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Screening of *Momordica dioica* for Hepatoprotective, Antioxidant, and Antiinflammatory Activities

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Abstract – The alcoholic extract of *Momordica dioica* roots significantly reduced CCl_4 induced hepatotoxicity in rats upon oral administration (200 mg/kg), as judged from the serum enzyme levels [serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), and alkaline phosphatase (ALP)]. These results were comparable with sylimarin (1.25 mg/kg, p.o.). The alcoholic extract inhibited the formation of oxygen derived free radicals (ODFR) *in vitro* with 4000 μg/ml ascorbic system. The alcoholic extract also significantly reduced carrageenan-induced paw edema when administered orally (200 mg/kg) and the activity was comparable with ibuprofen (200 mg/kg, p.o.).

Keywords – *Momordica dioica,* hepatoprotective activity, antioxidant activity, antiinflammatory activity, plethysmometer, carrageenan

Introduction

One of the causes for ill health is liver diseases. Free radicals damage the liver tissue through covalent binding and lipid peroxidation. Natural antioxidants can protect human body from free radicals (Osawa *et al.,* 1990). In India, plants are being used to cure liver disorders. A good number of Ayurvedic formulations are available in the market for this purpose (Subramonium *et al.,* 1998). *Momordica dioica* Roxb. (Cucurbitaceae) is a perennial dioiceous climber found throughout India (Sastri, 1962). The root extract has been used in ethno medical practices to treat fever and rheumatism (Satyavathi *et al.,* 1987a), bleeding piles (Satyavathi *et al.,* 1987b), inflammation caused by contact with urine of wall lizard (Nadakarni, 1976). Analgesic and anti-catatonic activities of this plant have already been reported (Vaidya *et al.,* 2001). The plant was shown to exhibit antifertility activity (Shreedhara *et al.,* 2001). The present study was aimed at screening and evaluating the ethanolic extract for hepatoprotective, anti-oxidant, and antiinflammatory activities of *Momordica dioica* roots.

Experimental

Materials – *Momordica dioica* roots were collected from the farm house of Mr. Ganapaiah, agriculturist, Konehosur, Chordi Taluk, Shimoga district, Karnataka, India, during October-November 2004 and authenticated by Dr. S.N. Yognarasimhan, Head, Medicinal Plant Division, Regional Research Centre (Ayurvedic), Jayanagar, Bangalore, Karnataka, India by comparing with herbarium specimen No.KRK 4250 and 4905.

The shade dried roots were coarsely powdered and Soxhlet extracted with ethanol (1 : 10 w/v) for 20 h (yield: 12%). The extract was suspended in Tween 80 (0.1%) for screening. Phytochemical screening (Wagner *et al.,* 1984; Harborne, 1973; Stahl, 1969) gave positive tests for carbohydrates, flavonoids, saponin glycosides and negative test for alkaloids, steroids, triterpenoids, tannins, proteins, lignans, chalcones, and irridoids.

Male Wistar rats (150-200 g) procured from the animal house, National College of Pharmacy, Shimoga, India were used for the tests. The animals were maintained under standardized environmental conditions (25 °C - 30 °C, 45% - 50% relative humidity, 12 h dark/light cycle) and were fed with standard rat feed (Hindustan lever limited, Bangalore) and water ad libitum.

Hepatoprotective activity – Animals were divided into four groups of six rats each. Animals in group 1 (normal group) were administered with vehicle (0.1% Tween 80 p.o.), for fifteen days. Group 2 animals (intoxicated control group) received vehicle (0.1% Tween 80 p.o.) for seven days. Group 3 animals (standard group) were administered with sylimarin (1.25 mg/kg, p.o.) for 7

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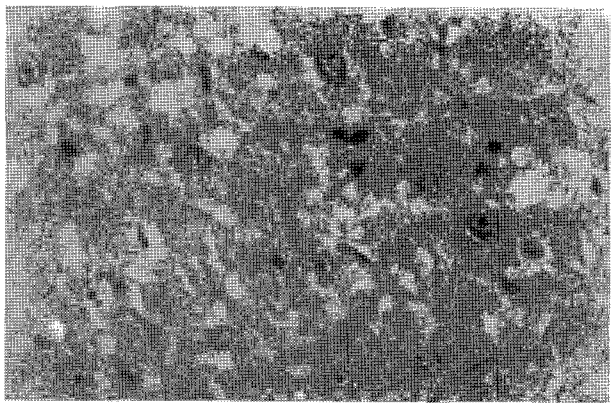

Fig. 1. Section of liver of the control rat with normal hepatocyte, sinusoid, and portal tract.

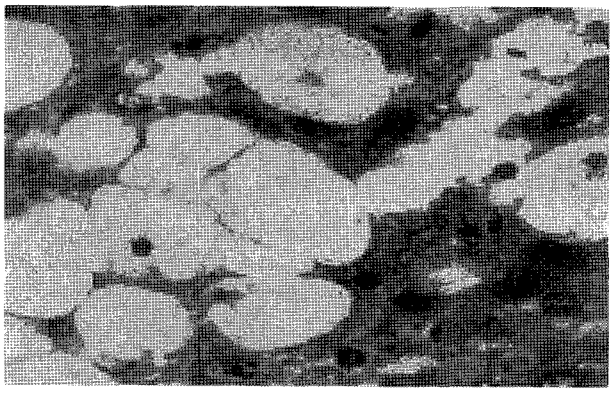

Fig. 2. Section of liver exposed to carbontetrachloride, showing marked fatty change around portal tract and hepatocyte with fatty changes and peripherally pushed nuclei.

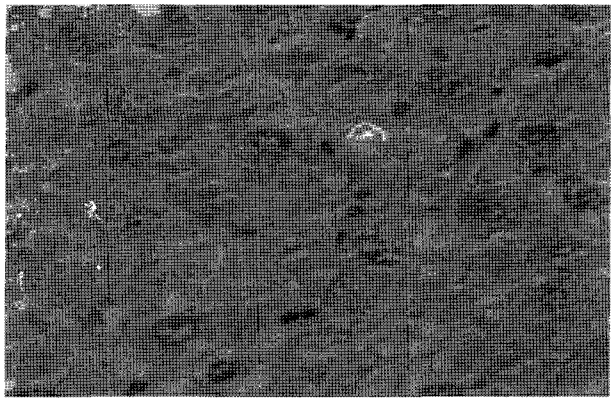

Fig. 3. Section of liver exposed to carbontetrachloride, and treated with silymarin showing normal hepatocyte and mild inflammation.

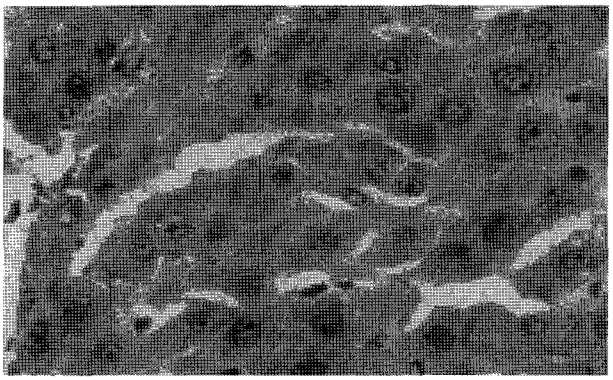

Fig. 4. Section of liver exposed to carbontetrachloride, and treated with ethanolic extract-soluble part showing mild congestion and inflammation.

days. Alcoholic extract of the root (200 mg/kg p.o.) were administered to animals of group 4 (test group) for 7 days.

CCl_4 (0.4 ml/kg, i.p.) in liquid paraffin (Nishigaki *et al.*, 1992) was administered to all the animals belonging to groups 2, 3, and 4 from 8^{th} day to 14^{th} day. Blood sample were withdrawn on 15^{th} day by heart puncture and serum was separated by centrifugation (3000 rpm at 4 °C for 10 min). The liver was immediately removed for histopathological studies. Serum glutamate oxaloacetate transaminases (SGOT) and serum glutamate pyruvate transaminases (SGPT) activities were measured by the method as described by Kind and Chandra (Kind and King, 1980; Chandra *et al.*, 1987). Alkaline phosphatase (ALP) activity was determined (Reitman and Frankel, 1957). The results and the observations of the histopathological studies are shown in Table 2 along with the photographs (Fig. 1 - 4).

Antioxidant activity – The effect on the super oxide radical production was evaluated using diphenyl picryl hydrazide (DPPH) reduction method. The reaction mixture contained different concentrations of ascorbic acid (from 12.5 μg to 400 μg/ml, 0.5 ml), *Momordica dioica* extract (from 12.5 μg/ml to 400 μg/ml, 0.5 ml) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were kept for 15 min and the optical density was measured at 517 nm in UV-visible spectrophotometer (Model 1601 Shimazdu make).

Antiinflammatory activity – Antiinflammatory activity against carrageenan induced acute inflammation (Winter *et al.*, 1962). Animals were divided into three groups of six rats each. Group 1, serving as control received only the vehicle, (0.1% Tween 80, 1 ml, p.o.), group 2 was treated with ibuprofen (200 mg/kg, p.o) and group 3 was treated with *Momordica dioica* extract (200 mg/kg, p.o). All the rats were administered 1 h before the injection of carrageenan. After 1 h, all the groups were injected with carrageenan (0.1%, 0.05 ml, s.c) in the hind paw in the left subplantar region. Paw volume was measured immediately and after 3 h using Plethysmometer.

Data were analysed by Student's t-test. Differences below the 0.05 level ($p < 0.05$) were considered as statistically significant.

Table 1. Effect of the alcoholic extract of *Momordica dioica* roots on DPPH radical scavenging

concentration μg/ml	% scavenging activity
400	77.30
200	76.87
100	68.59
50	65.77
25	63.40

Results and Discussion

Antioxidant study – The ethanolic extract of *Momordica dioica* root was found to scavenge the super oxide generated by ascorbic system at the dose of 12.5 μg/ml (Table 1). Free radical oxidative stress has been implicated in pathogenesis of a wide variety of clinical disorders, resulting usually from efficient natural antioxidant defenses. Potential antioxidant therapy therefore should include either natural free radical scavenging antioxidant enzymes or agents which are capable of augmenting the activity of these enzymes. Reactive oxygen species (ROS) has received considerable attention in the recent past because of its role in several pathological conditions including cancer, aging and atherosclerosis. ROS produced in vivo include super oxide radical $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). $O_2^{\cdot-}$ and H_2O_2 can interact in presence of transition metal ions to yield a highly reactive oxidizing species, the hydroxyl radical (Shinmoto *et al.*, 1992). If human disease is believed to be due to the imbalance between oxidative stress and antioxidant defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements (Bhattacharya *et al.*, 1999). DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, and then losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm (Govindarajan *et al.*, 2003). The extract strongly scavenged DPPH radical and the scavenging was found to be dose dependent.

Hepatoprotective activity (Fig. 1-4) – A remarkable elevation in serum GOT (167.5 ± 2.6), GPT (147.83 ± 3.31), and ALP (488.67 ± 2.46) were found in group 2. The above enzyme activity (85.33 ± 2.76, 63.0 ± 1.29, 423.67 ± 3.18 respectively) was considerably decreased in the animals of group 4 and was comparable with group 3 (Table 2). This was well supported by histopathological studies of the liver which showed corresponding arrest of necrosis with slight fatty and hydropic changes in the group treated with the extract. Simultaneous degenerative changes were also observed along with the nuclei pushed to the periphery in the animals of group 2 (Fig. 1-4, Table 2). Toxins like CCl_4, which produce hepatic necrosis, are believed to do so by forming highly reactive free radicals or other unstable intermediates in the process of metabolic degradation (Rang and Dale, 1987). There is a body of

Table 2. Effect of the alcoholic extract of *Momordica dioica* roots (p.o. for 7 days) on serum enzymatic changes in CCl_4 induced hepatotoxicity in rats including liver histopathology

group	treatment	dose mg/kg	SGOT (u/l)	SGPT (u/l)	ALP (u/l)	histopathology
1	normal (vehicle)	Tween 80 0.1%, 0.2 ml p.o.	50.17 ± 2.57	45.83 ± 2.65	429.83 ± 3.81	normal hepatocyte (Fig 1)
2	intoxicated control	CCl4 0.4 ml/kg, i.p.	167.5 ± 2.60	147.83 ± 3.31	488.67 ± 2.46	degeneration with peripherally pushed nuclei (Fig 2)
3	positive control sylimarin	1.25 p.o.	58.83 ± 1.70*	63.0 ± 1.29*	423.67 ± 3.18*	normal hepatocyte (Fig 3)
4	*Momordica dioica*	200 p.o.	85.33 ± 2.76*	80.67 ± 3.71*	430.33 ± 3.39*	normal hepatocyte with mild congestion (Fig 4)

* $p < 0.05$, Student's t-test (compared to control).

Table 3. Effect of alcoholic extract of *Momordica dioica* roots on carrageenan induced paw edema in rats

treatment	dose (mg/kg, p.o.)	increase in paw volume	% decrease in paw volume
control	------	1.26 ± 0.213	------
ibuprofen	200	0.44 ± 0.004*	65*
Momordica dioica	200	0.61 ± 0.023*	51.6*

* $p < 0.001$, Student's t-test (compared to control).

evidence to show that drugs which can inhibit protein synthesis can protect liver from CCl_4-induced necrosis. The other possible mechanism is the ability of these drugs to inhibit the microsomal enzymes, thereby inhibiting or slowing down the metabolic degradation. Significant recovery from necrosis was observed within 8 days after stopping CCl_4 in animals treated with sylimarin and alcoholic extract of the root of *Momordica dioica*. This was confirmed by restoration of liver enzyme levels almost to control levels.

Antiinflammatory activity – A remarkable reduction in the paw volume was observed in the group treated orally with 200 mg/kg of the extract and was comparable with the standard (Table 3). The process of inflammation generally consists of three phases. Dilatation and increased permeability of small blood vessels resulting in oedema and swelling, emigration of leucocytes from venules and capillaries, cellular infiltration and a general mopping up reaction, and proliferation of fibroblast and synthesis of new connective tissue to repair the injury. A number of mediators have been identified that initiate the early development (first phase) of certain experimentally induced inflammatory processes. These are considered to be released in a sequential manner. Thus, there is an initial release of histamine and 5-hydroxytryptamine (5-HT) producing an increased vascular permeability followed by release of kinins, further contributing to the increased vascular permeability and finally, the prostaglandins and slow reacting substance (SRS) are released to maintain the increased vascular permeability reproduced by histamine, 5-HT and kinins (Smith *et al.,* 1974). The biochemical events accompanying the second phase are not well understood. Many factors are implicated as the regulators of phagocytosis including calcium chemo toxin, leucocyte promoting factor, complement factor (Vinegar *et al.,*1973). As the exudative phase of inflammation subsides, the initial stages of the reparative or third phase are set in motion. The fibroblast, which is the dominant cell in the wounded zone, first proliferates then synthesizes extra cellular material including new collagen fibers and acidic mucopolysaccharides, which are laid down to form the new connective tissue matrix.

From the overall results, we can conclude that *Momordica dioica* root extract possess beneficial action against liver damage induced by CCl_4 through free radical scavenging activity. Certain flavonoids are reported (Di carlo *et al.*, 1999) to exhibit antiinflammatory, antioxidant, and hepatoprotective activities. Phytochemical analysis of the extract shows the presence of carbohydrates, saponins (triterpenoids), and flavonoids and hence we can speculate that these constituents might be responsible for the observed protective effects

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References

Bhattacharya, A., Chatterjee, A., Ghosal, S., and Bhattacharya, S.K., Effect of bioactive tannoid principles of *Emblica officinalis* (amla), *Indian J. Exp. Biol.* **37**, 676-680 (1999).

Chandra, T., Sadique, J., and Soma Sundaram, S., Effect of *Eclipta alba* on inflammation and liver injury, *Fitoterapia* **58**(1), 23-32 (1987).

Di Carlo, G., Mascolo, N., Izzo, A.A., and Capasso, F., Flavonoids; old and new aspects of a class of natural therapeutic drugs, *Life Sci.* **65**, 337-353 (1999).

Govindarajan, R., Rastogi, S., Vijayakumar, M., Rawat, A.K.S., Shirwaikar, A., Mehrorotra, S., and Pushpangadan, P., Studies on the antioxidant activity of *Desmodium gangeticum, Biol. Pharm. Bull.* **26**, 1424-1427 (2003).

Harborne, J.B., Phytochemical Methods, Chapman & Hall Ltd., London, pp. 52-105 (1973).

Kind, P.R.N. and King, E.J., Methods of Practical Clinical Biochemistry, Heinman, London, pp. 23-58 (1980).

Nadkarni, K.M., Indian Materia Medica, Popular Prakashan, Bombay, pp. 807 (1976).

Nishigaki, I., Kuttan, R., Oku, H., Ashoori, F., Abe, H., and Yagi, K., Supressive effect of curcumin on lipid peroxidation induced in rats by CCl_4 or super (60) CO irradiation, *J. Clin. Biochem. Nutr.* **13**, 23-29 (1992).

Osawa, T., Kuvakishi, S., and Namiki, M., Antimutagenesis and Anticarcinogenesis Mechanisms II, Plenum, New York, pp. 139-153 (1990).

Rang, H.P. and Dale, M.M., In Text book of Pharmacology, English language book society, Churchil Livingstone, pp. 69 (1987).

Reitman, S., and Frankel, S., A colorimetric method for the determination of sGOT and sGPT, *American J. Clin. Path.* **28**, 56-63 (1957).

Sastri, B.N., The Wealth of India (Raw Materials), CSIR, New Delhi, pp. 408 (1962).

Satyavathi, G.V., Raina, M.K., and Sharma, M., Medicinal Plants of India Vol. I, ICMR, New Delhi, pp. 317 (1987a).

Satyavathi, G.V., Gupta, A.K., and Tandon, N., Medicinal Plants of India Vol. II, ICMR, New Delhi, pp. 267 (1987b).

Shinmoto, H., Dosako, S., and Nakajima, I., Anti-oxidant activity of bovine lactoferrin, *Biosci. Biotech. Biochem.* **56**, 2079-2080 (1992).

Shreedhara, C.S., Pai, K.S.R., and Vaidya, V.P., Postcoital antifertility activity of the root of *Momordica dioica* Roxb., *Indian J. Pharm. Sci.* **63(6)**, 528-531 (2001).

Smith, J.H., Ford-Hutchinson, A.W., Elliot, P.N.C., and Bolam, J.P., Carrageenin-induced paw oedema in the Rat, *J. Pharm. Pharmac.* **26**, 692-698 (1974).

Stahl, E., Thin Layer Chromatography-A Laboratory Hand Book, Springer International, New York, pp. 206-258 (1969).

Subramonium, A., Evans, D.A., Rajasekharan, S., and Pushpangadan, P., Hepatoprotective activity of *Trichopus zeylanicus, Indian J. Exp. Biol.* **36(4)**, 385-389 (1998).

Vaidya, V.P., Shreedhara, C.S., and Pai, K.S.R., Analgesic and anticatatonic activity of the root of *Momordica dioica* Roxb., *Kuvempu Univ. Sci.* J., **1**(1), 13-18 (2001).

Vinegar, R., Trauz, J.F., and Selph, J.L., Concurrent evaluation and resolution in an acute inflammatory model of rat rrageenin-induced pleurisy, *Proc. Soc. Exp. Biol. Med.* **143**, 711-714 (1973).

Wagner, H., Baldt, S., and Zgainski, E.M., Plant Driug Analysis, Springer Verlag, Berlin/New York, pp. 126-169 and 291-305 (1984).

Winter, C.A., Risley, E.A., and Nuss, G.W., Carrageenan-induced edema in rats, *Proc. Soc. Exp. Biol.* **111**, 544-547 (1962).

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