Original Article

GC-MS ANALYSIS AND ANTIULCER ACTIVITY OF ETHANOL EXTRACT OF TUBERS OF *MOMORDICA TUBEROSA* COGN. (CUCURBITACEAE) IN RATS

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ABSTRACT

70% ethanol extract of tubers of *Momordica tuberosa* (TMT) showed the presence of saponins and triterpenoids. GC-MS analysis of the extract showed presence of 42 compounds with Isopulegol, a monoterpene and a steroid, Androstane as main constituents. Many fatty acid derivatives like myristic acid, margaric acid, oleic acid etc. were also present. The extract was then tested for its antiulcer activity using three different experimental models of ulcers in rats viz., aspirin, ethanol and pyloric ligation induced ulcers in rats. The LD_{50} in rat was 200mg/kg. One fifth and one tenth of maximum dose i.e.40 and 20mg/kg were used to assess the antiulcer activity. In pylorus ligation model, parameters like ulcer index, volume of gastric juice, free acidity, total acidity and pH were measured. The extract showed antiulcer activity in a dose dependant manner. The 40mg/kg dose reduced ulcer to an extent of 95% in aspirin model and 82% in pyloric ligation method. The results suggest the anti-ulcer activity of tubers of *Momordica tuberosa,* probably due to its antioxidant nature.

Key Words: Anti-oxidant, Aspirin, Ethanol, Free acidity, Pyloric ligation, Total acidity.

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INTRODUCTION

Precise etiology of ulcer is not clear and a satisfactory regimen remains elusive. Ulcers are believed to be imbalance in offensive factors like acid and pepsin and defensive factors like mucin secretion, tissue glutathione (GSH), cell proliferation, prostaglandins (Goel & Bhattacharya,1991 and Itoth & Guth, 1985). Peptic ulcer being one of the most

rampant gastrointestinal disorders continues to occupy the key position in concern of both, clinical practitioners and researchers. As a consequence, many synthetic drugs are explored offering newer and better options for treatment of peptic ulcer. However, these drugs differ from H_2 receptor antagonists, proton pump inhibitors or cytoprotective agents such as sucralfate. Unfortunately, most of these drugs confer simpler to severe side effects like arrhythmias, gynaecomastia, enterochromaffin like cell (ECL) hyperplasia and hematopoietic changes (Akhtar et al., 1992). Thus, there is an urgent need to search an indigenous drug with fewer side effects to have a better and safer alternative for the treatment of peptic ulcer. In this context, extensive studies and research has been undertaken which mainly focuses on search of antiulcer agents of plant origin. The participation of reactive oxygen species in the etiology and pathologies of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer has been reported earlier (Repetto & Llesuy, 2002). Several studies have shown alterations in the anti-oxidant status following ulceration, implying that free radicals may be associated with ethanol induced gastric mucosal damage in rats (Pihan et al., 1987 & Mizui et al., 1987). Drugs with multiple mechanisms of protective action, including antioxidant properties, may be beneficial in minimizing tissue injury in human disease (Barry, 1991). The plant *Momordica tuberosa* Cogn. (Cucurbitaceae) growing abundantly in and around Raichur is traditionally used as abortifacient (Kirtikar & Basu, 1991). Fruits of the plant possess hypoglycemic activity (Kameshwar Rao et al., 2003).The tubers have anti implantation activity (Koneri et al., 2006). Fruits contain citric acid and maleic acid (Parvati & Kumar, 2002) and are antioxidant and hepatoprotective (Swamy & Jayaveera, 2007). We have earlier reported the in vitro and in vivo antioxidant and hepatoprotective property of the tubers in rats in CCl_4 induced toxicity (Kumar et al., 2008). Since the fruits of *M.tuberosa* are reported to contain Vitamin C (Parvati & Kumar, 2002), a known antioxidant, it was hypothesized that the tubers may also contain antioxidant principles and may possess antiulcer property. Hence, they were selected for phytochemical screening and evaluation for antiulcer 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, ethanol and water (Kokate, 1996). Similarly, 70% ethanol 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 (Kokate, 1996 & Khandelwal, 2005).

GC-MS analysis of 70 % ethanol extract of tubers

The GC-MS analysis of 70% ethanol extract was carried out in a GC-MS Model GC-MS-QP2010 Plus. Make: SHIMADZU gas chromatograph fitted with ZB-624 30 m X 1.4 mm ID 0.25µm film thickness or equivalent column. Carrier gas was helium with a flow rate of 2.5 mL/min; column temperature initially was at 120°C for 2 min. Then rose to 250°C at the rate of 10°C per minute maintained at 250°C for 20 minutes; injector temperature was 220°C, detector temperature 260°C, Volume injected was 1µL with liquid injector of 70% ethanol extract in ethanol (1g in 5 mL ethanol); The Mass spectra operating parameters were, ionization potential, 70 eV; ion source temperature; 250°C, Solvent delay 3.0 min , Program Run time: 31 minutes and scan range 30-350 amu, EV voltage 3000 volts.

The identification of components present in the 70% ethanol extract was based on direct comparison of the retention times and mass spectral data by computer matching with the commercial library Wiley 229.

Animals

Albino rats (150 – 200 g) and mice (20 – 25 g) of both sex were obtained from Sri. Venkateshwara Enterprises, Bangalore and housed in plastic animal cages in groups of 6 - 8 animals with 12:12 h 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.

Ethanol induced ulcer

The ulcers were induced by ethanol (Luis et al., 2002). Five groups of six animals each were fasted for 18h and water was given *ad-libitum*. The first group animals served as negative control and received saline. The animals of second group served as positive control and received ethanol 100% (1mL/200g) for inducing gastric ulcers. The animals of third group orally received standard drug, lansaprazole (8 mg/kg). Animals of group four and five received the TMT at 20 mg/kg and 40 mg/kg dose po, respectively. All the animals in group three, four and five received ethanol 100% (1mL/200g) after 60min of respective treatments. The animals were sacrificed by cervical dislocation after one hour of ethanol administration and stomach was incised along the greater curvature and examined for ulcers. The number of ulcers per stomach was noted by using simple microscope and score was given (Kulkarni, 2002) viz. 0 for normal coloured stomach, 0.5 for red colouration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcer > 3 but < 5mm and 3 for ulcer > 5mm.Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated.

Pylorus-ligated rats' method

In the pylorus ligation method (Shay, et al., 1945), albino rats were divided into five groups of six animals each and fasted for 18h. Care was taken to avoid coprophagy. The treatment schedule and grouping was similar to ethanol model. The pyloric ligation was performed under light ether anesthesia 60 min after respective treatments. The animals were not given water during the post operative period. After 4h stomachs were dissected out, contents were collected into tubes for estimation of volume, acid content and pH. The stomachs were cut open along the greater curvature and ulcer index and percentage of protection were determined as mentioned in ethanol model.

Estimation of Acid content and pH

The gastric juice was collected 4h after pylorus ligation; volume was measured and centrifuged for 5 min at 2000 rpm. The pH of the gastric juice was recorded. Then the contents were subjected to analysis for free and total acidity. Free acidity was determined by titrating with 0.01mol/L NaOH, using Topfer's reagent as indicator until solution turns to yellowish orange and noted the volume of NaOH (this volume represents free acidity) then titration was continued with phenolphthalein as indicator until red tinge as end point. This volume corresponds to total acidity**.**

Aspirin induced ulcer method

In the aspirin-induced ulcers (Banerjee et al., 2005), five groups of albino rats (150–175 g), with each group consisting of six animals were used. The first group served as a control group and received only saline for 8 days, the second group served as positive control administered with Aspirin (200mg/kg), the third group served as the standard, received Lansaprazole (8 mg/kg), fourth and fifth group received TMT 20 and 40 mg/kg. The groups III to V received lansaprazole, TMT 20 and 40 mg/kg for 8 days orally respectively. After 8 days of treatment animals were fasted for 24 h and ulcer was produced by aspirin 1 % (200 mg/kg, in CMC suspension) po. Animals were sacrificed after 4 h of treatment under over dose of anaesthesia with ether and stomach was dissected out. The stomach was cut open along the greater curvature and ulcer index and percentage of protection were determined as mentioned in other models.

Statistical Analysis

Data was shown as the mean ± SEM and analysed by one way ANNOVA followed by students'' test. The level of significance for all experiments was $P<0.05$*, 0.01** and 0.001***.

RESULTS AND DISCUSSION

The preliminary phytochemical investigations showed the presence of sterols in the pet ether extract, saponins, cardiac glycosides, triterpenoids and bitters in the ethanol extract and carbohydrates and constituents of ethanol extract in aqueous extract. The phytoconstituents present in the 70% ethanol extract were similar to that of ethanol and aqueous extracts. The presence of saponins was confirmed by the persistent foam test and haemolysis test. GC-MS analysis of the extract showed presence of fragments like

cyclopentane acetic acid, myristic acid, margaric acid, a monoterpene isopulegol, thymin and arachidic acid. A steroid of androstane derivative was present up to 2.88% and a phenol derivative, 2-methoxy-4-vinylphenol was present at 1.13%. A complete analysis report is given in table 1.

Pretreatment with 70% ethanol extract of tubers of *M.tuberosa* cogn. protected the animals from alcohol, aspirin and pylorus ligation induced gastric ulcerations. Gastro protection offered by TMT at 40 mg/kg dose was almost equivalent to lansaprazole 8mg/kg in aspirin model. The percentage of reduction in ulcer index and percentage of protection at different doses is tabulated in table 3 and reduction in both total and free acidity together with pH changes in table 2.

Table 1 - GC-MS analysis of 70% ethanol extract of tubers of *M. tuberosa*

No.	Retention	Name	% Area	Time (Min)
		I. Aliphatic compounds		
1.	9.131	Methyl isovalerate		0.36
2.	10.32	Methyl isobutytrate		0.30
3.	10.75	1-Decanol,2-Hexyl		0.61
4.	11.06	Dichloroacetic acid,4-Hexadecyl ester	0.43	
5.	12.54	Oleic acid		1.76
6.	13.14	Chloroacetic acid,tetra decyl ester	0.62	
7.	13.39	Ethyl undecenoate		1.42
8.	13.96	Myristic acid		0.75
9.	14.25	Stenol		0.38
10.	14.74	Margaric acid	0.43	
11.	15.02	Pentadecanoic acid		0.99
12.	15.71	Arachidic acid	2.67	
13.	16.96	6-tetradecanesulfonicacid,butyl ester	3.05	
14.	18.02	Pentafluoropropionic acid,heptadecyl ester	0.67	
15.	18.35	Ethyl linoleate	2.67	
		II.Aromatic compounds, plant acids and esters		
1.	5.364	Cyclopentane acetic acid	2.48	
2.	6.579	2-n-Propylthiane	2.90	
3.	8.864	2-Furanocarboxyaldehyde,5-hydroxy methyl	1.72	
4.	9.819	1-Ethyl-2-Pyrrolidinone	0.39	
5.	10.46	Ethanol 2-(3,3-dimethylcyclohexylidene)-(Z)-	0.36	
6.	11.15	1-Isopropenyl-3-Propenyl-Cyclopentane	0.60	
7.	11.33	Bicyclo[2.2.1 Heptane,2-(1-Buten-3-yl)-3-Deutero	0.20	

8.	11.82	2,5,Cyclohexadiene-1,4-dione,3-hydroxy-2-methyl-5-(1- methylethyl)	1.73	
9.	13.07	1H-Cycloprop[e]azulen-4-ol-decahydro-1,1,4,7-Tetramethyl-[1ar-(1a alpha,4 alph	0.57	
10.	13.65	Ethyl N-(o-anisyl) formimidate	0.66	
11.	14.67	delta.2-tetrazaboroline,1,4,5,triethyl	0.76	
12.	19.38	Cyclohexanone,5-ethenyl-5-methyl-4-(1-methyl ehtenyl)-2-(1-methylethylidene)-c	6.12	
		III. TERPENE DERIVATIVES		
1.	14.89	Linalool oxide	0.70	
2.	19.12	(-)-isopulegol		10.62
		IV. PHENOLIC COMPOUNDS		
1.	8.754	2- Methoxy-4-vinylphenol	1.13	
		V. HYDROCARBONS		
1.	15.31	(trans)-2-nonadecene	0.91	
2.	22.20	n-Pentatriacontane		1.13
3.	23.67	n-octadecane	4.65	
		VI. STEROID COMPONENTS		
1.	11.66	Androstan-17-one,3-ethyl-3-hydroxy-(5 alpha)-	2.88	
		VII. CYCLOOLIGISILANES		
1.	16.17	Hexa-T-butyl cyclotrisilane		15.30
		VIII. PYRIMIDINE BASE		
1.	5.716	Thymin	1.14	

Table 3– Effect of *M.tuberosa* on gastric secretion following pyloric ligation induced ulcer in rats

Treatment	Dose	Volume (mL)	pH	Total acidity (mol/L)	Free acidity (mol/L)
Control	--	8.28±0.573	2.28±0.79	123.97±4.581	98.65±1.63
Lansoprazole	08 mg/kg po	1.91±0.197***	5.95±0.32***	63.37±2.24***	55.20±1.50***
TMT	20 mg/kg po	5.21± 0.207	2.5±0.093	78.39±11.08***	67.08±5.02**
TMT	40 mg/kg po	2.71±0.247	2.4±0.045	72.50±5.55***	75.35±10.72*

Data represents mean ± SEM of six rats per treatment.TMT: 70%ethanol extract of tubers. Level of significance *P< 0.05 v/s control, **P< 0.01 v/s control, ***P< 0.01 v/s control

Table 3: Effect of *M.tuberosa* on induced gastric ulcers in rats

Treatment	**Dose**	**Ethanol ulcers (1 mL/kg)**		**Aspirin ulcers (200 mg/kg)**		**Pyloric ligation ulcers**	
	mg/kg	Ulcer index	% protection	Ulcer index	% protection	Ulcer index	% protection
Control	---	3.083±0.611	---	3.75 ±0.629	---	2.83±0.380	--
Lansaprazole	8mg/kg	0.084±0.083	97.77	0.084±0.083	97.77	0.084±0.083	97.77
TMT	20mg/kg	1.25±0.512	59.45	0.333±0.333***	91.12	1.583±0.083*	44.06
TMT	40mg/kg	1.17±0.641	62.15	0.167±0.105***	95.54	0.5 ±0.316***	82.33

Data represents mean ± SEM of six rats per treatment.TMT: 70%ethanol extract of tubers.* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (Vs. Control) respectively.

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NSAIDS are known to induce peptic ulcer by not only denaturing mucous glycoprotein but also by free radical formation (Shirwaikar et al., 2006). Similarly, ethanol is also known to produce free radicals and induce peptic ulcer (Soll, 1990). Pyloric ligation induced ulcers are due to increased presence of acid and pepsin in the stomach (Pillai et al., 1982). Also, high turnover rate of mucin forms a protective barrier to epithelial digestion. The action can also be explained in light of the hydroxyl radical scavenging action of mucin (Grisham et al., 1987).

Although in most of the cases, the etiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (Goel & Bhattacharya,1991 and Itoth & Guth, 1985).To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury in vivo (Akhtar et al., 1992). Lipid peroxidation is a free radical mediated process, which has been implicated in the variety of disease states. It involves the formation and propagation of free radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids, which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore membrane lipids are susceptible to proxidative attack (Cheesman, 1993). We have earlier reported (Kumar et al., 2008) a significant decrease in lipid per oxidation by 70% ethanol extract of *M. tuberosa* in the CCl_4 model of hepatotoxicity. In the present study, the ethanol extract of tubers of *M.tuberosa* has shown significant protection against ulcers in dose dependant manner in all the experimental models of ulcers. In addition, gastric volume and total acidity were reduced significantly but, to our surprise, without much change in pH. Research is on to ascertain the reasons for the same.

Though we have not studied the active principles responsible for its antiulcer property, it is likely that antioxidant principles, saponins and triterpenoids present in the ethanol extract of *M.tuberosa* may be involved in this action. Some saponins produced in legumes, namely, group B soya saponins, contain an antioxidant moiety attached at C23 (Yoshiki et al., 1998).This unique sugar residue 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), allows saponins to scavenge superoxides by forming hydro peroxide intermediates, thus preventing bio-molecular damage by free radicals (Yoshiki & Okubo, 1995). Hence, the antiulcer activity of TMT could be attributed to the antioxidant potential of saponins and triterpenoids present in it. There is a claim (Kirtikar & Basu, 1991) and report (Koneri et al., 2006) that the plant is an abortifacient. Therefore, its use as antiulcer agent in pregnancy may be contraindicated.

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