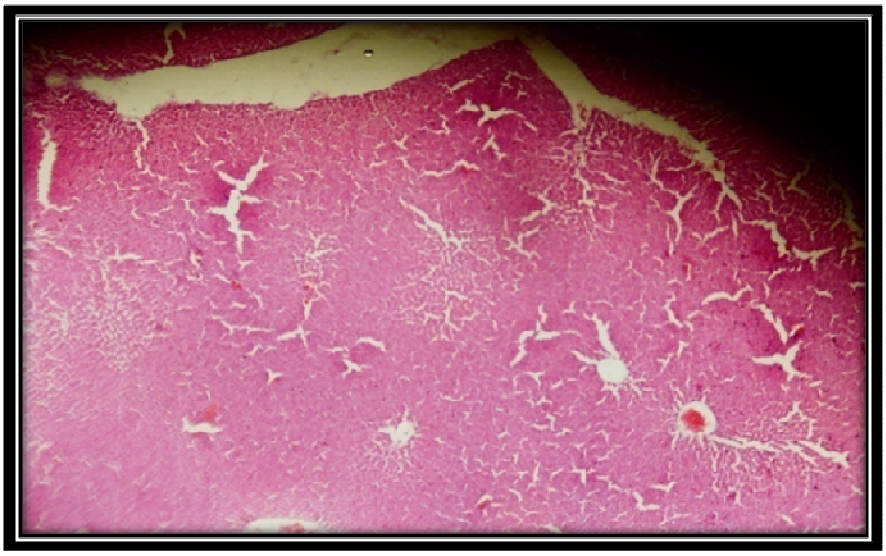


Fig 2. Section of the liver tissue of rats treated with Paracetamol showing dilation of central vein, peri portal inflammation, foci of loss of architecture & nuclear enlargement of hepatocytes.



Histopathological studies, showed acetaminophen to produce extensive vascular degenerative changes, peri portal inflammation and mild nuclear enlargement in hepatocytes. Treatment with different doses of *X. americana* Linn. extracts produced mild degenerative changes and absence of inflammation when compared to with hepatic control groups. All these results indicate a Hepatoprotective potential of the extracts.

Fig 3. Section of the liver tissue of silymarin treated rat showing normal hepatocytes, portal triad showing portal vein, portal artery and hepatic duct.

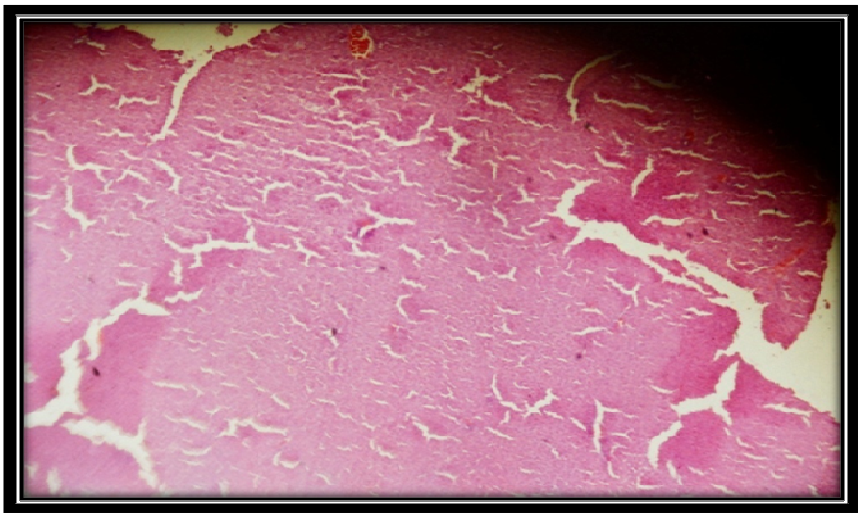
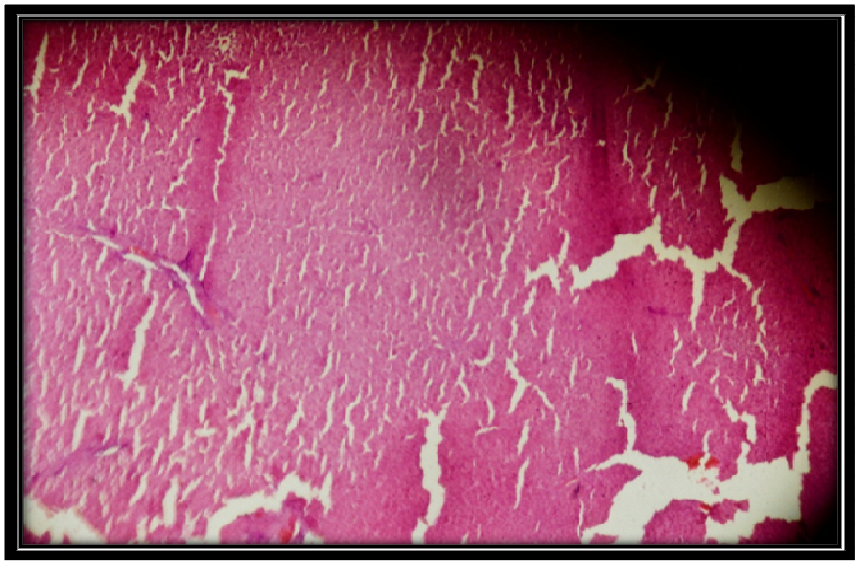
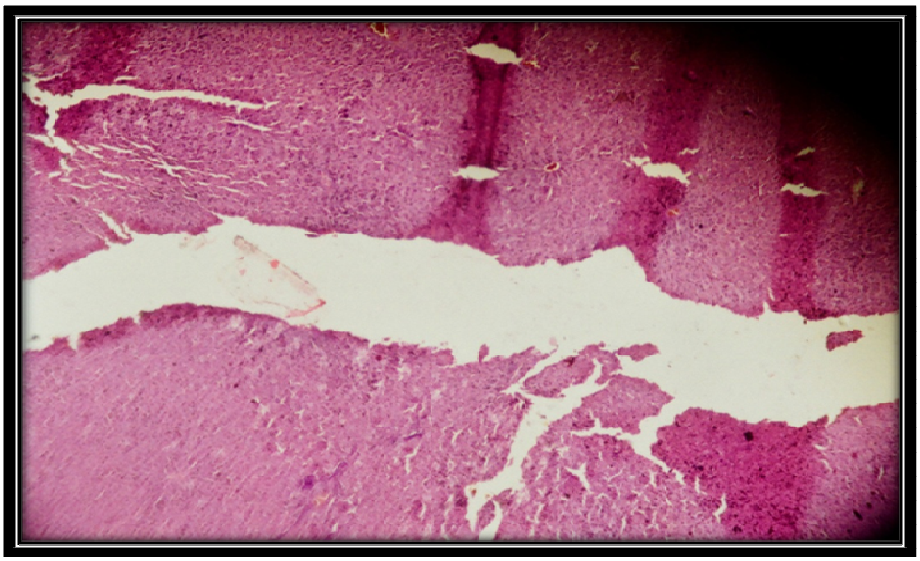


Fig – 4 Section of liver tissue of rat treated with Paracetamol + aqueous extract of *X.americana* Linn. showing mild peri portal inflammation only.

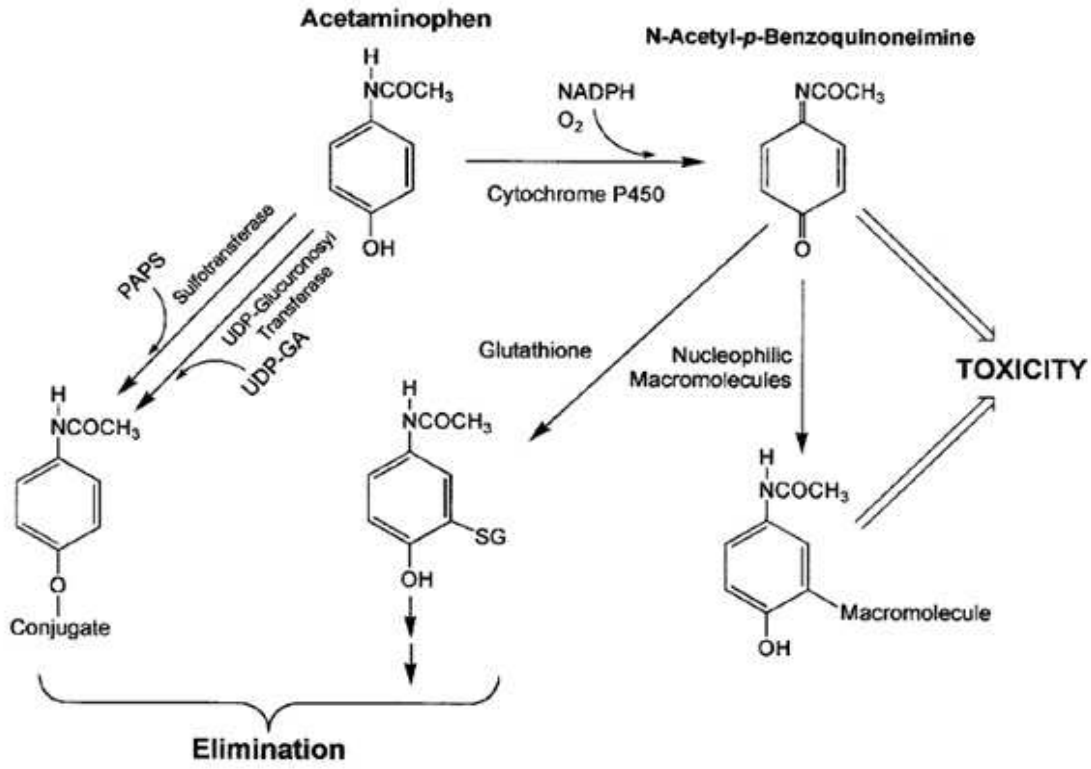


Acetaminophen (Paracetamol) [14] a widely used antipyretic- analgesic drug produces acute hepatic damage on accidental over dosage. It is established that fraction of acetaminophen converted via the cytochrome P_{450} pathway to a highly toxic metabolite; N-acetyl –P-benzoquinamine (NAPQ1)

Fig – 5 Liver section of a rat treated with Paracetamol + aqueous extract of *X. americana* Linn. (High dose) showing almost normal cell architecture



DISCUSSION



Mechanism of acetaminophen induced hepatoxicity.
Acute administration of Paracetamol high dose (500mg/kg) caused a marked hepatocellular injury which was clearly evidenced from the significant elevation in the levels of AST, ALT, ALP, total bilirubin and decrease in total protein (Group2), which are reliable markers of hepatotoxicity. The aqueous leaf extract of *Ximenia americana* Linn. at doses of 200 and 400mg/kg/p.o. reduced the elevated levels significantly (group 4, 5) and stabilized the deficient protein levels and was found to be statistically significant ($P<0.05$) on comparison with the normal control group. The activity exhibited by aqueous extract of *Ximenia americana* Linn. was comparable with the standard drug Silymarin (group 5). Silymarin provided a better inhibition of the elevated AST, ALT, ALP and total bilirubin induced by Paracetamol and also exhibited protein levels similar to the normal control group.

CONCLUSION

The results obtained from the levels of the hepatic marker enzymes showed significant hepatoprotective activity of aqueous extracts of *Ximenia americana* Linn.when compared to Silymarin. However, further studies are required such as detailed Phytochemical examination of the active constituents will provide the principle(s) responsible for the activity and to elucidate their mechanism of action.

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