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HEPATOPROTECTIVE ACTIVITY OF *TABERNAEMONTANA DIVARICATA* LINN. AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of hydroalcoholic extract of *Tabernaemontana divaricata* Linn.against paracetamol induced liver damage in rats. The hydroalcoholic extract of *Tabernamontana divaricata* Linn. (600mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the hydroalcoholic extract of *Tabernaemontana divaricata* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Keywords: Tabernaemontana divaricata, Paracetamol, hepatoprotetive, hepatotoxicity

INTRODUCTION

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [1]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders [2]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. *Tabernaemontana divaricata* Linn (Apocyaceae) is an herbaceous perennial weed growing wild in the hot region of India. *Tabernaemontana divaricata* has been claimed to be useful as diuretic, anthelmintic, antidiabetic, expectorant and hepatoprotective in traditional system of medicine [3]. Antimicrobial and cytotoxicity activity [4], diuretic [5], urolithiasis [6] and anti-inflammatory [7] activity of *Tabernaemontana divaricata* has been reported. The study was conducted to establish the traditional use of *Tabernaemontana divaricata* as hepatoprotective against paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals: Male Wistar rats weighing between 150 – 220 gm were used for this study. The animals were obtained from animal house, SRM College of Pharmacy, Potheri, Tamilnadu, India.

The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s.

Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC.

Plant Material: The fresh plants were collected in rural areas of medicinal plants research unit,Tambaram,Chennai Tamilnadu. The plant was identified by a Botanist, and voucher specimen was deposited in the Department of Botany, After authentification, the plants were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of Extract: The coarse powder plant material was extracted with ethanol: water (1:1) by using soxhlet apparatus. The solvent were removed under reduced pressure to get semisolid mass. Standard methods were used for preliminary phyto chemical screening of the extract was performed to know the phytoconstituents in the extract [8], it was found that the extract contains alkaloid, flavonoids, glycosides, steroid, and tannins.

Hepatoprotective Activity: A total of 24 animals were equally divided into 4 groups of six each. Group – I served as normal control received 0.5% (CMC) carboxy methyl cellulose solution [9] (1 ml/kg) once daily for 3 days. Group – II served as paracetamol control, administered with paracetamol (3gm/kg) as single dose on day3. Group –III received, *Tabernaemontana divaricata* extract (200 mg/kg) once daily for 3 days. Group – IV served as reference control, received Silymarin (25mg/kg) once daily for 3 days.

Group III and IV received paracetamol (3gm/kg) as single dose on day 3, thirty minutes after the administration of *Tabernaemontana divaricata* and Silymarin respectively. All the test drugs and paracetamol were administered orally by suspending in 0.5% CMC solution.

After 48h of paracetamol feeding, the blood was collected by direct cardiac puncture under light ether anesthesia and serum was separated for the estimations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) [10,11] and bilirubin. [12]

Statistical Analysis: The values were expressed as mean ± 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.

RESULTS

The results of hepatoprotective activity of hydro-alcholic extract of *Tabernaemontana divaricata* on Paracetamol treated rats are shown in Figure I and II. The hepatic enzymes ALT, AST, ALP and bilirubin in serum was significantly ($P < 0.001$) increased in paracetamol treated animals when compared to control. The hydro-alcholic extract of *Tabernaemontana divaricata* treatments significantly ($P < 0.01$) reversed the levels of AST, ALP and bilirubin ($P < 0.01$) and ALT($P<0.001$) when compared to paracetamol alone treated rats. Silymarin (25 mg/kg) treated animals also showed significant decrease in AST ($P<0.01$), ALT, ALP and bilirubin ($P<0.001$) levels when compared to paracetamol alone treated rats.

DISCUSSION

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses [13]. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome [14] or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity [15,16].

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 AST [17].

Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present higher concentration in cytoplasm. When there is heaptopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage [18].

The elevated level of these entire marker enzymes observed in the group II, paracetamol treated rats in this present study corresponded to the extensive liver damage induced by toxin. The reduced concentrations of ALT, AST and ALP as a result of plant extract administration observed during the present study might probably be due in part to the

presence of flavonoids. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines [19].

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hypatocyte. Decrease in serum bilirubin after treatment with the extract in liver damage induced by paracetamol, indicated the effectiveness of the extract in normal functional status of the liver.

CONCLUSION

The hydro-alcoholic extract has shown the ability to maintain the normal functional statues of the liver. From the above preliminary study, we conclude that the hydro alcoholic extract of *Tabernaemontana divaricata*, is proved to be one of the herbal remedies for liver ailment.

Table I: Effect of *Tabernaemontana divaricata* on serum ALT, AST and ALP of paracetamol induced hepatotoxicity in rats

Treatment	Dose(mg/kg)	ALT (U/L)	AST(U/L)	ALP(U/L)
Control	1 ml/kg	157.66 ± 4.6	74.2 ± 2.92	188.4 ± 3.16
Paracetamol	3gm/kg	227.5 ± 6.8[a]	176 ± 4.7[a]	578.8 ± 8.9[a]
Extract	200 mg/kg	174.25 ± 6.73[a]	107 ± 5.42[b,c]	299.75 ± 11.89[a,c]
Silymarin	25mg/kg	151.4 ± 6.63[c]	122 ± 2.5[c]	414 ± 12.89[c]

Values are expressed as mean ± SEM; [a]P<0.001; [b]P<0.01,[c]P< 0.001
Data were analysed by using one way ANOVA followed by Dunnet's 't' test.

REFERENCES

1. Guntupalli M. J. Ethnopharmacol. 2006; 103: 484–490.
2. Chatterjee TK. Medicinal plants with hepatoprotective properties. In: Herbal Options. 3rd Edn. Books and Allied (P) Ltd. Calcutta. 2000; 135.
3. Kiritikar KR and Basu BD. Indian Medicinal Plants. International book distributors.Dehradun, India. 1996. 2064 – 2065.
4. Dulaly Chowdhury. Fitoterapia. 2002; 73; 92-94.
5. Udupihille M and Jiffry MTM. Indian J Physiol and Pharmacol. 1986; 30: 91-97.
6. Rao SG. Indian Drugs. 1985; 22: 640-643.
7. Vetrichelvan. Diuretic and anti inflammatory activities of *Aerva lanata* in rats. 2000; 62:300-302.
8. Zapesochnaya G. Canthin-6-one and beta-carboline alkaloids from Aerva lanata. Planta Medica. 1992; 58: 192-196.
9. Harborne JB. Phyto – Chemical Methods: A guide to modern techniques of plant analysis" 2nd edn; Chapmanand hall, New York. 1984. 85.
10. Reitman S and Frankel S. Am. J. Clin. Pathol. 1957; 28: 56.
11. Kind PRN and King EJ. J Clin Pathol. 1954; 7: 322.
12. Jendrassik L and Grof P. Biochemische Zeitschrift. 1938; 297: 81-89.
13. Boyd EH and Bereczky GM. Br J Pharmacol. 1966; 26: 606-614.
14. Dahlin D. Proc Natl Acad Sci. 1984; 81: 1327-1331.
15. Moron MS, Depierre JW and Mannervik B. Biochem Biophys Acta. 1979; 582: 67-78.
16. Gupta AK. J. Pharmacol. Toxicol. 2006; 1: 82-88.
17. Shah M. Indian Drugs. 2002; 39: 333-337.
18. Nkosi CZ, Opoku AR and Terblanche SE. Phy.the.Res.2005; 19: 341–345.
19. Gupta AK and Misra N. American Journal of Pharmacology and Toxicology. 2006; 1: 17-20.