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Research Article

HEPATOPROTECTIVE ACTIVITY OF *TRICHOSANTHES CUCUMERINA* L.

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ABSTRACT

Objective: This study investigated the hepatoprotective activity of ethanolic extract of *Trichosanthes cucumerina* L. (EETC), in paracetamol induced hepatotoxic studies carried out in male albino rats and included to produce toxicity of the formulation.

Methods: Albino rats (175 g) were taken and divided into four groups liver necrosis was induced by intraperitoneal injection of paracetamol (3 mg/kg b.w, p.o). The hepatoprotective activity of EETC was evaluated by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP). The serum levels of total proteins and bilirubin were also estimated. The histological studies were carried out to support the above parameters. Here Silymarin was used as standard drug.

Result: Administration of EETC (150 mg/kg, p.o.) markedly prevented paracetamol-induced elevation of levels of SGPT, SGOT, ALP, and bilirubin. The decreased level of total proteins due to hepatic damage induced by paracetamol was found to be increased in EETC treated group.

Conclusion: The results are comparable to the of silymarin. A histopathological study of liver exhibited almost normal architecture when compared with paracetamol treated group. Hence the hepatoprotective activity of EECT was proved.

Keywords: *Trichosanthes cucumerina* L., Serum glutamate oxaloacetate transaminase, Serum glutamate pyruvate transaminase, Sylimarin and Paracetamol.

INTRODUCTION

In the last few decades, there is a tremendous growth in the area of herbal medicine. It is coming popularized in developing as well as in the developed countries due to its natural origin and also considering its lesser side-effects [1]. Herbal remedies provide a lot of drugs for the treatment of internal diseases, which are considered to be stubborn and incurable by other system of medicines.

It aims both to prevention and cure the diseases [2]. In an ancient system, the traditional medicines such as Siddha, Ayurveda, Chinese and Japanese have been approved for the prevention diagnosis and treatment for liver disorders. This effort is to prove scientific insight behind the traditional adoption. Better therapeutic effect, less toxicity, good patient compliance and cost efficiency are important reasons for choosing a drug from natural sources [3]. Ayurvedic and herbal medicinal products contain a combination of a number of chemical compounds that may give the predictable activity in amalgamation.

Trichosanthes cucumerina L. (F. Cucurbitaceae) are used to treat liver disorders. It is one of the ingredients in various Ayurvedic formulations used, especially for the treatment of liver disorders and also in other diseases [4]. This study was to evaluate the hepatoprotective effect of ethanolic extract of *T. cucumerina* L. (EETC) which acute hepatotoxicity was induced by paracetamol treatment.

METHODS

Drugs and chemicals

All reagents used in the procured were analytical grade.

Paracetamol tablet (Sun Pharmaceuticals Ltd.) purchased from a Drugstore. Total bilirubin, direct bilirubin, total proteins, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate were assayed by using kits from Ecoline Diagnostic, New Delhi.

Plant collection

Fresh leaves of *T. cucumerina* L. was collected from the field of Komarapalayam and authenticated by Dr. P. Satyanarayana, Scientist D and Head Office In Charge, Southern Regional Centre, TNAU Campus, Coimbatore. Voucher specimen (No: JKKNCP/0102/12) has been deposited in the Department of Pharmacognosy, JKK Nataraja College of Pharmacy, Komarapalayam, Tamil Nadu, India.

Preparation of plant extracts

The dried leaves of extracted with alcohol and then alcoholic extract of each plant were subjected to solvent extraction.

Ethanol extract of EETC

Fine powdered leaves of *T. cucumerina* L *was* extracted successively with petroleum ether and ethanol (60-80°C) using soxhlet apparatus. The extract was filtered and evaporated to separate solvent and residue. The semisolid residue thus obtained was stored in a desiccator until further use.

Animals

Albino rats either sex weighing between 175±25 g were used in this evaluation. These rats aged between 2 and 2.5 months were procured from animal house located in JKK Nataraja College of Pharmacy, Komarapalayam. They were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic condition under natural light and dark schedule and were fed on a standard laboratory diet. Food and water were given ad libitum.

Experimental protocol

Acute oral toxicity study

Acute oral toxicity study was followed by using Organization of Economic Co-operation and Development (OECD) Guidelines - 423 - Fixed dose procedure .

Acute toxicity study was performed for EETC according to the acute toxic classic method as per OECD (423) guidelines 5, albino rats were used

Table 1: Hepato protective report of EETC

S.No	Particulars	SGPT (U/ml)	SGOT (U/ml)	ALP (U/ml)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (mg/dl)
1.	Control	63.73±5.33	135.03±12.21	170.33±22.22	0.653±0.13	0.31±0.17	6.98±0.19
2.	Paracetamol (3 g/kg)	282.58±10.13*	419.65±25.93*	436.51±27.07*	2.14±0.90*	1.89±0.12*	2.56±0.24*
3.	Sylimarin (100 mg/kg)	68.31±7.44†	171.06±17.75†	175.88±22.84†	0.94±0.17†	0.34±0.16†	5.87±0.25†
4.	EETC (150 mg/kg)	138.11±10.96†	218.83±14.12†	226.10±17.39†	1.09±0.50†	0.79±0.13†	4.9±0.24†

Values are expressed as mean±SD of six animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. N=6 *p<0.01 as compared with control, †p<0.01 as compared with standard, SD: Standard deviation, EETC: Ethanolic extract of *Trichosanthes cucumerina* L., SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase

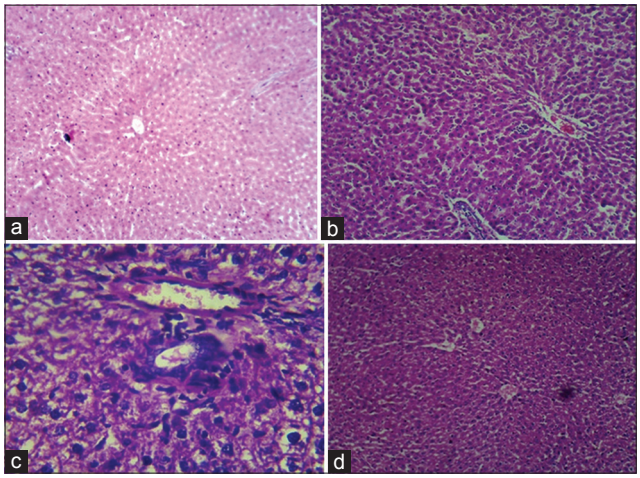

Fig. 1: Histopathology of liver sections, (a) Group I (control), (b) Group II (paracetamol induced), (c) Group III (sylimarin), (d) Group IV (Ethanolic extract of *Trichosanthes cucumerina L.)*

for acute toxicity study. The animals were kept in fasting condition for overnight providing only water, and then the extract was administered orally at the doses of 5, 50, 300 and 2000 mg/kg and observed for 16 days. If the death was observed in 2 out of 3 animals, then the dose administered was concluded as toxic dose. Animals are not shown signs of toxicity, including mortality; nature, severity, and duration of effects up to the dose level of 2000 mg/kg for the extract.

Hepatoprotective activity [4-12]

Group 1: Normal control rats, which received 0.5% carboxy methyl cellulose solution (1 ml/kg) 1 time daily for 7 days.

Group 2: Hepatotoxient, administered with paracetamol (3 g/kg) a single dose on day 7.

Group 3: Standard drug control, receives silymarin (100 mg/kg) once daily for 7 days (standard).

Group 4: Receives EETC (150 mg/kg) once daily for 7 days.

Group 3 and 4 receives paracetamol (3 g/kg) as a single dose on 7th day, after 30 minutes administration of drug extract and silymarin, respectively.

Assessment of hepatoprotective activity [13-19]

In the present study, the hepatoprotective activity was evaluated biochemically and histopathologically. After 24 hrs of drug treatment, the animals were dissected under ether anesthesia from each rat the blood sample was withdrawn from the carotid artery in the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 minutes at room temperature. Serum from blood was separated by centrifugation at 7000 rpm for 10 minutes. The separated serum was used for the estimation of some biochemical parameters such as alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), bilirubin and protein.

For the histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique. Sections (5 µm) of livers stained with eosin and hemotoxylin , observed microscopically for histopathological studies.

RESULTS AND DISCUSSION

The effects of EETC of SGOT, SGPT, alkaline phosphatase, total bilirubin, direct bilirubin and total protein levels in rats with paracetamol-induced liver damage were summarized in Table 1. Administration of paracetamol (3 g/kg body weight, orally) after 24 hrs resulted in a significant (p<0.01) elevation of hepatospecific serum markers such as SGPT, SGOT, total bilirubin, direct bilirubin and total protein in the paracetamol group (Group II) in comparison (Groups III and IV) and paracetamol with the control group (Group I). On administration of the silymarin group (Group III) and EETC (Group IV), the serum markers were restored to the normal levels.

Histopathology analysis

The light microscopy examination of the transverse section of paracetamol treated and extract treated rats livers were shown in Fig. 1a-d. Fig. 1b shows the liver of paracetamol intoxicated rats shows a wide necrosis across the cells. The liver sections of the paracetamol intoxicated rats showed necrosis, ballooning and degeneration in the hepatic plates and loss of the cellular boundaries and karyolysis. Accumulation of neutrophils also found. In the liver section of standard drug sylimarin treated rats, normal hepatocytes and lobular structure are observed in hepatocytes which may be due to the effective mechanisms in Fig. 1c.

Fig. 1d (EETC) shows the histological architecture of treated liver sections with a mild degree of degeneration and necrosis and indicated the moderate effect. The hepatocytes nucleases are at the recovery stage, and there are very minimal numbers of neutrophils. infiltration of lymphocytes and fatty changes.

REFERENCES

1. Padh H, Patel B. Herbal drugs. Curr Sci 2001;81:15.
2. Thaibinh T. Herbal medicine. Indian J Pharm Educ 1998;32:104-6.
3. Chandira M, Jayakar B. Formulation and evaluation of herbal tablets containing *Ipomoea digitata* Linn. Extract. Int J Pharm Sci Rev Res 2010;3:101-10.
4. Nadkarni KM. Indian Materia Medica. Mumbai: Popular Prakashan Pvt. Ltd.; Vol. I . 2002. p. 223-5.
5. Vishnu SN, Gaikwadkuldeep G, Deepak BG. Hepatoprotective activity of *Cassia alata Linn.* in rifampicin indued liver injury in rats. Adv Pharm Toxicol 2010;11:27-36.
6. Ahamed SM, Jayaveera KN. Rao V, Tukura N. Hepatoprotective activity and antioxidant activity of ethanolic extract of *Feronialimonia* leaves on paracetamol induced hepatic injury in rats. Adv Pharm Toxicol 2010;11:47.
7. Gujrati V, Patel N, Rao VN, Nandakumar K, Gouda TS, Shalam MD, Shantakumar SM. Hepato protective activity of alcoholic and aqueous extract of leaves of *TylophoraIndica Linn* in rats. Adv Pharm Toxicol 2009;10(1):136-42.
8. Praveen TK, Dharmaraj S, Bajaj J, Dhanabal SP, Manimaran S, Nanjan MJ, *et al.* Hepatoprotective activity of petroleum ether, diethyl ether, and methanol extract of Scoparia dulcis L. against CCl4-induced acute liver injury in mice. Indian J Pharmacol 2009;41(3):110-4.
9. Slater TF. Biochemical Mechanism of Liver Injury. London: Academic Press; 1965. p. 1-54.

10. Sallie R, Tredger JM, Williams R. Drugs and the liver. Part 1: Testing liver function. Biopharm Drug Dispos 1991;12(4):251-9.
11. Ashok SK, Somayaji SN, Bairy KL. Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. Indian J Pharmacol 2001;33(2):260-6.
12. Galighor AE, Kozloff EN. Biochemistry and molecular biology. In: Essentials of Practical Micro Technique. 2nd ed. New York: Lea and Febiger; 1976.
13. Galighor AE, Kozloff EN. In: Essentials of Practical Micro Technique. 2nd ed. New York: Lea and Febiger; 1976.
14. Das S, Roy P, Auddy RG, Mukherjee A. Silymarin nanoparticle prevents paracetamol-induced hepatotoxicity. Int J Nanomedicine 2011;6:1291-301.
15. Koch RR, Glende EA Jr, Recknagel RO. Hepatotoxicity of bromotrichloromethane – bond dissociation energy and lipoperoxidation. Biochem Pharmacol 1974;23(20):2907-15.
16. Carvalho M, Carvalho F, Bastos ML. Is hyperthermia the triggering factor for hepatotoxicity induced by 3,4-methylenedioxymethamphetamine (ecstasy)? An *in vitro* study using freshly isolated mouse hepatocytes. Arch Toxicol 2001;74:789-93.
17. Shibayama Y. Role of lipid peroxidation in enhancement of endotoxin hepatotoxicity. Exp Toxicol Pathol 1992;44(4):205-8.
18. Shtukmaster S, Ljubuncic P, Bomzon A. The effect of an aqueous extract of *Teucrium polium* on glutathione homeostasis *in vitro*: A possible mechanism of its hepatoprotectant action. Adv Pharmacol Sci 2010;2010:938324.
19. Iriadam M, Musa D, Gumushan H, Baba F. Effects of two Turkish medicinal plants *Artemisia herba-alba* and *Teucrium polium* on blood glucose levels and other biochemical parameters in rabbits. J Cell Mol Biol 2006;5:19-24.