HEPATOPROTECTIVE EFFECT OF ETHANOL EXTRACT OF TUBERS OF *MOMORDICA TUBEROSA* cogn. IN THIOACETAMIDE INDUCED HEPATIC DAMAGE.

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Summary

The study was aimed at assessing the *invivo* antioxidant and hepatoprotective activity of 70% ethanol extract of tubers of *Momordica tuberosa* (TMT) against thiacetamide (100 mg/kg, sc) induced hepatic damage in albino rats. The invivo antioxidant activity was determined by estimating the tissue levels of GSH and lipid peroxidation. The degree of hepatoprotection was assessed by estimating levels of biochemical markers like SGPT, SGOT, Bilirubin (Total and Direct), ALP, and Triglycerides. LD50 studies in rats were carried out up to a dose of 200mg/kg .One fifth and one tenth of maximum dose, 40 and 20mg/kg were used to assess the protective property in thioacetamide model of hepatotoxicity in rats. The extract at a dose of 20 and 40 mg/kg produced significant effect by decreasing the activity or level of serum enzymes, bilirubin, cholesterol, triglycerides and tissue lipid peroxidation, while it significantly increased the levels of tissue GSH in a dose dependent manner. The effects of extract were compared with standard, Silymarin at 100 mg/kg dose. These results suggested 70% ethanol extract of the tubers at 40mg/kg to possess hepatoprotective activity against thioacetamide induced hepatic damage and significant antioxidant activity in rats.

Key words:
GSH, hepatoprotective, lipid peroxidation, *Momordica tuberosa,* thioacetamide (TAA).

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Introduction

Largest organ in the body liver plays a pivotal role in regulating internal chemical environment. It is involved in several vital functions, viz. metabolism, secretion and storage. It has a great capacity to detoxicate toxic substances, xenobiotics, drugs and synthesize physiologically vital principles.

Liver diseases are worldwide problem. Management of liver diseases has become a critical concern in medical science. Very few drugs available in allopathy system of medicine are not free from side effects. So, there is an enormous scope for the herbs in the management of liver diseases. Search for herbs available locally for treating hepatitis is continuing to reduce the cost of treatment. The plant, *Momordica tuberosa* Cogn. (Cucurbitaceae) grows abundantly in the wet fields around Raichur, India. Literature survey of the plant indicated few reports, suggesting insufficient phytochemical as well as pharmacological profile of the plant. Traditionally the plant has been used as abortifacient .[1] There is report citing anti hyperglycemic activity of the plant [2].

The plant *Momordica tuberosa* belongs to family Cucurbitaceae, originating in tropical regions of India and South East Asia as climber. It is very well known by the name Athalkkai in Tamil and Karchikai in Kannada. Literature survey of the plant indicated insufficient work done on it. The fruits of this plant, contains Vitamin C,[3]a known antioxidant and possesses hepatoprotective [4] and anti diarrhoeal property[5].Tubers of the Plant are reported to possess anti implantation activity[6].

Thioacetamide was originally used as fungicide to protect the decay of organs[7].It is recognized as a potent hepatotoxicin and caracinogen in rats[8].Therefore, in present study thioacetamide was used to induce hepatotoxicity in rats for assessing the organ protective property of the extract.

Materials and Methods

Chemicals

Standard Silymarin was obtained from Microlabs,Bangalore, all solvents used were from Nice company,Mumbai. Trichloroacetic acid and Thiobarbituric acid procured from Loba chemie, chemical kits for estimation of SGOT,SGPT,Triglycerides,Total and Direct Bilirubin,cholesterol, serum creatinine were obtained from M/S Accucare company. Alkaline phosphate kit obtained from M/S Span diagnostics and Thioacetamide from S D Fine chemicals, Mumbai. All glasswares used in the study were of Borosil. All other chemicals used were analytical grade. Glass double distilled water was used in all experiments.

Plant material

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

Preparation of extracts

The coarse powder of shade dried tubers of *M.tuberosa* was extracted successively with pet. ether (60-80), chloroform, alcohol and water[9].Similarly 70% ethanol extract of the tubers (TMT) was also prepared after defatting the drug. The obtained extracts were dried under reduced pressure by using Rota-flash evaporator. All extracts obtained were screened for the presence of phytoconstituents by using the qualitative tests.[9,10]

Animals

Albino rats (150-200g) and mice (18-25 g) of either sex were used for the study, obtained from Sri Venkateshwara Enterprise, Bangalore**.** Animals were kept in standard plastic animal cage in groups of 6-8, with 12 hr of light and dark cycle in the institutional animal house. The animals were fed with standard rodent diet and provided water *ad libitum. After* one week of acclimatization the animals were used for further experiments. Approval from the institutional animal ethical committee for use of animals was obtained as per the Indian CPCSEA guidelines prior to the experiment.

Toxicity studies

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

Thioacetamide induced hepatotoxicity [11]

Healthy albino rats were divided into 5 groups of 6 animals each. Group-I and Group II, which served as normal, received normal saline (1ml/ kg) for 9 days. Group III received 100 mg / kg silymarin (standard drug) orally for 9 days. Group IV and Group V received 20 mg/kg and 40 mg /kg TMT (orally). But on 9th day 30 minutes after administration of saline, Silymarin and test extract, animals of group II-V received 100mg/kg thioacetamide (s.c). The animals were fasted for 12 hr before administration of thioacetamide. Blood samples were collected for biochemical analysis and sacrificed the animals after 24 hr of thioacetamide injection.

Biochemical studies

The blood was obtained from all animals by puncturing retro–orbital plexus. Collected blood centrifuged (2000 rpm for 10 mins) to get clear serum and was subjected to various biochemical studies like SGPT [12], SGOT [12], ALP [13,14], bilirubin (total and direct) [15], serum Cholesterol [16] and serum triglycerides [17].

In vivo lipid peroxidation [18]

The degree of lipid peroxide formation was assayed by monitoring thiobirbituric reactive substance formation. Combine 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 umol of lipid phosphate) with 20 ml of TCA-TBA-HCL (Stock solution of 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid and mixed thoroughly).Solution was heated for 15 mins and cooled. Then precipitate was removed by centrifugation at 1000 rpm for 10 mins and absorbance of sample was determined at 535 nm against a blank that contained all reagents but no lipid.

In vivo tissue GSH [19]

Glutathione measurement was performed using a modification of Ellamn procedure. Tissue sample were homogenized in ice-cold trichloroacetic acid (1gm tissue in 10 ml 10%

TCA) in an ultra trux tissue homogenizer. The mixture was centrifuged at 3000 rpm for 10 min. Then 0.5ml of supernatant was added to 2ml of (0.3M) disodiumhydrogenphosphate solution. Later 0.2ml of dithiobisnitrobenzoate (0.4mg/ml in 1% sodium acetate) was added and absorbance was read at 412 nm.

Statistical analysis

Results were expressed as mean of # SEM (n-6). Statistical analysis was performed with one-way ANOVA followed by Tukey-Kramer multiple comparisons test. P value less than 0.05 was considered to be statistically significant.

Results and Discussion

Phytochemical screening

Preliminary phytochemical investigations showed the presence of sterols in the pet ether extract, saponins, cardiac glycosides, triterpenoids and bitters in alcohol extract and carbohydrates and constituents of alcoholic extracts in aqueous extract. The phytoconstituents present in the 70% ethanolic extract were similar to that of ethanol and aqueous extracts. The hydro ethanol extract is known to dissolve most of the polar constituents than ethanol itself. Hence, 70% ethanolic extract of the tubers of *Momordica tuberosa* (TMT) was selected.

Effect of TMT on serum enzymes, bilirubin, cholesterol and triglycerides

Increased levels of SGPT, SGOT, total and direct bilirubin and ALP were observed in thioacetamide treated group. The treatment with TMT restored the elevated levels of biomarker enzymes of hepatitis to the near normal levels in a dose dependant manner. There was no significant rise in total cholesterol and triglycerides levels in thioacetamide treated group. Dose dependent effect was observed with the 70% ethanolic extract. The changes in biochemical markers are shown in table 1 and hepatoprotective effect of 40mg/kg of TMT was comparable to 100mg/kg dose of standard drug Silymarin.

In vivo lipid per oxidation

The treatment with TMT significantly reduced the lipid per-oxidation induced by thioacetamide in a dose dependant manner. Silymarin 100 mg/kg showed 65.91% inhibition, whereas 20 mg/kg of TMT showed 51.86% inhibition and 40mg/kg has shown 63.19% inhibition which was almost near to standard Silymarin. The results are tabulated in table 2.

Table 1 Effect of 70% ethanolic extract of *Momordica tuberosa* tubers on biochemical markers in thioacetamide induced hepatotoxicity

	Biochemical parameters Mean ± SEM						
	SGPT U/L	SGOT U/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Total Cholesterol mg/dl	ALP IU/L	Triglycerides mg/dl
Negative control (1mlDistWater po)	75.78 ± 2.818	87.38 ± 6.121	0.93 ± 0.047	0.28 ± 0.023	112.74 ± 3.585	129.68 ± 5.013	176.94 ± 3.667
Thioacetamide(positive control) (1mldistwater po+100mg/kg sc)	299.58 ± 10.722	403.35 ± 8.667	2.38 ± 0.116	0.61 ± 0.070	190.54 ± 11.128	469.35 ± 7.218	215.87 ± 10.264
Thioacetamide + Silymarin (100mg/kg sc +100mg/kg po)	98.33 ± 4.043***	106.13 ± 6.927***	1.05 ± 0.061***	0.29 ± 0.030***	125.28 ± 5.656***	146.62 ± 7.408***	174.37 ± 9.903**
Thioacetamide+70%ethanol extract(100mg/kgsc+20mg/kg po)	147.39 ± 0.208***	143.78 ± 0.434***	1.16 ± 0.061***	0.44 ± 0.018	161.38 ± 0.393**	193.72 ± 0.063***	195.16 ± 0.078
Thioacetamide+70%ethanol extract(100mg/kgsc+40mg/kg po)	100.06 ± 0.385***	109.11 ± 0.215***	1.02 ± 0.025***	0.28 ± 0.01***	127.12 ± 0.084***	151.06 ± 0.215***	179.28 ± 0.153**

Values are the mean ± SEM of six rats/treatment.
Significance *P<0.05, **P <0.01 and *** P<0.001 compared to Thioacetamide treatment.

Table 2 Effect of 70% ethanolic extract of tubers of Momordica tuberosa on invivo lipid peroxidation in Thioacetamide induced Hepatotoxicity.
[Values expressed as absorbance are mean ± SEM from 6 animals in each group. Figures in parentheses are % increase (+) or decrease (−) over Thioacetamide treated group].

Treatment	Absorbance Mean ±SEM	% Inhibition
Negative control (1 ml Distilled water)	0.237 ± 0.008	---------
Positive control Thioacetamide(100mg/kg sc)	0.754 ± 0.008	---------
Thioacetamide+ Standard(Silymarin) (100mg/kg s.c.+100mg/kg p o)	0.259 ± 0.003***	65.91
Thioacetamide+ 70%Ethanolic extract (100mg/kg s c + 20mg/kg po)	0.363 ±0.0008***	51.86
Thioacetamide +70%Ethanolic extract (100mg/kg s c + 40mg/kg po)	0.279 ± 0.001***	63.19

Significance***P<0.001, compared to Thioacetamide treatment.

In vivo GSH

There was a marked depletion of GSH level in thioacetamide treated groups. Silymarin 100 mg/kg increased tissue GSH by 77.47%. Treatment with 70% ethanolic extract showed a dose dependent increase in the levels of GSH. However, at both doses, the GSH level increase was less compared to the standard silymarin. Effect of TMT on GSH levels is shown in table 3.
Estimation biochemical markers such as SGPT, SGOT, ALP, bilirubin, total cholesterol and triglycerides serve to indicate the extent of liver damage. Levels of these parameters are elevated with thioacetamide administration due to its hepatotoxic nature and treatment with the test extract restored them to the near normal. The effect of test extract was comparable to standard, Silymarin at 100 mg/kg.

Here the elevation of SGOT level is more than SGPT as the former is also present in nephron, adding to SGOT released with TAA treatment[20]. So, the release of SGOT from nephron to serum causes its increased level. Hence, SGPT, which is specific only to the liver function, is a better parameter for detecting liver damage [21]. There was no significant rise in total cholesterol and triglycerides level. But, the extract showed significant reduction in their levels too. Thioacetamide may increase the synthesis of fatty acids and decrease the release of hepatic lipoproteins. Chronic thioacetamide exposure produces cirrhosis in rats [22] and is metabolized by liver CYP 450 $2E_1$ to thioacetamide-5-oxide, a potential hepatotoxic [23,24]. The thioacetamide-5-oxide is responsible for the change in cell permeability, increased intracellular concentration of calcium, increase in nuclear volume and enlargement of nucleoli and also inhibits mitochondrial activity which leads to cell death [25]. In vivo administration of thioacetamide to rodents results in cell death in centrilobular zones both by apoptosis and necrosis. The cellular changes induced by apoptosis occur after a cascade of cell signaling and caspase mediated events and are triggered by two major pathways: extrinsic and intrinsic [25]. The extrinsic pathway implicates death ligands such as Fas ligand, TNFα, TRAIL and their receptors. The intrinsic pathway includes apoptotic

stimuli induced by cytotoxic drugs or oxidative stress which target mitochondria [26]. In addition to this, the available reports suggest cirrhosis associated with an increased extent of lipid per oxidation with long term use of TAA [27]. Enhanced lipid per oxidation leads tissue damage and failure of antioxidant defence mechanism. GSH widely distributed in cells and present in high concentration in liver (Aftab *et al*., 2002) protects the cell against free radical, peroxides and other toxins. So the depletion of GSH level in tissue subsequently leads to tissue damage. In this study, TAA treatment produced the depletion of GSH level. But, the extract significantly increased the level of GSH and decreased the extent of lipid peroxidation. These results suggest possible free radical scavenging and anti oxidant property of TMT and hence, its hepatoprotective property. The activity may be attributed to the presence of saponins and triterpenoids in the extract. Further investigation is on in the lab to isolate, characterize and screen the active principles possessing antioxidant and hepatoprotective property.

Table3 Effect of 70% ethanolic extract of tubers of *Momordica tuberosa* on invivo GSH levels in Thioacetamide induced Hepatotoxicity.
[Values expressed as absorbance are mean ± SEM from 6 animals in each group. Figures in parentheses are % increase (+) or decrease (−) over thioacetamide treated group].

Treatment	Absorbance Mean ±SEM	% Inhibition
Negative control (1 ml Distilled water)	0.921 ± 0.007	---------
Positive control Thioacetamide(100mg/kg sc)	0.506 ± 0.010	---------
Thioacetamide+ Standard(Silymarin) (100mg/kg s.c.+100mg/kg p o)	0.898 ± 0.009***	77.47
Thioacetamide+ 70%Ethanolic extract (100mg/kg s c + 20mg/kg p o)	0.792±0.009***	56.52
Thioacetamide +70%Ethanolic extract (100mg/kg s c + 40mg/kg p o)	0.886 ±0.003***	75.09

Significance ***P<0.001, compared to Thioacetamide treatment.

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