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EVALUATION OF ANTI-ULCER ACTIVITY OF THE EXTRACT OF *TALINUM TRIANGULARE* IN RATS

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Abstract:
Preventive and therapeutic activities of Hydroalcoholic extract of Talinum triangulare leaves against ethanol-induced ulcer in wistar rats is the aim of this study. Fresh plants of T. triangulare were purchased from a local market in Bhopal,M.P and were identified by a botanist. The extraction was done using Soxhlet apparatus and (Ethanol: water; 80:20) as the solvent. The process was allowed to run for a total of 18 hours. Five groups (n = 6) of Wistar rats were used to study the anti-ulcer activity of Talinum triangulare Hydroalcoholic extract. PBS, Hydroalcoholic extract, omeprazole and ethanol were administered to the animals per orally (p.o). Group 1 received PBS (10 mL/ kg) all over the experimental period (11 days) and served as control. Group 2 received PBS (10 mL/ kg) for 10 days and on the 11th day received absolute ethanol (5 mL/ kg) and served as ulcer control. Group 3 and 4 were respectively administered with 300 and 400 mg/kg Talinum triangulare Hydroalcoholic extract, group 5 with omeprazole (8 mg /kg) for 10 days. All the groups were fasted for 24 h and again administered with the extract or drug at respective dose. After 30 min of this treatment, animals of groups 2-5 were administered with 5 mL/ kg ethanol to induce ulcer. After 15 min of ethanol administration, all the animals were sacrificed using anesthetic ether. Gastric volume was measured by pylorus ligation method. Each stomach was opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (20 X). The length and width (mm) of ulcer on the gastric mucosa were measured by plane glass square (10×10 mm).

Key words: *Anti-ulcer Activity, Talinum triangulare, Pylorus ligation method*

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INTRODUCTION:

Excessive acute or chronic alcohol consumption poses a serious health hazard and can result into several metabolic disorders in hepatic and extra-hepatic diseases [1]. Alcohol is a commonly used hepatotoxin in experimental hepatopathy. Although the pathogenesis of alcohol-induced liver disease is not clearly defined, there is evidence that ethanol-induced liver injury is due to oxidative stress that leads to fibrosis and impaired liver functions [2].

Alcohol overuse is also characterized by central nervous system (CNS) intoxication symptoms, impaired brain activity, poor motor coordination, and behavioral changes. Excessive alcohol consumption commonly causes hepatic, gastrointestinal, nervous and cardiovascular injuries leading to physiological dysfunctions . Cellular disturbances resulting from excessive alcohol consumption results in increased formation of oxidative stress biomarkers such as malondialdehyde (MDA); reduction in the level of reduced glutathione level and a decrease in the activities of antioxidant enzymes [3,4].

Free radicals and reactive oxygen species (ROS) have been implicated in the oxidative damage of bimolecular and various organs of the body. Studies have shown the crucial role free radicals play in the pathogenesis of many human diseases namely, cardiovascular and pulmonary diseases, some types of cancer, immune/autoimmune diseases, inflammation, diabetes, cataracts and brain dysfunction such as Parkinson and Alzheimer [5].

However, the deleterious effect of free radicals can be corrected by antioxidants – both enzymatic and nonenzymatic. Oxidative stress is known to arise when there is an imbalance between free radical production (especially reactive oxygen species; ROS) and endogenous antioxidant defense system. This shift in balance is associated with oxidative damage to a wide range of biomolecules including lipids, proteins, and nucleic acids, which may eventually impair normal functions of various tissues and organs [6].

There is an increasing global interest concerning the use of medicinal plants in the prevention and treatment of different pathologies. The beneficial effects of plants are attributed to the presence of secondary metabolites such as polyphenols, tannins, terpenoids, alkaloids, flavonoids. Considering the central role played by free radicals in the initiation and progression of many diseases, the use of natural products with antioxidant constituents has been proposed as an effective therapeutic and/or preventive strategy against diseases and the search for potent and costeffective antioxidants of plant origin has since increased [7]. Many plants have been shown to possess antioxidant potentials. This has thus raised interest in the investigation of commonly consumed plants for their phytochemicals with nutritional and chemotherapeutic potentials. Therefore, the need to argument synthetic chemotherapeutic compounds with natural products is the drive for the exploitation of natural products from plants; as they may have little or no side effects yet meeting the nutritional, chemotherapeutic and economic needs [8]. Moreover, despite the efforts of pharmaceutical companies in the production of synthetic antibiotics, there yet exists a marked increase in pathogen population exacerbated by multi drug resistant microorganisms. Consequently, there is increased research into phytochemicals for the effective therapeutics combat of this menace. The therapeutic effects of plant-based drugs have been documented to be due to the phytochemicals that constitute the plants [9].

Preliminary phytochemical studies on *T. triangulare* revealed the presence of omega -3-fatty acids and high levels of essential nutrients like minerals (such as calcium, potassium and magnesium), soluble fibres (such as pectin) and vitamins (such as C, α and β tocopherols and βcarotene) which are required for growth and development . The leaf extracts of waterleaf have been proved to possess remarkable antioxidant activity and high kaempferol content. Waterleaf is a mucilaginous vegetable with high oxalate content and is rich in saponins. Cooking or blanching removes nearly most of the soluble oxalate. Furthermore, the leaves serve as sauce, condiment, spice, softening of soups and for flavouring in foods [10]. T. triangulare leaves have been implicated medically in the management of cardiovascular diseases like stroke and obesity. According to traditional medicine the leaves of waterleaf are used to treat polyuria, internal heat, measles, gastrointestinal disorders,hepatic ailments and cancer . Airaodion et al. [11] has reported its hypoglycemic and hypolipidaemic activities in Wistar rats. This study therefore sought to investigate the preventive and therapeutic activities of its Hydroalcoholic extract against ethanol-induced Ulcer in Wistar rats.

MATERIAL AND METHODS:

Fresh plants of *T. triangulare* were purchased from a local market in Bhopal, M.P and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room

temperature in an open laboratory space for 21 days and milled into powder using an electronic blender. The extraction was done using Soxhlet apparatus and (Ethanol: water; 80:20) as the solvent. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol and water was evaporated in a rotary evaporator at 35 OC .The extract was preserved in the refrigerator until when needed.

Experimental designer:

Animals:

Wistar rats (180-200 g) and Swiss albino mice (males; 20–25 g) were used in the present study. The animals were procured from College of Veterinary Science and Animal Husbandry Mhow, Indore (M.P), India. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

Acute toxicity study:

Five groups (n = 5) of male albino mice were used in the acute toxicity study of *T. triangulare*Hydroalcoholic extract. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, and 3000 mg /kg) of the extract. A group of animals which received equal volume of PBS served as control. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days.

Anti-ulcer (ulcer-preventive) activity study:

Five groups (n = 6) of Wistar rats were used to study the anti-ulcer activity of *T. triangulare* Hydroalcoholic extract. PBS, Hydroalcoholic extract, omeprazole and ethanol were administered to the animals per orally (p.o). Group 1 received PBS (10 mL/ kg) all over the experimental period (11 days) and served as control. Group 2 received PBS (10 mL/ kg) for 10 days and on the 11th day received absolute ethanol (5 mL/ kg) and served as ulcer control. Group 3 and 4 were respectively administered with 300 and 400 mg/kg *T. triangulare* Hydroalcoholic extract, group 5 with omeprazole (8 mg /kg) for 10 days. All the groups were fasted for 24 h and again administered with the extract or drug at respective dose. After 30 min of this treatment, animals of groups 2-5 were administered with 5 mL/ kg ethanol to induce ulcer. After 15 min of ethanol administration, all the animals were sacrificed using anesthetic ether. Gastric volume was measured by pylorus ligation method. Each stomach was opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (20 X). The length and width (mm) of ulcer on the gastric mucosa were measured by plane glass square (10×10 mm). The Ulcer Area (UA) was calculated. The % of protection (P %) availed to the animals through various treatments were calculated using the formula:

$$P\% = \frac{\text{UA ulcer control} - \text{UA treatment}}{\text{UA ulcer control}} x100$$

Grouping of animals:

Group-I Control Received PBS 10 ml/kg, p.o
Group-II Ulcer Control Received PBS 10 ml/kg, p.o up to 10 days and on 11th day receives absolute alcohol 5 ml/kg, p.o
Group-III Received HATT 300 mg/kg, p.o
Group-IV Received HATT 400 mg/kg, p.o
Group-V Received Omeprazole 8 mg/kg, p.o

Bio-Chemical estimation:

Estimations in Gastric Juice:

The various biochemical parameters like carbohydrate content viz. fucose, hexosamine, total hexoses, and sialic acid and total carbohydrates were estimated. Gastric volume, pH, free and total acidity and total proteins were also evaluated [12]

Gastric volume:

The gastric juice was centrifuged, allowed to decant, and taken into a glass syringe of graduation 0.01 ml. The volume of gastric juice was measured.

Determination of pH:

Using the pH meter the pH of the gastric juice was measured.

Determination of free acidity and total acidity

0.01 N NaOH was standardized using oxalic acid as the primary standard. Free acidity was estimated by titrating 1 ml of gastric juice with 0.01 N Sodium hydroxide using topfer's reagent as indicator until the red colour changes to yellowish orange. The volume of sodium hydroxide consumed was noted which corresponded to free acidity. Titration with 0.01N NaOH was continued using phenolphthalein as indicator until the yellowish orange colour changed

to red. The amount of NaOH consumed was noted and corresponded to total acidity.

Acidity was calculated by using the following formula, and expressed as mEq/l/100g.

$$\text{Acidity} = \frac{\text{Volume of NaOH x Actual Normality of NaOH X 100}}{0.1} \text{meq/lit/100gm}$$

Estimation of Total Proteins [13]:
An alcoholic precipitate was prepared by adding 9.0 ml of 90% alcohol to 1 ml of gastric juice. To 0.1 ml of this protein mixture, 1 ml of 0.1N NaOH was added. 0.4 ml phenol reagent was added to 0.05 ml of the reaction mixture and was kept for ten minutes to complete the reaction. Absorbance was measured at 610 nm against blank in a spectrophotometer. The amount of protein present in the gastric juice was calculated from standard curve prepared with bovine albumin and was expressed in term of µg/ml of gastric juice.

Estimation of Total Carbohydrates [14]:
An alcoholic precipitate containing the dissolved mucosubstances was prepared by adding 9 ml of 90% alcohol to 1 ml of gastric juice. The mixture was allowed to decant and the supernatant layer was discarded. The precipitate containing the mucosubstances was dissolved in 0.5 ml of 0.1N sodium hydroxide and 1.8 ml of 6N HCl was added. This mixture was boiled on a water bath, neutralized and diluted with distilled water to get a final volume of 4.5 ml. This solution was used for the estimation of carbohydrates like total hexoses, hexosamine, sialic acid and fucose as follows.

Estimation of Fucose [15]:
Procedure:
The blank and the sample tubes containing 0.4 ml of distilled water and 0.4 ml of hydrolysate was mixed carefully in a water bath with 1.8 ml sulphuric acid mixture. The reaction was allowed to take place for 3 minutes by heating on a water bath. After cooling the tubes 0.1 ml of cysteine reagent was added to the blank and to one of the tubes containing the hydrolysate (unknown) while cysteine reagent was not added to the test-tube containing the hydrolysate (unknown blank). The reaction was allowed to continue for 90 min. The absorbance was read spectrophotometrically at 396 and 430 nm using distilled water as blank.

The optical density for the fucose in the hydrolysate was calculated. The readings, which were taken at 396 and 430 nm, were noted and the difference calculated. Then the values without cysteine were subtracted from this and evaluated.

Estimation of Hexosamine [16]:
Procedure:
0.5 ml of acetyl-acetone reagent was added to 0.5 ml of the hydrolysate fraction. The reaction was allowed to take place by heating for 20 min in a boiling water bath. The mixture was allowed 58 to cool and 1.5 ml of 90% alcohol and 0.5 ml of Ehrlich's reagent was added. After 30 min the intensity of colour development was measured spectrophotometrically at 530 nm against blank. The amount of hexosamine present in the sample was estimated from the standard curve prepared by using D (+) glucosamine hydrochloride and concentration was expressed in µg/ml of gastric juice.

Estimation of Total Hexoses [17]:
Procedure:
The reaction mixture containing 0.4 ml of hydrolysate and 3.4 ml of orcinol reagent was heated for 15 min. in the boiling water bath. The tubes were cooled to room temperature and the colour developed was measured at 540 nm against the blank. The amount of hexoses present in the sample was determined from the standard curve of D (+) galactose - mannose and has been expressed in µg/ml of gastric juice.

Estimation of Sialic Acid [17]:
Procedure:
A reaction mixture containing 0.5 ml of the hydrolysate in 0.1N H2SO4 and 0.2 ml of sodium periodate was mixed and allowed to stand for 20 min. 1 ml of sodium arsenite solution was added and mixed by shaking. 3 ml of Thiobarbituric acid was added and the mixture was heated on a boiling water bath for 15 min. After cooling, 4.5 ml of cyclohexanone was added, thoroughly shaken and centrifuged. The pink colour formed in the supernatant layer was pipetted out and intensity of colour was measured spectrophotometrically at 550 nm. A standard curve was prepared using sialic acid and the amount of sialic acid present in the sample was determined expressed in µg/ml of gastric juice.

Statistical Analysis:
All the results obtained from various activities, as described above, were analyzed statistically by using $P<0.05$ were considered significant.

RESULTS:

Acute Toxicity Study:

Administration of the different extractives of Hydroalcoholic extract of *Talinum triangulare*in mice at doses of 250, 500 mg/kg and 3000 mg/kg by oral gavages did not reveal any adverse effects or signs of toxicity.

Observations twice daily for fourteen days also did not reveal any drug related observable changes or mortality. Accordingly, the acute oral LD50 of the extractives was concluded to exceed 3000 mg/kg body weight, the highest dose tested in the study. No mortality was found and all the behavioral parameters were normal during the study.

Evaluation of anti ulcer activity Hydroalcoholic extract of *Talinum triangular:*

Effect of the Hydroalcoholic extract of *Talinum triangulare* on Ethanol Induced Gastric Ulcers in Rats

Ethanol administration in rats (1 ml/200g b.w.) induced ulceration of the gastric mucosa of the control group, characterised by haemorrhagic gastric lesions. The Hydroalcoholic extract of *Talinum triangulare* caused a reduction in the severity of these lesions induced by ethanol which was evident by a significant ($p<0.01$) reduction in the ulcer index and an increase in the percentage protection of ulcers when compared with the control group.

At 300 mg/kg b.w. the Ethanolic leaves extract of *Talinum triangulare* showed a protection index of 26.47% and at 400 mg/kg b.w. the extractives showed protection index of 70.21% .The results were comparable to Omeprazole (8 mg/kg) which reduced the ulcer index 69.23% significantly .

Table 1. Effect of the Ethanolic leaves extract of *Talinum triangulare* on Ethanol Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg b.w)	Gastric Volume(ml)	Ulcer area(mm^2)	Protection (%)
Control	PBS 10 ml/kg	1.51±0.07	0.00±0.00	NA
HATT	300 mg/kg	3.46±0.30	485.36±7.00	26.47%
HATT	400 mg/kg	2.11±0.30	297.42±0.28	55.33%
Omeprazole	8 mg/kg	2.47±0.30	154.87±5.21	70.21%

Data are expressed as mean SEM., n=6 in each group. *p<0.05, **p<0.01 - One way ANOVA followed by Dunnett's test:

Effect on Gastric Volume:

Gastric volume in the Hydroalcoholic extract of *Talinum triangulare* treated groups indicated that there was no significant decrease in the volume of the gastric juice at 300 mg/kg (3.46), but at 400 mg/kg (2.11) there was a significant decrease in comparison to the control group ($p<0.05$). Omeprazole at 8 mg/kg (2.47) caused a significant decrease in gastric volume.

Effect on pH of Gastric Juice:

The Hydroalcoholic extract of *Talinum triangulare* at 300 mg/kg and 400mg/kg significantly increased the pH 3.04 and 3.86 respectively of gastric juice ($p<0.01$) and was comparable to the standard drug Omeprazole at 8 mg/kg was 6.31

Effect on Free Acidity and Total Acidity:

Estimation of gastric juice of Hydroalcoholic extract of *Talinum triangulare* treated groups indicated that there was a significant decrease in the free acidity and total acidity of the gastric juice. Rats treated with 300 mg/kg and 400 mg/kg of Hydroalcoholic extract of *Talinum triangulare* showed a significant decrease in free acidity and total acidity ($p<0.01$) was (34.32 & 32.55) and (36.32& 36.22) was comparable to that of the Omeprazole at 8 mg/kg treated group ($p<0.01$) of rats was 18.13and 26.25respectively.

Table 2. Effect of Hydroalcoholic extract of *Talinum triangulare* on Antisecretory Parameters

Treatment	**Dose (mg/kg b.w)**	**pH**	**Free Acidity (mEq/l/ 100g)**	**Total Acidity (mEq/l/ 100g)**
Control	PBS 10 ml/kg	1.51±0.45	57.01±4.6	76.32±2.5
HATT	300 mg/kg	3.04±0.35	36.32±0.47	36.22±2.61
HATT	400 mg/kg	3.86±0.61	34.32±2.65	32.55±2.55
Omeprazole	8 mg/kg	6.31±1.21	18.13±5.2	26.25±2.58

Values are mean ± S.E.M, n=6, NS-not significant, *p < 0.05 and **p < 0.01 Vs control, One way ANOVA followed by Dunnett's test:

Effect on Total Proteins:

The results indicated that the total protein content was significantly decreased in the group treated with Hydroalcoholic extract of *Talinum triangulare* when compared to the control. A dose of 300 and 400 mg/kg showed significant decrease in the total protein content (p<0.01) 410.19±2.14 and 301.54±2.01 respectively compared to the control group ie 450.21±0.36.

Table 3. Effect of Hydroalcoholic extract of *Talinum triangulare* on Total Proteins and C/P

Treatment	**Dose (mg/kg b.w)**	Total proteins (µg/ml)	C/P
Control	PBS 10 ml/kg	450.21±0.36	0.65
HATZ	300 mg/kg	410.19±2.14**	1.8
HATZ	400 mg/kg	301.54±2.01**	3.54
Omeprazole	8 mg/kg	233.47±2.5**	4.36

Values are expressed in terms of mean ± S.E.M, **p<0.01- One way ANOVA followed by Dunnett's test

Effect of Hydroalcoholic extracts of *Talinum triangulare* on Total Carbohydrates:

Total carbohydrates content (hexose, hexosamine, fucose and sialic acid) and C/P ratio of the Hydroalcoholic extract of *Talinum triangulare* treated group indicated that there was a significant increase in the total carbohydrate content (p<0.01) and C/P ratio and was comparable to the Omeprazole treated group of rats.

Table 4. Effect of Hydroalcoholic extracts of *Talinum triangulare* on Total Carbohydrates

Treatment & Dose (mg/kg)	Total Carbohydrates (µg/ml)			
	Total Hexose	Hexosamine	Fucose	Sialic acid
Control	150.21±0.33	170.23±0.87	70.87±0.58	23.02±0.10
HATZ	280.54±1.25**	230.46±0.58**	80.55±1.21**	43.66±1.29**
HATZ	310.21±1.11**	260.54±0.12**	86.89±1.22**	38.25±1.55**
Omeprazole	480.56±0.36**	420.11±0.82**	160.36±0.27**	51.36±0.98**

Values are expressed in terms of mean ± S.E.M, **p<0.01 Vs control- One way ANOVA followed by Dunnett's test

CONCLUSION:

In the present study, we also observed flattening of the mucosal folds which suggests that the gastro protective effect of *Talinum triangulare* extract might be due to a decrease in gastric motility. It is reported that the changes in the gastric motility may play a role in the development and prevention of experimental gastric lesions. Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume. Ethanol produces a marked the gastric irritants on rugal crest. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration. The acute toxicity profile of *Talinum triangulare* extract could be considered favorable judging from the absence of adverse clinical manifestations in experimental animals after 14 days of observation. It is concluded that the extract has no acute toxicity and that the oral lethal dose for male and female rats is in excess of 5 g/kg. In conclusion, *Talinum triangulare* extracts could significantly protect the gastric mucosa against ethanol induced injury. Such protection was shown to be dose dependent as ascertained by the reduction of ulcer areas in the gastric wall as well as the reduction or inhibition of edema and leucocytes infiltration of sub mucosal layers and protection was most prominent at a dose of 400 mg/kg leaf extract. Further studies are required to determine the active ingredients responsible for the mechanism of anti-ulcer of *Talinum triangulare* extracts.

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