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Phytochemical Investigation and Hepatoprotective Activity of Coscinium Fenestratum Colebr., A Rare Endangered Spp., from Western Ghats of India

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ABSTRACT

Ethanolic extract of stem bark of Coscinium fenestratum was investigated for hepatoprotective activity against CCl4 induced liver damage. Various biochemical parameters were studied to evaluate the hepatoprotective activity of crude extract. Serum total bilirubin, total protein, AST, ALT and ALP were determined to assess the effect of the extract on CCl4 induced hepatic damage. The study was also supported by histopathology of liver sections. Results of this study revealed that the serum markers in animals treated with CCl4 showed elevated concentration indicating severe hepatic damage. Blood samples from the animals treated with ethanolic extract showed significant reduction in the serum markers indicating the effect of plant extract in restoring the normal functional ability of the hepatocytes. Two doses of extract tested, hepatoprotective activity was more pronounced at the dose of 100mg/ml. This study reveals that ethanolic extract of C.fenestratum could afford a significant protection against CCl4 induced hepatocellular injury.

Keywords: Phytochemical Investigation, Hepatoprotective Activity, Cosinium fenestratum Colebr.

INTRODUCTION

Liver diseases have been one of the major causes of morbidity and mortality all over world. Drug induced liver injury (DILI) is one of the most common causative factor that poses a major clinical and regulatory challenge. The manifestations of DILI are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminate hepatic failure. Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Bioactive compounds extracted from plants collectively known as secondary metabolites (Valko et.al. 2007). Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (Prashith et.al 2009). Free radicals contribute to more than one hundred disorders in humans such as cellular damage, cancer, aging, hepatitis and a variety of antioxidants are intimately involved in these diseases prevention. C.fenestratum belongs to Menispermaceae family and it is a critically endangered plant found in Western Ghats of India. Stem and roots of this plant are used as crude drugs for treating liver disorder (Keshavamurthy.1994). The plant is used in traditional system in opthalmopathy, inflammation, ulcers, skin disease, abdominal disorders, fever and general debility as strong anti-feeding in hypotensive activities snake bite, wounds to relieve pain. It has been reported that the main components in the stem of C.fenestratum are protoberberine alkaloids (Prashith et.al 2009). Berberine and its derivative have been reported to exert profound influences on the nervous system including the anti-amnesic effect against memory defect induced by scopolamine. A minor alkaloid, 12,13-dihydro-8-oxo-berberine as well as berberine, oxyberberine, tetrahydroberberine, sitosterol and stigmasterol were reported (Pinho et.al 1992).

MATERIALS AND METHODS

Collection and identification of plant material:

The stem bark of C.fenestratum (voucher Cf/1452), was purchased from local shops of Udupi, Karnataka, identified and authenticated by the corresponding author and a voucher specimen is deposited in Departmental herbaria.

Extraction and phytochemical screening:

Plant material was powdered mechanically and subjected to soxhlation using ethanol for 48hours. Extract was filtered, concentrated in vacuum using rotary flash evaporator and dried in the desiccators. Yield (27.2% w/w) was recorded, stored in refrigerator and preliminary phytochemical screening was done.

Animals: Wistar albino rats of either sex each weighing 150-200 g was procured from the National College of Pharmacy, Shivamogga and maintained at standard housing conditions. The animals were fed with commercial diet and water add libitum during. The study was permitted by the Institutional Animal Ethical Committee with Reg No. 144/1999/CPCSEA/SMG.

EVALUATION OF HEPATOPROTECTIVE ACTIVITY:

Acute toxicity studies: Review of literature revealed that, it is non-toxic up to 2500mg. Acute toxicity study was conducted for plant extract by stair case method (Tushar et.al 2008). Hence 100mg/kg/b.w was selected for the evaluation of anti hepatotoxic activity.

The animals were divided into four groups of six rats each. The animals in group-I served as control and received the vehicle 1ml/kg/day of 1 % w/v gum tragacanth p.o. for 14 days. All the animals of group-II to IV received 0.1ml/kg/day of CCl4 i.p, for 14 days. Group-III animals received the standard drug silymarin at dose of 100mg/kg/day p.o. for 14 days. C.fenestratum extract was administered to the animals of group-IV in the dose of 100 mg/kg/day p.o. for 14 days. The CCl4, silymarin and the extract were administered concomitantly to the respective group of animals.

The animals of all the groups were sacrificed on 14th day under light ether anaesthesia. Blood sample of each animal was collected separately by carotid bleeding and allowed to coagulate for 30min at 37°C and centrifuged at 2500rpm for 10min and was subjected to biochemical investigation viz., total bilirubin, total protein (Purushothaman et.al 1977) and serum ALT, AST and ALP (Balandrin et.al 1988). Results of biochemical estimations were recorded as mean ± SE of six animal in each group. The data was subjected to one way ANOVA followed by Dunnett's test. p values ≤ 0.001 was considered as statistically significant.

Histopathology:

The liver samples were excised from the experimental animals of each group and washed with the normal saline. Ini-

tially the materials were fixed in 10% buffered neutral formalin for 48hours. Then with bovine solution for 6hours and were processed for paraffin embedding. 5µm thickness sections were taken using microtome, processed in alcohol-xylene series and was stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes (Ulican et.al 2003 & Wattanathorn et .al 2006).

The effect of ethanolic extract of C.fenestratum on CCl4 induced liver damage in rats with reference to biochemical changes in serum is shown in Table-II. At the end of 14 days treatment, blood samples of CCl4 treated animals showed significant increase in the levels of total bilirubin (2.380±0.13), ALT (446.70±1.15), AST (2313.7±11.23) and ALP (1406.1±2.68) but the total protein level decreased (5.629±0.26) reflecting the liver injury caused by CCl4. Whereas blood samples from the animals treated with Crude drug of C.fenestratum showed decreased levels of total bilirubin (0.73±0.02), ALT (201.20±1.41), AST (186.13±2.02) and ALP (93.26±1.29) and significant increase in total protein (8.10±0.01), indicating the recovery of hepatic cells (Bessey et.al 1964). The animals group treated with the Sylimarin also showed significant reduction in serum markers and increase in total protein content. Liver sections of the animals treated with extract of C.fenestratum exhibited significant liver protection against CCl4 induced liver damage as evident by the presence of normal hepatic cords absence of necrosis and fatty infiltration (Javasinghe et.al 2003).

DISCUSSION

C.fenestratum extract is effective in fighting oxidative stress due to hepatic damage. Another study to assess the antioxidant activity of the alcoholic stem extract of C.fenestratum was investigated in streptozotocin-nicotinamide induced type-2 diabetic rats. Diabetic rats administered with alcoholic extract of C.fenestratum showed significant increase in the activity of enzymatic antioxidants, such as catalase, glutathione peroxidase, glutathione synthetase, peroxidase and superoxide dismutase and in nonenzymatic oxidants ascorbic acid, ceruloplasmin and tocopherol. The antioxidant activity of C.fenestratum could be due to the presence of berberine and phenolic compounds (Singh et.al 1990 & Peng. et.al 1997)

Immediate injury to the plasma membrane by non-metabolized CCl4 leads to loss of intracellular enzymes and electrolytes and entry of ions from the extracellular environment occurs due to prompt metabolism of CCl4 to the damaging metabolite (trichloromethyl, trichloroperoxyl radical) in the endoplasmic reticulum (Malhotra et.al 1989)

This organelle, the site of the earliest and greatest concentration of the metabolite, suffers peroxidative disruption of membranes and dissociation of the lipoprotein transport system from the lipids, which require transport. The result is steatosis. Subsequently, as the metabolite accumulates and leads to secondary free radicals, peroxidative injury of mitochondrial, lysosomal and plasma membranes occurs and presumably leads to necrosis.

Results indicated that the crude drug provide significant protection against CCl4 induced hapatotoxicity in rats. The CCl4 is biotransformed by the cytochrome p-450 system to produce the trichloromethyl free radicals, which intern covalently binds to cell membranes and organelles to elicit lipid peroxidation (Venukumar et al 2002). Plant secondary metabolites have the ability to induce microsomal enzymes either by accelerating the excretion of CCl4. Phytoconstituents like flavonoids, triterpenoids, saponins (Punitha et.al 2005) and alkaloids (Tran et.al2001) are known to possess hepatoprotective activity. Phytochemical investigations of extract revealed the presence of alkaloids, phenols, saponins, glycosides, flavonoids, triterpenoids, sterols and tannins. C.fenestratum possesses significant protective effect against hepatotoxicity induced by CCl4 which may be attributed to the individual or combined action of phytoconstituents present in it. The component(s) of the extract responsible for this effect however was not investigated. Further investigations are needed for identification of active compounds responsible for hepatoprotective activity. The present finding provides scientific evidence to the ethnomedicinal use of this plant genetic resource by the tribal group of Western Ghats in treating jaundice.

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RESULTS:

TABLE I : Phytochemical Investigation..

Serial No.	Secondary Metabolite	Coscinium fenestratum
1	Flavonoids,	+
2	Alkaloids	+
3	Glycosides	+
4	Steroids	+
5	Quinones	Nd
6	Terpenes	+
7	Tanins	+
8	Saponins	+

Note: + detectable, Nd- undetectable

TABLE II : Effect of the stem bark of Coscinium fenestratum ethanol extract on CCl4 induced hepatotoxicity in rats.

Group (N)	Total Bilirubin (mg/dl)	Total Protein (gm%)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (1% w/v Gum tragacanth p.o.)	0.466±0.01	9.380±0.076	142.24±0.320	57.60±0.432	174.4±1.135
CCl4 (0.1ml/kg/day i.p.)	2.380±0.13*	5.629±0.26*	2313.7±11.23*	1406.1±2.68*	446.70±1.15*
CCl4 + Silymarin	0.630±0.01	8.22±0.02	178.40±1.87	65.13±1.21	188.10±1.26
CCl4 + Ethanol stem bark extract of Coscinium fenestratum	0.73±0.02	8.10±0.01	186.13±2.02	93.26±1.29	201.20±1.41

ANOVA					
F	8.14	10.62	1021.12	100.13	11.05
df	4,25	4,25	4,25	4,25	4,25
P	<0.001	<0.001	<0.001	<0.001	<0.001

N=six animals in each group. Values are expressed as mean ±SE.

REFERENCES

1. Valko,M., Leibfritz,D., Moncol,J., Cronin,M,T,D., Mazur,M., Telser,J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry and Cell Biology, 39: P 44-84. | 2. Prashith,K,T,R., Syed Murthuza., Sudharshan,S,J., Chinmaya,A., Valleesha,N,C. 2009. Phytoconstituents and antioxidant activity of Drypetes roxburghii wall seed, Coscinium fenestratum Colebr and Nardostachys jatamansi DC. Global J. Pharmacol, 3(1): P53-58. | 3. Keshavamurthy,K,R. 1994. Medicinal plants of Karnataka; Karnataka Forest Department (Ed.). Bangalore, India. P223-224. | 4. Prashith,K,T,R., Syed Murthuza, Sudharshan,S,J., Chinmaya,A., Valleesha,N,C., Rajeshwara,A,N. 2009. Central nervous system (CNS) depressant and Analgesic activity of Nardostachya jatamansi DC. Coscinium fenestratum Colebr. J. Pharmacy of Research. 2(11): P1716-1719. | 5. Pinho,M,M,P., Pinto,M,M,M., Kijjoa,A., Phraradai,K., Diaz,J,G., Herz,W. 1992. Protoberberine alkaloids from Coscinium fenestratum. Phytochemistry. 31(4): P1403-1407. | 6. Tushar,K,V., George,S,. Remashree,A,B,. Balachandra,I,. 2008. Coscinium fenestratum (Gaertn.) Colebr. A review on this rare endangered and highly traded medicinal species, Journal of Plant Science 3: P133-145. | 7. Purushothaman,K,K., Sarada,A., Connolly,J,D. 1977. Malabaricones A-D novel diarylnonanoids from Myristica malabarica Lam. Journal of Chemical Society. 5: P587-588. | 8. Balandrin,M,J., Klocke,J,A. 1988. Medicinal, aromatic and industrial materials from plants. In YPS Bajaj (Ed.). Biotechnology in Agriculture and Forestry. Medicinal and Aromatic plants, 4: P1-36. | 9. Ulican,O., Greksak,M., Vancova,O., Zlatos,L., Galbavy,S., Bozek,P., Nakano,M. 2003. Hepatoprotective effect of Rooibos tea (Aspalathus linearis) on CCl4-induced liver damage in rats. Physiol. Res. 52: P461-466. | 10. Wattanathorn,J., Uabundit,N., Itarat,W., Mucimapura,S., Laopatarakasem,P., Sripanidkulchai,B. 2006. Neurotoxicity of Coscinium fenestratum stem, a medicinal plant used in traditional medicine. Food Chem. Toxicol. 44: P1327-1333. | 11. Bessey,O,A., Lowery,D,M., Brock,M,J. 1964. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J Biol Chem. 164: P321-329. | 12. Javasinghe,U,L., Kumarihamy,B,M., Bandara,A,G,. Waiblinger,J., Kraus,W. 2003. Antifeeding activity of some Sri Lankan plants. Nat. Prod. Res. 17 (1): P5-8. | 13. Singh,G,B., Singh,S., Bani,S., Malhotra,S. 1990. Hypotensive action of a stem of Coscinium fenestratum, stem extract. J. Ethnopharmacol. 38: P151-155. | 14. Peng,W,H., Hsieh,M,T., Wu,C,R. 1997. Effect of long-term administration of berberine on scopolamine-induced amnesia in rats. Japan. J. Pharmacol. 74 (3): P261-266. | 15. Malhotra,S., Taneja,S,C., Dhar,K,L. 1989. Minor Alkaloid from Coscinium fenestratum. Phytochemistry. 28(7): P1998-1999. | 16. Venukumar,M,R., Latha,M,S. 2002. Antioxidant effect of Coscinium fenestratum in carbon tetrachloride treated rats. Indian J. Physiol. Pharmacol. 46: P223-228. | 17. Punitha,I,S,R., Rajendran,K., Shirwaikar,A., Shirwaikar,A. 2005. Alcoholic Stem Extract of Coscinium fenestratum regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. Evid Based Complement Alternat Med. 2(3): P375-81. | 18. Tran,Q,I., Adnyana,I,K., Tezuka,Y., Nagaoka,T., Tran,Q,K., Kadota,S. 2001. Triterpene saponins from Vietnamese ginseng (Panax vietnamensis) and their hepatocyteprotective activity. J Nat Prod. 64: P456-461. |