Indian Journal of Experimental Biology
Vol 46, July 2008, pp. 510-513

Antioxidant and hepatoprotective activity of tubers of *Momordica tuberosa* Cogn. against CCl_4 induced liver injury in rats

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Received 4 October 2007; revised 13 May 2008

Hydro alcoholic extract of tubers of *M. tuberosa* was subjected to preliminary phytochemical screening and evaluated for *in vitro* and *in vivo* antioxidant and hepatoprotective activity against CCl_4 induced liver damage in rats. Pretreatment with 70% ethanolic extract of *M. tuberosa* reversed CCl_4 induced elevation of levels of serum biomarkers to near normal levels, suggesting that the tubers of *M. tuberosa* possess hepatoprotective property and this property may be attributed to the antioxidant property of the plant.

Keywords: Antioxidant, CCl_4, Hepatoprotective, *Momordica tuberosa.*

Hepatic system is the major organ system involved in the metabolism, detoxification and excretion of various endogenous and exogenously administered/ingested substances like xenobiotics, pollutants, etc. This physiological activity of the liver results in the generation of highly reactive free radicals, which covalently bonds with membrane lipids causing lipid peroxidation. Lipid peroxidation alters the membrane permeability and causes tissue damage. Since, the liver is involved in various biochemical reactions, it is prone to be attacked by the free radicals and cell necrosis results. However, inbuilt antioxidant systems like superoxide desmutase (SOD), tissue glutathione (GSH) etc. protect the tissues from free radical attack. Excessive release of reactive oxygen species power over this system resulting in organ damage. Strengthening of inbuilt protective mechanisms or exogenous administration of antioxidants may be useful in protecting the organs. Inspite of phenomenal growth of allopathic system of medicine, synthetic antioxidant/ organsprotectant are not available. Hence, researchers worldwide are engaged in searching for organ protective i.e. hepatoprotective drugs from herbal origin.

The plant *Momordica tuberosa* Cogn. found to be growing abundantly in and around Raichur, is traditionally used as abortifacient[1]. The fruits and tubers of the plant possess hypoglycemic activity[2]. The tubers also have abortifacient activity[3]. Fruits are reported to contain citric acid and maleic acid[4]. The plant is relatively virgin and phytochemical and pharmacological profiles of tubers are incomplete. Since the fruits of *M. tuberosa* are reported to contain Vitamin C[4], a known antioxidant, it is hypothesized that the tubers may also contain antioxidant principles and hence, they have been selected for phytochemical screening and evaluation of antioxidant and hepatoprotective properties.

Materials and Methods

Plant material— The tubers of *M. tuberosa* were collected from the suburban fields of Raichur during January and were identified and authenticated by Prof. Srivatsa, Retired Professor of Botany, L.V.D. College, Raichur. A Herbarium specimen (VLCP-02/05) was deposited in the Department of Pharmacognosy, V.L. College of Pharmacy, Raichur.

Preparation of extract— The coarse powder of shade-dried tubers of *M. tuberosa* was extracted successively with petroleum ether, chloroform, alcohol and water as per Kokate[5]. Similarly, 70% ethanolic extract (TMT) was also prepared after defatting of the drug. The obtained extracts were dried under reduced pressure by using Rota-flash evaporator.

Preliminary phytochemical screening— All extracts obtained were screened for the presence of phytoconstituents by using the qualitative tests[5, 6].

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Animals — Albino rats (150–200 g) and mice (20–25 g) of either sex were obtained from Sri Venkateshwara Enterprises, Bangalore and housed in plastic animal cages in groups of 6 – 8 animals with 12:12 hr of light:dark cycle under standard husbandry conditions. The animals were fed with standard rodent diet and provided water *ad libitum*. The animals were used for the study after one week of acclimatization. The approval of Institutional Animal Ethical Committee was obtained prior to the experiments.

Antioxidant activity — TMT was screened for reducing power activity as per Oyaizu[7]. Different concentrations of TMT were prepared such that each ml contains 20, 40, 60, 80 and 100 µg and 1 ml of each solution was mixed with phosphate buffer (2.5 ml. 0.2 *M*, *p*H 6.6) and potassium ferricynide 1% (2.5 ml). The mixture was incubated for 20 min at 50° C. Trichloroacetic acid 10% (2.5 ml) was added and centrifuged at 3000 rpm for 10 min. Distilled water (2.5 ml) was added to 2.5 ml of supernatant followed by 0.1% ferric chloride (0.5 ml) and absorbance was measured at 700 nm. The increase in the absorbance was directly proportional to the increase in reducing power. The percentage increase in absorbance was calculated.

The measurement of super oxide anion scavenging activity of TMT was performed using the method of Nishimiki, modified by Ilhams *et al*[8]. Various concentrations of TMT solutions were prepared such that each 0.1 ml contains 20, 40, 60, 80 and 100 µg. Nitroblue tetrazolium (NBT) solution (1 ml, 156 µM NBT in 100 m*M* of phosphate buffer, *p*H 7.4) and nicotinamide adenine dinucleotide (NADH) (1 ml, 468 µM in100 m*M* phosphate buffer *p*H 7.4) were mixed with 0.1 ml of various concentrations of TMT. The reaction was initiated by adding 10 µl of phenazine methosulphate (PMS) solution (60 µ*M* PMS in 100 m*M* phosphate buffer, *p*H 7.4) to the above mixture and incubated at 25°C for 5 min. The absorbance was measured at 560 nm. The absorbance was inversely proportional to the super oxide anion scavenging activity. The % decrease in the absorbance in the reaction mixture was calculated.

Hydroxyl radical generation by phenyl hydrazine was measured by 2-deoxy ribose degradation assay as explained by Halliwell and Gutteridge[9]. Solutions of 1 m*M* deoxy ribose and 0.2 m*M* phenyl hydrazine HCl were prepared in 50 m*M* phosphate buffer (*p*H 7.4). Deoxy ribose 0.6 ml (1 m*M*) and 0.4 ml TMT (varying doses 20, 40, 60, 80, and 100 µg) or sodium metabisulphate (25 µg Std.) were mixed and phosphate buffer was added to make the volume to 1.6 ml. The reaction mixture was incubated for 10 min and 0.4 ml of 0.2 m*M* phenyl hydrazine HCl was added and incubated for 1 hr and 1ml each of 2.8% TCA and 1% (w/v) of thiobarbituric acid were added. The reaction mixture was heated for 10 min on a boiling water bath. The tubes were cooled and absorbance was measured at 532 nm. The absorbance of the reaction mixture was inversely proportional to the hydroxyl radical scavenging activity. The % decrease in absorbance was calculated.

Acute toxicity of the TMT was determined by using albino mice as per the OECD guidelines 420 (fixed dose method). The LD_{50} of TMT was found to be 200 mg/kg. Therefore 1/10th (20 mg/kg) and 1/5th (40 mg/kg) doses were selected for further study.

The hepatoprotective activity of TMT was assessed by using the method of Suja *et al*[10]. The albino rats of either sex were divided into 5 groups of 6 animals each and were fasted for 24 hr with water *ad libitum*. The animals of group I and II received 1 ml of saline whereas animals of group III received silymarin (100 mg/kg), IV and V received TMT (20 and 40 mg/kg, po) respectively for 5 days. Animals of group I received 1 ml/kg of liquid paraffin on 2nd and 3rd day, whereas animals of group II, III, IV and V received 2 ml of CCl_4: Paraffin (1:1) subcutaneously on 2nd and 3rd day, 30 min after treatment with vehicle/silymarin/TMT. The blood samples were collected by cardiac puncture and the animals were sacrificed and liver tissues were collected for further analysis.

Blood samples collected were used for the estimation of biochemical markers of hepatic injury viz. serum glutamate pyruvate transaminase (SGPT) [11], serum glutamate oxaloacetate transaminase (SGOT)[11], alkaline phosphatase (ALP)[12], serum bilirubin[13], cholesterol[14,15] and triglycerides[16]. Liver tissue collected were analysed for tissue glutathione (GSH)[17] and lipid peroxidation[18].

Statistical analysis — The results were expressed as mean ± SE. Statistical analysis was performed with One-Way analysis variance (ANOVA) followed by Student's *t* test. $P < 0.05$ was considered to be statistically significant.

Results and Discussion

The preliminary phytochemical investigations have shown the presence of sterols in the pet ether extract, saponins, cardiac glycosides, triterpenoids and bitters

in the alcoholic extract and carbohydrates and constituents of alcoholic extracts in water extract. The phytoconstituents present in the 70% ethanolic extract were similar to that of ethanolic and aqueous extracts. The presence of saponins was confirmed by the persistent Foam test and Haemolysis test as reported by Kokate[5] and Khandelwal.[6]

The antioxidant activity of TMT in three *in vitro* models was studied (Table 1). TMT has shown significant and dose dependant antioxidant activity in all the models of the study. The reducing power activity of TMT at 50 and 100 µg were significantly higher than the standard, sodium metabisulphate. In other models of antioxidant activity (super oxide anion scavenging and hydroxyl radical scavenging activities) the TMT at 100 µg dose showed comparable activity to that of standard i.e. sodium metabisulphate 25 µg. (Table 1).

Administration of CCl_4 enhanced the serum biochemical markers as indicated by their serum levels in group II (Table 2). In addition, CCl_4 depleted the levels of tissue GSH and enhanced the lipid peroxidation. This is in conformity with the earlier report[19]. Pretreatment with TMT (20 and 40 mg/kg) reduced the elevated levels of biochemical markers to near normal levels in a dose dependant manner (Table 2). Similarly treatment with TMT prevented the depletion of tissue GSH and reduced lipid peroxidation (Table 3).

The mechanism of hepatic damage by CCl_4 is well documented. CCl_4 is metabolized by CYP 450 enzyme system to trichlormethyl radical ($CCl_3^{\bullet}$). This in turn reacts with molecular oxygen and gets converted to trichloromethyl peroxy radical. This radical forms covalent bonds with sulfhydryl group of several membrane molecules like GSH leading to their depletion and causes lipid peroxidation. The lipid peroxidation initiates a cascade of reactions leading to tissue necrosis[17]. TMT has significantly scavenged reactive oxygen species as indicated in Table 1. Similarly the test extract significantly reduced the elevated serum biochemical markers of

Table 1— *In vitro* antioxidant effect of 70% ethanolic extract of tubers of *M. tuberosa*

[Values expressed as absorbance are mean ± SE from 3 animals in each group. Figures in parentheses are % increase (+) or decrease (−) over control].

Treatment	Reducing power	Superoxide anion scavenging	Hydroxyl radical scavenging
Control	0.147 ± 0.001	0.540 ±0.0005	0.352 ± 0.0005
Std. (25µg)	0.233 ± 0.002 (+58.50)	0.127 ± 0.002 (-76.08)	0.118 ± 0.003 (- 66.47)
TMT (20µg)	0.161 ± 0.001 (+09.52)	0.313 ± 0.001 (-42.03)	0.294 ± 0.005 (-16.47)
TMT (40µg)	0.195 ± 0.003 (+32.65)	0.261 ± 0.001 (-51.66)	0.237 ± 0.004 (-32.67)
TMT (60µg)	0.236 ± 0.002 (+60.54)	0.224 ± 0.001 (-58.51)	0.187 ± 0.001 (-46.87)
TMT (80µg)	0.260 ± 0.001 (+76.87)	0.190 ± 0.0008 (-64.81)	0.141 ± 0.001 (-59.94)
TMT (100µg)	0.288 ± 0.002 (+95.91)	0.151 ± 0.001 (-72.03)	0.108 ± 0.005 (-69.31)

All values are significant at $P < 0.001$. Std. = sodium metabisulphate

Table 2 — Effect of 70% ethanolic extract of tubers of *M. tuberosa* on biochemical parameters after CCl_4 induced hepatic injury in wistar rats

[Values are mean ± SE from 6 rats in each group]

Treatment	SGPT U/l	SGOT U/l	ALP U/l	Total bilirubin mg/dl	Direct bilirubin mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
Control (saline 0.5 ml po × 5 days)	055.56 ± 3.33	052.21± 5.62	122.29 ± 6.49	0.93 ± 0.03	0.19 ± 0.02	110.88 ± 10.77	171.22 ± 7.20
CCl_4 (CCl_4 2ml/kg sc)	312.38 ± 1.46	318.42 ± 13.54	235.88 ± 8.21	4.89 ± 0.45	1.45 ± 0.22	172.63± 10.35	190.34 ± 7.52
Silymarin (100mg/kg po 5 days) + CCl_4	065.39 ± 8.70*	071.21 ± 6.84*	095.68 ± 9.48*	1.55 ± 0.30	0.25 ± 0.02*	118.25 ± 8.98*	145.48 ± 8.25*
TMT (20mg/kg po × 5 days) +CCl_4	162.23 ± 6.54*	126.13 ± 9.69*	146.89 ± 9.03	2.13 ± 0.21*	0.48 ± 0.01*	157.93 ± 17.07*	177.20 ± 7.18
TMT (40mg/kg po × 5 days) +CCl_4	084.91 ± 2.91*	079.67 ± 4.68*	102.43 ± 4.43*	1.60 ± 0.19*	0.27 ± 0.01*	128.91 ± 5.09*	143.73 ± 3.80*

* $P \leq 0.01$ versus CCl_4 group

Table 3 — Effect of 70% ethanolic extract of *M. tuberosa* on the tissue levels of GSH and lipid peroxidation in the CCl_4 treated rats

[Values expressed as absorbance are mean ± SE from 6 animals in each group. Figures in parentheses are % increase (+) or decrease (−) over CCl_4 treated group].

Group	Dose (mg/kg)	Tissue levels of GSH	Tissue lipid peroxidation
Control(Water)	0.5 ml po	0.905 ± 0.002	0.228 ± 0.005
CCl_4(1:1 in liquid paraffin)	2 ml/kg, sc	0.503 ± 0.005	0.590 ± 0.011
Silymarin + CCl_4	100 mg/kg, po	0.902 ± 0.006*(+79.32)	0.192 ± 0.013*(-67.45)
TMT + CCl_4	20 mg/kg, po	0.729 ± 0.017*(+44.93)	0.267 ± 0.007*(-54.74)
TMT + CCl_4	40 mg/kg, po	0.858 ± 0.007*(+70.57)	0.184 ± 0.01* (-68.81)

* $P \leq 0.01$ versus CCl_4 group,

hepatic injury (Table 2). It is apparent from the present results that the antioxidant property of TMT prevented the formation of trichloromethyl peroxy radical thereby reducing tissue damage. This is further confirmed by the fact that TMT has shown significant restoration of GSH and reduced lipid peroxidation. Therefore the hepatoprotective activity of TMT may be due to its antioxidant potential. Since there are reports that the plants containing saponins possess antioxidant properties[19], the hepatoprotective and antioxidant properties of the test plant may be attributed to the presence of above constituents. There is a claim and report that the plant is an abortifacient[1,3]. Therefore, its use as hepatoprotective agent in pregnancy may be contraindicated.

It may be concluded from the present study that the TMT possesses hepatoprotective property and this may be attributed to the antioxidant principles that are present in the plant. Further studies are needed not only to confirm but also to isolate and characterize the active principle responsible for the activity. In addition, it is necessary to evaluate the toxicological aspect and also its usage in pregnancy.

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