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HEPATOPROTECTIVE ACTIVITY OF *TAGETS ERECTA* AGAINST CARBON TETRACHLORIDE-INDUCED HEPATIC DAMAGE IN RATS

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Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways of growth, fight against disease, nutrient supply, energy provision and reproduction (1). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drugs, which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease (2, 3). Thus, 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. However, there is not much drugs available for the treatment of liver disorders (4, 5). Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4)-induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts (6, 7).

The plant *Tagetes erecta* Linn., locally known as Genda Phul (Marigold) belongs to the family Asteraceae (Compositae). It is a stout, branching herb, native of Maxico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics including Bangladesh and India (8). It is very popular as a garden plant and yields a strongly aromatic essential oil (tagetes oil), which is mainly used for the compounding of high-grade perfumes (9). Different parts of this plant including flowers are used in folk medicine to cure various diseases. Leaves are used as antiseptic and in kidney troubles, muscular pain, piles and applied to boils and carbuncles. The flower is useful in fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints and is also employed in diseases of the eyes (8, 10). They are said to purify blood and flower juice is given as a remedy for bleeding piles and also used in rheumatism, colds and bronchitis (8, 10). Phytochemical studies of its different parts have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids and triterpenoids (11). The plant *Tagetes erecta* has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics,syringic acid, methyl-3,5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallate (10).

EXPERIMENTAL

Plant material

Fresh flowers of *Tagetes erecta* were collected from the adjoining areas of Cuttack, Orissa, India during December-January, 2009 and identified taxonomically in Dept. of Botany, Dhenkanal College, Dhenkanal, Orissa, India. The fresh flowers were washed under running tape water to remove adhered dirt then shade dried and pulverized in a mechanical grinder to obtain coarse powder.

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Preparation of extract

The powdered plant material was extracted with 80% ethanol in water using Soxhlet apparatus. The solvent was removed under reduced pressure, to obtain dry extract which gave yellowish colored sticky residue. A portion of ethanolic extract was suspended in water and fractionated with petroleum ether, chloroform, ethyl acetate and *n*-butanol. All the fractions were dried by distillation under reduced pressure and kept in a dessicator for use.

Animals

Wistar albino rats (150–250g) of either sex were used for the activity study and procured from the animal house, Institute of Pharmacy and Technology Salipur, Cuttack, Orissa. The animals were housed in polypropylene cages and maintained at 24 ± 2^{o}C under 12 h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee, (Regd. No-1053/ac/07/ CPCSEA) as per the requirement of committee for the purpose of control and supervision on animal (CPCSEA) New Delhi.

Preliminary phytochemical screening

The extracts of *T. erecta* Linn. were subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids etc. (12, 13).

Evaluation of hepatoprotective activity

For experimentation, rats were divided into four groups consisting of six animals in each. Group one was kept on normal diet and was injected with 0.2 mL/kg, *i.p.* of liquid paraffin once daily and served as control; the second, third and fourth group received CCl_4 (1875 mg/kg b.w.) by oral route. Moreover, the third and fourth group received silymarin (100 mg/kg; *p.o.*) and extract of *T. erecta* (400 mg/kg, *p.o.*), respectively, once daily for seven days and on the seventh day, CCl_4 was given by oral route 30 min after the administration of silymarin and test drug. Animals were observed for any change in clinical signs and body weights. On the seventh day, blood was collected from the animals, by cardiac puncture, for hematological and biochemical analysis. The blood samples were dispensed into plain tubes and allowed stand for 3 h to ensure complete clotting. The clotted blood samples were then centrifuged at 3000 rpm for 10 min. The clear sera were aspirated and stored frozen for serum biochemical analysis. The indices determined included: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and alkaline phosphatase (ALP). All the hematological and biochemical studies, mentioned above, were carried out in Tarini Patholab, Salipur, Cuttack, Orissa, India (14, 15).

The histopathological studies of the dissected liver were carried in Prasanti Laboratories, Cuttack, Orissa, India. For these studies, sections of liver of each rat were immersed immediately in 250 mL of neutralized 10% (v/v) formalin. The tissues were kept in the fixative for 12 h, dehydrated with serial ethanol cycles (70% to absolute), and then embedded in paraffin. The paraffin-embeded tissue was cut into 5 µm sections. The tissue sections were deparafinized and stained with hematoxylin-eosin. Microscopic examinations were done at the magnification of 400×.

Statistical calculations

The data are presented as the mean ± SEM. Student's *t*-test is used for stastical analysis and $p < 0.05$ is considered significant.

RESULTS

The preliminary phytochemical screening reveals the presence of phytoconstituents which are as follows: general extract – steroids, lipids, terpenoids, flavonoids, saponins; petroleum ether fraction – lipids, steroids, terpenoids; chloroform fraction – triterpenoids, tannins, saponins, sterols; ethyl acetate fraction – flavonoids, lipids, saponins; and *n*-butanol fraction – carbohydrates and saponins.

The effect of ethanolic extract of *T. erecta* on serum transaminase, alkaline phosphatase, bilirubin levels in CCl_4-intoxicated rats are summarized in Table 1. There was a significant ($p < 0.001$) increase in serum ALT, AST, ALP and bilirubin levels in CCl_4-intoxicated group compared to the normal control group. Ethyl acetate fraction of *T. erecta* (EATE) at the dose of 400 mg/kg orally significantly decreased the elevated serum marker enzymes and level of bilirubin almost to the normal level compared to CCl_4-intoxicated group (Table 1).

The histological appearance of the hepatocytes reflect their damage conditions (16). Normal histological appearance is shown in Figure 1A. The hepatocytes of rat treated with a single dose of 1.25 mL CCl_4/kg, showed centrilobular necrosis and extensive fatty change was observed on the midzonal or entire lobe at 24 h after treatment (Fig. 1B). Effective hepatoprotective agent usually protects the hepatocytes from the histopathological

Table 1. Effect of ethanolic extract of *T. erecta* on some biochemical parameters of CCl_4-intoxicated rats.

Parameters	Normal control	CCl_4 control	EATE (400 mg/kg)	Silymarin (25 mg/kg)
ALT(U/L)	56.7 ± 9.2	178.3 ± 14.8^0	128.7 ± 11.2***	75.3 ± 7.7***
AST(U/L)	23.2 ± 2.2	60.5 ± 5.9^0	41.5 ± 5.1**	27.08 ± 3.68**
Bilirubin (mg/dL)	0.12 ± 0.04	0.44 ± 0.05	0.22 ± 0.05*	0.14 ± 0.03*
ALP(U/L)	141.8 ± 8.2	382.9 ± 12.9^0	0.43 ± 0.09	163 ± 10.9***

Values are the mean ± SEM (n = 6). CCl_4 control group compared with normal control group $^0p < 0.001$. Experimental groups compared with CCl_4 control group *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

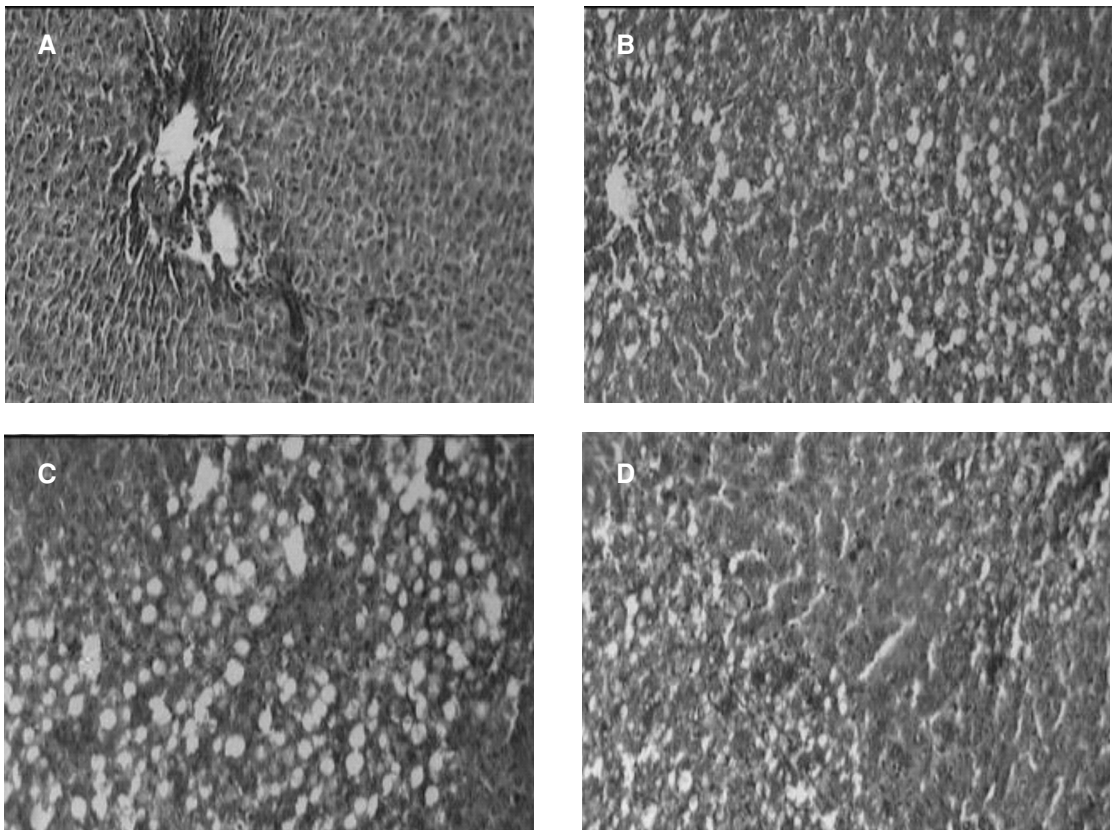

Figure 1. Histopathological appearance of liver cells of ethanolic extract of *T. erecta* on some biochemical parameters of CCl_4 intoxicated rats (staining with H + E, magnification ca. 400×). (**A**) Normal cells; (**B**) liver cells of rats treated with CCl_4 showed centrilobular necrosis and extensive fatty change was observed on the midzonal or entire lobe at 24 h after treatment; (**C**) liver cells of rats treated with CCl_4 and EATE showed a significant recognizability, (**D**) liver cells of rats treated with CCl_4 and silymarin showed no necrosis or fatty deposition but had only minimal portal inflammation

changes caused by toxic agents. Liver tissues of rats treated with CCl_4 and silymarin showed no necrosis or fatty deposition but had only minimal portal inflammation (Fig. 1D) reflecting good protection of the known hepatoprotective drug silymarin. Histological changes in the liver of rats treated with 400 mg/kg of EATE extract and CCl_4 showed a significant recovery except cytoplasmic vascular degenerations around portal tracts, mild inflammation and foci of lobular inflammation (Fig. 1C).

DISCUSSION AND CONCLUSION

Liver injury induced by CCl_4 is the best characterized system of xenobiotic-induced hepatotoxic-

ity and is commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs (16). The changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. It has been established that carbon tetrachloride accumulates in hepatic parenchymal cells and gets metabolically activated by cytochrome P-450 dependent monoxygenases from trichloromethyl free radical ($CCl_3^{\cdot}$). These free radicals alkylate cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides, leading to liver damage. Lipid peroxidation will initiate pathological changes such as depression of protein synthesis (17).

In assessment of liver damage by CCl_4 hepatotoxin, the determination of enzyme levels ALT and AST is more specific to the liver and better parameter for detecting liver damage. Necrosis and liver damage release the enzyme into circulation; therefore, it can be measured in the serum. High levels of serum AST indicate liver damage, due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in similar manner. Therefore, ALT is more specific to liver and a better parameter for detecting liver injury (18).

The serum ALP and bilirubin levels are also related to the status and function of hepatic cells. An increase in serum ALP is due to increased synthesis, in the presence of increasing biliary pressure (19, 20). The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis. Hyperalbuminemia is most frequent in the presence of advanced chronic liver diseases. Hence, a decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the generation process and the production of liver cells. Treatment with CCl_4 increases the levels of total lipids, total triacylglycerols and total cholesterol in liver (21, 22).

Phytoconstituents such as flavonoids, terpenoids and steroids etc., have received considerable attention in recent years due to their diverse pharmacological properties including hepatoprotective and antioxidant activity (14, 15). There has been growing interest in the analysis of certain flavonoids, terpenoids, and steroids stimulated by intense research into their potential benefits to human health. The presence of these phytoconstituents in *T. erecta* reported previously may be responsible for the observed hepatoprotective activity.

REFERENCES

1. Ward F.M., Daly M.J.: Hepatic Disease. in Clinical Pharmacy and Therapeutics. Walker R., Edwards C. Eds, pp. 195–212, Churchill Livingstone, New York 1999.
2. Sharma A., Chakraborti K.K., Handa S.S.: Fitoterapia 62, 229 (1991).
3. Subramonium A., Pushpangadan P.: Indian J. Pharmacol. 31, 166 (1999).
4. Karan M., Vasisht K., Handa S.S.: Phytother. Res. 13, 24 (1999).
5. Chaterrjee T.K.: Medicinal Plants with Hepatoprotective Properties, p. 143, Herbal Options, Books and Applied Allied (P) Ltd., Calcutta 2000.
6. Rubinstein D.: Am. J. Physiol. 203, 1033 (1962).
7. Suja S.R., Latha P.G., Pushpangadan P., Rajasekharan S.: J. Trop. Med. Plants 3, 191 (2002).
8. Kirtikar K.R., Basu B.D.: Indian Medicinal Plants, pp. 1385–1386, Lalit Mohan Basu. Allahabad 1987.
9. Manjunath M.B.L.: The Wealth of India, pp. 109–110, CSIR, New Delhi 1969.
10. Ghani A.: Medicinal plants of Bangladesh. Chemical constituents and uses, 2nd edn., pp.301–302, Asiatic Society of Bangladesh, Dhaka 1998.
11. Faizi S., Naz A.: Helv. Chim. Acta 87, 46 (2004).
12. Kokate C.K., Purohit A.P., Gokhale S.B.: Pharmacognosy. 37th edn., Nirali Prakashan, Pune 2007.
13. Khandelwal K.R.: Practical Pharmacognosy Techniques and Experiments, 2nd edn., pp.149–156, Nirali Prakashan, Pune 2000.
14. Defeduis F.V., Papadopoulos V., Drieu K.: Fundam. Clin. Pharmacol. 17, 405 (2003).
15. Manoj B., Aqueed, K.: Indian J. Exp. Biol. 41, 85 (2000).
16. Prophet E.P., Mills B., Arrington J.B., Sobin L. H.: Laboratory Methods in Histology, 2nd edn., p. 279, American Registry of Pathology, Washington D.C. 1992.
17. Faroon O., De Rosa C.T., Smith L.: Toxicol. Ind. Health 10, 4 (1994).
18. Zimmerman H.J., Seef L.B.: Enzymes in hepatic disease. in Diagnostic Enzymology, Goodly

E.E. Ed., pp. 24–26, Lea and Febiger, Philadelphia 1970.
19. Kamiyamma T., Sato C., Liu J.: Toxicol. Lett. 66, 7 (1993).
20. Recknagel R.: Pharmacol. Rev. 19, 145 (1967).
21. Williamson E.M., Okpako D.T., Evans F.J.: Selection, preparation and pharmacological evaluation of plant material, p. 13, J. Wiley, Chichester 1996.
22. Moss D.W., Butterworth P.J.: Enzymology and medicine, p. 139, Pitman Medical, London 1974.

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