
HEPATOPROTECTIVE ACTIVITY OF *VITEX NEGUNDO* LINN. BY PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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Objective: The present study was intended to evaluate the hepatoprotective activity of ethanolic extract of leaves of *Vitex negundo* Linn.. The *Vitex negundo* Linn. is a medicinal plant found throughout India.

Method: The ethanolic extract of *Vitex. Negundo* Linn. (300 mg/kg) was administered orally by suspending in tween-20 solution to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. The hepatoprotective activity was also supported by histopathological studies of liver tissue. An effective significant alteration in all biochemical parameters and histopathological sections was observed

Result: Since results of biochemical studies of blood samples of paracetamol treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by paracetamol and blood samples from the animals treated with the oral administration of ethanol extract produced a significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase, (AST), alkaline phosphatase (ALP) to the acute hepatotoxic induced rats. They exhibited a significant inhibition of hepatic toxicity by using various marker enzymes and the histopathological analysis.

Conclusion: From these results, concluded that the *Vitex negundo* Linn. was effective in protecting the liver against the injury induced by paracetamol in rats at the dose of 300 mg/kg/body weight. These results suggest that leaves of *Vitex negundo* Linn. may supports the hepatic cells protection.

KEY WORDS: *Vitex negundo* Linn., Paracetamol, Hepatoprotective, silymarin, SGOT

1. INTRODUCTION

Liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury (DILI) is one of the most common causative factor that poses a major clinical and regulatory challenge¹. Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant

based preparations which are employed for their treatment of liver disorders^{2,3}. Herbal medicines remain a popular alternative throughout the India. The phytochemical components of medicinal plants often act individually, additively or synergistically in improvement of health. Clinical research in this century has confirmed the efficacy of several plants in the treatment of liver disease⁴. After having analyzed the various chemical components present in leaves of *Vitex negundo* Linn., it is imperative that

focus shifts to the medicinal applications of the plant^{5,6}.

Aceta-aminophen (N-acetyl-p-aminophenol, Paracetamol) is widely used as analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses. It is established that following an oral therapeutic dose, a fraction of acetaminophen is converted via the cytochrome P-450 pathway to a highly toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) which is normally conjugated with glutathione and excreted in the urine as conjugates. Overdoses of acetaminophen deplete glutathione stores leading to accumulation of NAPQI, mitochondrial dysfunction, the development of acute hepatic necrosis. Also depletion of glutathione enhances the expression of tumour necrosis factor alpha (TNF α). TNF α primes phagocytic NADPH oxidase to the enhanced production of oxygen free radicals and contributes to the liver damage. Acetaminophen induced hepatotoxicity in rodents is a widely used animal model to assess hepatoprotective activity of new compounds. It is a powerful inducer of cytochrome P-450 and produces a highly reactive quinoneimine, which combines with sulphahydral groups of proteins and cause rapid depletion of intracellular GSH. Normally GSH contributes significantly to the intracellular antioxidant defensive system as it is a powerful consumer of superoxide singlet oxygen, and hydroxyl radicals. The breakdown of the GSH-dependent antioxidant defensive system increases the intracellular flux of oxygen apoptosis. The rise in serum levels of AST, ALT and ALP has been attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage⁷. The leaves of *Vitex negundo* Linn. are antibacterial, antitumor, astringent, febrifuge, sedative, tonic and

vermifuge. They are useful in dispersing swellings of the joints from acute rheumatism and of the testes from suppressed gonorrhoea. The juice of the leaves is used for removing foetid discharges and worms from ulcers, whilst oil prepared with the leaf juice is applied to sinuses and scrofulous sores. Leaves are anti-parasitical, vermifuge, pain reliever and insect repellents⁶. The present study was conducted to evaluate hepatoprotective activity of ethanolic extract against paracetamol-induced toxicity in rats.

2. MATERIAL AND METHODS

Chemicals- Paracetamol (S.D. Fine Chemicals, Mumbai), ethanol (Research Lab, Mumbai), Silymarin (Micro lab, Ltd), SGOT, SGPT, Acid phosphatase and Alkaline phosphatase test kits (Span Diagnostics Pvt. Ltd. Surat, India).

2.1 Plant collection & authentication

Fresh leaves of *Vitex negundo* Linn. were collected from Sangli, and Miraj areas during the month of November–December. The freshly collected plant was authenticated by the Botanist Dr. Smt. Yadav. U. S. from Botany department, of Willingdon College, Sangli.

2.2 Preparation of extracts

The leaves were washed and were shade dried to obtain coarse powder. The powdered leaves of known quantity was taken in a Soxhlet apparatus and extracted with absolute ethanol. The material was extracted continuously for 72h. The crude ethanol extract was then concentrated by distilling off the solvent under reduced pressure^{8,9} and subjected for further studies. The colour and consistency of the extract and its % yield value was calculated and reported.

2.3 Phytochemical screening

Ethanolic extract was subjected to preliminary organic qualitative analysis. Preliminary phytochemical study was

screened of ethanolic extract for the presence of carbohydrates, glycosides, alkaloids, steroids, flavonoids, tannins, fats and oils¹⁰.

2.4 Animal selection and procurement

Healthy male young adult animals Wistar albino rats commonly used laboratory strains employed. Male rats having the body weight (180-200g) were selected for study. At the commencement of its dosing, each animal body weight is should fall in an interval within 20% of the mean initial weight of any previously dosed animals. The temperature in the experimental animal room was maintained at 22°C (\pm 3°C). Although the relative humidity was maintained from 50-60%. Lighting was artificially maintained for 12 hrs dark and 12 hrs light. Animals were kept in the cages for at least 5 days prior to dosing for acclimatization to the laboratory conditions¹¹.

Wistar Albino rats used in the present studies was procured from the animal house of Appasaheb Birnale College of Pharmacy, Sangli. All animal used after approved by Institutional animal Ethics Committee and procedures were performed in accordance with Institutional animal Ethics Committee approved by CPCSEA (CPCSEA/ac/843/27/12/2004).

2.4 Selection of Dose: In order to decide the dose of plant extract it is essential to go through the toxicity study of the extract according to OECD guidelines. Hence for acute oral toxicity and LD50 was determined according to OECD guidelines 425¹¹. Acute oral toxicity was carried out by up and down regulation method for ethanolic extract of *Vitex negundo* Linn.

2.6 Animal grouping: The male Wistar albino rats were kept at polypropylene cage and further the animals were separated into four groups. Each group contains 6 no. of animals in a one group.

Treatment is given for 8 days.

- ❖ Group 1: Normal (Distilled water 10ml/kg p.o. for 8 days).
- ❖ Group 2: Control (Paracetamol 3gm/kg p.o. for 8 days)
- ❖ Group 3: Standard (Silymarin100 mg/kg+ Paracetamol 3gm/kg p.o. for 8 days)
- ❖ Group 4: Ethanolic extract of *Vitex negundo* Linn. 300 mg/kg + Paracetamol 3gm/kg p.o. for 8 days).

2.7 Hepatoprotective activity in paracetamol induced hepatotoxicity

The drug solution was prepared in Tween 20 solution and administered orally according to body weight of the animal. 200 mg/ml of ethanol extract was prepared and 1ml /100gm of body weight was administered to each animal for 8 days. After treatment, blood samples were removed from all animals by retro orbital puncture method. Serum was separated by centrifugation at 3000 rpm at 4°C for 10 min and used for measurement of various biochemical markers like aspartate and alanine aminotransferase (AST and ALT) activities, alkaline phosphatase (ALP) activity¹³ using commercially available kits. All the biochemical parameters were estimated.

Finally the animals were sacrificed 24h after last dose of Paracetamol administration on the 8th day and dissected the organ liver. Weight of each liver was taken and then histopathology of the liver samples was carried out^{12,13}.

3. STATISTICAL ANALYSIS

The values were expressed as mean \pm SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values <0.05 were considered significant.

4. RESULT

Vitex negundo Linn. is a short plant of mediterranean region and used as herbal remedy of folklore usage. An ethanolic extract of *Vitex negundo* Linn. leaves was prepared by soxhlet extractor. The colour and consistency of extract blackish and semisolid later crystalline. Yield value of extract is 13.40%. Preliminary phytochemical screening of ethanolic extract was carried out and this study showed the presence of carbohydrates, fats, oils, polyphenolic compounds, tannins, alkaloids, flavonoids, irridoid glycosides, steroids, volatile oils, gums, mucilage and saponins.

Acute oral toxicity and LD₅₀ determination of the plant extract was done according to OECD guidelines 423 for deciding the dose of the plant extract.

LD₅₀ Data:

Test type: Limit test

Limit dose: 2000 mg/kg

Assumed LD₅₀: None.

No mortality was observed at limit dose 2000 mg/kg hence LD₅₀ value is greater than 2000 mg/kg of body weight.

Acute oral toxicity:

Body weight changes: No change in body weight.

No mortality is observed at limit dose of 2000 mg/kg.

For evaluation of hepato-protective activity of ethanolic extract of *Vitex negundo* Linn. the following parameters were determined.

1] Biochemical parameters

2] Liver weight

3] Histopathological changes

Experiments were carried to study biologically measurable proof for the hepatoprotectivity of ethanolic extract of *Vitex negundo* Linn. in Wistar albino rats. An experimental liver damage was induced with Paracetamol at the single dose 3gm/Kg of body weight. Blood samples and liver were collected and evaluated biochemically

for ACP, ALT, SGPT and SGOT. Further the liver was histopathologically studied for hepatocyte protection level. The administration of Paracetamol to the rats resulted in a marked increase in ACP, ALT, SGPT and SGOT.

4.1 Histopathology of the Paracetamol Model

Hepatoprotective effect of ethanolic extract of *Vitex nigundo* Linn. was further confirmed by histopathological examination of the livers of normal, control, Paracetamol treated silymarin and Paracetamol plus ethanolic extract treated groups are showed in following fig.1

DISCUSSION

Paracetamol is a common analgesic and antipyretic. Several studies have demonstrated the induction of hepatocellular damage by acetaminophen higher doses in experimental animals and humans¹⁴. For screening of Hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted by the kidney. Moreover, paracetamol hepatotoxicity has been attributed to the formation of toxic metabolites, when a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite N acetyl- p-benzoquinoneimine (NAPQI)¹⁵. Toxic metabolites (N-acetyl-p-benzoquinoneimine) can alkylate and oxidise intracellular GSH, which results in liver GSH depletion subsequently leads to increased lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid and ultimately, liver damage due to higher doses of paracetamol^{16,17}. Reactive metabolites can exert initial cell stress through a wide range

of mechanisms including depletion of glutathione (GSH) or binding to enzymes, lipids, nucleic acids and other cell structures¹⁸. Normally, AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. ALT is more selectively a liver paranchymal enzyme than AST¹⁹.

Administration of 3 gm/kg body weight of Paracetamol to experimental animals for 1day produced statistically significant rise in the enzymes levels, namely SGOT (166.17 KA units/ml), SGPT (111.83 KA units/ml), ACP (4.6752 KA units), ALP (16.4480 KA units) indicating the chemical induced hepatocellular toxicity. The inhibitory effect of the ethanolic extract of *Vitex negundo* Linn. on hepatotoxicity was compared to that of positive control group. The significant protection in the biochemical parameters like SGOT (129.34), SGPT (74.50), ACP (3.0564) and ALP (11.8121) against Paracetamol induced elevations in pretreatment of the animals with the 300 mg/kg p. o. of the ethanolic extract of leaves of *Vitex negundo* Linn. Further there was increase in weight of the liver treated with the Paracetamol (10.69 gm) is seen as compared to the normal (7.02 gm). The treatment with the ethanolic extract of *Vitex negundo* Linn. retains the liver weight (7.12 gm) near to the normal.

Liver section of control rat showing a normal hepatic architecture wall brought about from the central vein. The liver samples of Paracetamol treated rats showed gross necrosis of the centrilobular hepatocytes characterized by gross necrosis, degeneration, karyolysis and eosinophilic infiltration which are significantly prevented by treatment with the ethanolic

extract of *Vitex negundo* Linn. that showed the hepatoprotective activity. The histopathological pattern of the livers of the rats treated with Paracetamol plus extract showed minimal necrosis in centrilobular and regeneration of hepatocytes.

A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties. Administration of leaves of ethanolic extract of *Vitex negundo* Linn. that showed significant hepatoprotective activity; while qualitative phytochemical investigations on the ethanolic extract of *Vitex negundo* Linn. also showed test positive for flavonoids by chemical tests. Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties and was found to be useful in the treatment of liver damage²⁰.

The administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of Paracetamol. The results indicated that the ethanolic extract of *Vitex negundo* Linn. has significant hepatoprotective activity. The obtained results indicated a high degree of protection against the hepatotoxic effect of Paracetamol. The serum enzyme levels were significantly ($P < 0.05$) declined in Paracetamol treated group.

CONCLUSION

The inhibitory effect of the *Vitex negundo* Linn. on hepatotoxicity was compared to that of positive control group. The hepatoprotective effect was further confirmed by histopathological examination of the livers of all groups. Qualitative phytochemical investigations of ethanolic extract of *Vitex negundo* Linn. also showed positive for flavonoids by chemical tests, the flavonoid constituents of the plant possess antioxidant properties and was found to be useful in the treatment of liver

damage. A significant index and values were observed in the acute assays; an effective significant alteration in all biochemical and histopathological sections was observed. It was concluded that ethanolic extract of *Vitex negundo* Linn. leaves were having the hepatoprotective activity which support the hepatic cell protection. However the mechanism of action and the active component which is responsible for the actual hepatoprotectivity is not well known. From the available data's and the experimental results the present study was suggested that the ethanolic extract of *Vitex negundo* Linn. could have a preventive activity towards Paracetamol induced hepatotoxicity in albino rats. However further exploration is needed in order to elucidate the components responsible for hepatoprotection.

5. ACKNOWLEDGEMENT

The authors are thankful to the Principal Prof. D. D. Chougule and Management of Appasaheb Birnale College of Pharmacy, Sangli for providing facility to carry out the work.

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Table 1: Tabulation of response data (i.e. animals showing signs of toxicity including nature, severity, duration of effects and mortality) and dose level for each animal.

Extract	No. of animals used	Limit dose (mg/kg)	Sign of toxicity	Duration of effect	Mortality
Ethanollic extract	Five rats (female)	2000	No	No	No

Table no. 2: Acute oral toxicity results for rats

Extract of <i>Vitex negundo</i> Linn.	Dose (mg/kg)	Short term result
Ethanollic extract	2000	00000

(0 = survived)

Table No.3: Effect of ethanollic leaves of *Vitex negundo* Linn. on Paracetamol induced hepatotoxicity.

Groups	SGOT(units/ml)	SGPT(units/ml)	ACP(KA units)	ALP (KA units)

Normal (Distilled water 10ml/kg)	83.67 ±1.856	67.00 ±2.053	2.6130 ±0.0853	10.2566 ±0.0701
Control (Paracetamol 3gm/kg)	166.17 ±1.347	111.83 ±2.167	4.6752 ±0.0988	16.4480 ±0.6181
Standard (Silymarin 100mg/kg + paracetamol 3gm/kg)	120.17 ±2.056*	73.84 ±1.740*	2.9703 ±0.0711*	11.0038 ±0.2348*
Test (Ethanol extract 300mg/kg + paracetamol 3gm/kg)	129.34 ±0.9545*	74.50 ±1.945*	3.0564 ±0.0942*	11.8121 ±0.0617*

Significance evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t-test control verses all. $P < 0.05$ is considered as criterion for significance. Values are mean \pm SEM, (n=6) SGOT-Serum Glutamate Oxaloacetate Transaminase, SGPT- Serum Glutamate Pyruvate Transaminase, ACP-Acid Phosphatase, ALP- Alkaline Phosphatase



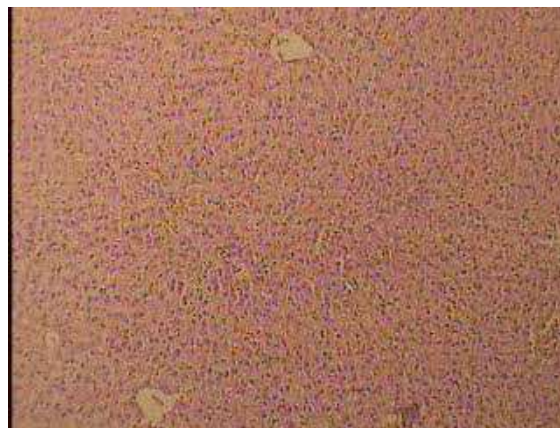
A) Control (Paracetamol (3 gm/kg))



B) Test Ethanol extract (300 mg/kg)



C] Normal group (Distilled water)



D] Standard silymarin (100mg/kg)

Fig. 1: Representative photomicrographs of histopathological changes showing effect of test materials on the rats intoxicated with Paracetamol.