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Effect of *Tridax procumbens* Extracts on Ethanol Induced Gastric Ulceration in Wistar Rats

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The effect of *Tridax procumbens* extracts on ethanol induced gastric ulceration in Wistar rats was studied. Twelve Wistar rats weighing 150-250 g were used for the study. Gastric ulceration was induced with 0.2 mL kg^{-1} b.wt. of the necrotizing agent (80% ethanol-in- 0.1N HCl) and were treated as follows: Group 1 (control) was treated with 0.2 mL kg^{-1} b.wt. of the vehicle (placebo), groups 2 and 3 were treated with 100 and 250 mg kg^{-1} b.wt. of the extracts, respectively and group 4 was treated with 250 mg kg^{-1} b.wt. of the standard drug, omeprazole. The experiments lasted for 21 days. Acute toxicity test (Median lethal dose, LD_{50}), gastric juice pH, gastric acid concentration, gastric ulcer scores, gastric ulcer index, percentage ulcer protection (% ulcer inhibition), activity levels of superoxide dismutase (SOD), catalase and malondialdehyde (MDA) were determined. The results showed that the extracts highly statistically ($p<0.05$) increased the gastric juice pH ranging from 3.7±0.36 to 4.47±0.15 compared with pH value of 1.46±0.14 in the control. The percentage ulcer protection in the extract treated groups ranged between 49.5-97.6% in a dose dependent manner. Inhibition of gastric ulceration in the extract (97.6%) and omeprazole (98.7%) treated rats was similar at a dose of 250 mg kg^{-1}. The control did not exhibit ulcer protection. Activities of superoxide dismutase (SOD) were 87.4±2.44 and 91.53±1.53 µmol mg^{-1} at doses of 100 and 250 mg kg^{-1} of extract, respectively. There was no statistical difference ($p>0.05$) between SOD of the extracts (91.53±1.43 µmol mg^{-1}) and omeprazole (92.03±1.44 µmol mg^{-1}) both at a dose of 250 mg kg^{-1}. Malondialdehyde (MDA) was 43.6±0.66 and 33.73±1.17 µmol mg^{-1} in the extracts and 42.63±2.14 µmol mg^{-1} in omeprazole treated rats. The distilled water treated rats had MDA of 66.87±2.14 µmol mg^{-1}. The results of the study therefore, suggest that *Tridax procumbens* extracts possess anti-ulcerogenic and antioxidant properties against 80% ethanol-in-0.1N HCl induced gastric ulceration in Wistar rats.

Key words: *Tridax procumbens* extracts, gastric irritants, gastric ulceration, gastric mucosa

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INTRODUCTION

The gastric mucosa is continuously exposed to potential gastric irritants such as ethanol, hydrochloric acid, infected bacterial products and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). It appears that these gastric irritants damage the gastric mucosa by inducing not only necrosis but also apoptosis in gastric mucosal cells (Okwuosa *et al*., 2011). Peptic ulcers result from an imbalance between factors that can damage the gastro duodenal mucosal lining and defense mechanisms that normally limit the injury. Aggressive factors include gastric juice (including hydrochloric acid, pepsin and bile salts refluxed from the duodenum), *H. pylori* and NSAIDs. *Helicobacter pylori* contribute to mucosal injury by multiple mechanisms.

Injury or damage to bodily tissue is associated with the process of inflammation. Inflammatory response mediators such as prostaglandins stimulate the gastric proton pump which is the final step to secrete gastric acid (Aperia *et al*., 2009). Therefore, the introduction of H_2-receptor antagonists (or H_2-receptor blockers) followed by Proton Pump Inhibitors (PPIs) reflects a major medical breakthrough in the treatment of gastric ulceration and gastro esophageal reflux diseases.

Current treatment regimens involve the use of proton pump inhibitor and two antibiotics in *H. pylori* elimination therapy (Stepehn, 2007). But drug resistance has significantly affected their efficacy. Therefore, the search for an ideal anti-gastric ulceration drug continues and has been extended to herbal therapy. The use of herbs to treat diseases and for the maintenance of integrity of gastric mucosa is almost universal within the tropics (Borrelli and Izzo, 2000). These herbs are used for their availability, affordability, efficacy and safety (Watcho *et al.*, 2011).

Tridax procumbens is a flavonoid plant of open waste places, native of central tropical America, now widely occurring in the tropics (Gamboa-Leon *et al.*, 2014). In humans, *Tridax procumbens* have been used for treatment of boils, blisters and cuts by local healers (Nalleua *et al*., 2013) among many other ailments. However, literature on anti-ulcerogenic properties of *Tridax procumbens* is scanty. This could provide useful information and data for the treatment and management of mucosal lesion. Therefore, this study investigates the healing effects of *Tridax procumbens* extract on 80% ethanol–in-0.1 NHCL induced gastric ulceration in Wistar rats.

MATERIALS AND METHODS

All glass wares used in this study were washed with detergent and rinsed with deionized water. All reagents were standard reagents of analytical grade and were products of Sigma Chemical Co. St. Louis MO, USA or BDH Chemical Ltd, Poole, England.

Plant collection and preparation of extract: Fresh leaves of *Tridax procumbens* plant were obtained from the Botanical gardens of the Benue State University, Makurdi, identified and voucher specimen deposited in the Department of Plant Sciences and Biotechnology, Benue State University Makurdi. The harvested leaves were air-dried in a well-ventilated room for 21 days before milling.

Fifty grams of powdered leaves were macerated in 1000 mL of 50% ethanol for 48 h. The extract obtained was strained, filtered, evaporated to dryness on a rotator evaporator (Model 349/2 Corning/England) and stored in the refrigerator until required. 2.5 g of the 50% ethanol extract was dissolved at 4°C in normal saline (0.85% w/v NaCl) and made up to 100 mL with ethanol. Appropriate dilutions were made from the stock for the study.

Experimental design-treatment regimen: Twelve Wistar rats weighing between 150-250 g obtained from the Central Animal House, College of Health Sciences, Benue State University, Makurdi were randomly divided into 4 groups of 3 rats each and allowed to acclimatize for two weeks prior to the commencement of the study. The animals were housed under standard laboratory conditions and fed with standard animal feeds (Livestock Feeds Ltd, Makurdi) and water *ad libitum*. Ethical regulations were followed in accordance with National and International guidelines for the protection of animal welfare during the experiments.

Gastric ulceration was induced by administration of 0.2 mL of the necrotizing agent (80% ethanol-in-0.1N HCl) orally after a 24 h fast. Group 1 served as the control and was administered 0.2 mL of the placebo with food and water. Groups 2 and 3 were treated 100 and 250 mg kg^{-1} b.wt. of the extracts, respectively. Group 4 was treated with 250 mg kg^{-1} b.wt. of omeprazole. The doses of *Tridax procumbens* extracts used in this study had been used previously (Watcho *et al.*, 2011). Placebo, extracts and omeprazole were administered orally for 3 weeks. Biochemical parameters investigated were acute toxicity test (median lethal dose LD_{50}), gastric acid secretion, percentage ulcer inhibition (% ulcer protection), superoxide dismutase activity, catalase assay and lipid peroxidation assay.

Acute toxicity test (median lethal dose LD_{50}): The acute toxicity test was determined using the procedure described by Preston *et al.* (1987). Briefly, 2.5 g of *Tridax procumbens* extract was dissolved in 100 mL of deionized water and 5 mg kg^{-1} b.wt. of the extract was

administered orally, corresponding to 1/10th of the oral LD_{50} of the extract. The percentage mortality was plotted against dose of the extract on a special probability-log graph paper from which the LD_{50} value was determined.

Gastric acid secretion assay and gastric ulcer scoring: Gastric acidity assay was performed using the method described by Heeba *et al*. (2009). The animals were fasted for 24 h with free access to water. Then, each animal was given 0.2 mL of the necrotizing agent (80% ethanol-in-0.1N HCl). The animals in groups 1 to 4 were given appropriate treatment for 21 days by the oral route. Then, the animals were fasted for 24 h after the last day of experiment and were euthanized by inhalation of over dose of chloroform and their stomachs opened along the greater curvature and gastric content drained into centrifuge tubes and gastric pH determined using standard method. Similarly, gastric ulcer scoring was determined with the method of Raji *et al*. (2004). The ulcers were viewed with the aid of a magnifying lens (x10) and each given a severity rating [< 1 mm = 1 (pin point), >1 mm < 2 mm = 2 and>2 mm<3 mm = 3] (Okwuosa *et al.*, 2011). The gastric lesions formed were scored and the mean Ulcer Index (U.I) and percentage inhibition (% ulcer protection) of gastric ulceration were calculated as follows:

$$\text{Ulcer Index (U.I)} = \frac{\text{Mean degree of ulceration} \times \text{group of ulceration (\%)}}{100}$$

$$\text{Ulcer protection (\%) (Ulcer Inhibition (\%))} = \frac{\text{U.I in control} - \text{U.I in test}}{\text{U.I in control}} \times 100$$

Determination of superoxide dismutase (SOD) activity, catalase assay and malondialdehyde assay: Homogenate sample of gastric tissue was made and divided three portions. The levels of Superoxide Dismutase (SOD) activity was determined by the method described by Okwuosa *et al.* (2011), which involves inhibition of epinephrine autoxidation in an alkaline medium at 480 nm using spectrophotometer (Jenway, Essex, England) using one portion of the homogenate of gastric tissue. The rate of autoxidation of epinephrine was noted at 45 sec intervals in all groups. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation, as 1 unit of SOD specific activity.

The second portion of the homogenate of gastric tissue was used for catalase assay was carried out with the method described by Raji *et al.* (2011). Briefly, 0.5 mL of the homogenate of gastric tissue was mixed with 0.5 mL of 0.1M H_2O_2, 1 mL of 0.1 M H_2SO_4 and 7 mL of 0.0l M potassium permanganate. Absorbance was read at 480 nm within 30-60 sec against distilled water and the result was expressed in μmol mg^{-1} protein.

The malondialdehyde assay-lipid peroxidation assay was carried out with the procedure described by Raji *et al.* (2011). The 2 mL of TBA reagent and 1 mL of trichloroacetic acid (TCA) were mixed with 2 mL of homogenate of gastric tissue. The mixture was heated at 60°C for 20 min. It was then cooled and centrifuged at 4000 rpm for 10 min. The absorbance of the supernatant was read at 540 nm using spectrophotometer (Jenway, Essex, England).

Statistical analysis: Predication equations were derived using multi-variant regression analysis. The results were expressed as Mean±SD. Statistical significance between the groups was determined by analysis of variance (ANOVA). $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The anti-ulcerogenic property of *Tridax procumbens* extracts in Wister rats was studied. Results from the study shows the extracts caused a high statistically significant increased ($p<0.05$) in gastric pH, gastric fluid and percentage ulcer protection but decreased ulcer scores and gastric acid secretion in a dose dependent manner when compared to the control (Table 1-3). It appears that the extracts inhibited the secretion of prostaglandins, which in turn, increases gastric mucosal blood flow, bicarbonate secretion, gastric pH and gastric wall mucus secretion (Wallace, 2008). The results also showed that the extracts at a doses of 100 and 250 mg kg^{-1}, enhanced the gastric acid secretion of 10.83±0.55 and 14.93±0.43 mL, respectively. These results were similar to those produced by the omeprazole but different from that of control group (4.63±0.35) Table 2. The pH value was lowest in control (1.46±0.36), 4.47±0.15 and 5.53±0.21 in extract and omeprazole treated rats at 250 mg kg^{-1} b.wt. Low pH is an index of high gastric acidic. The similarities in activity between the extract and omeprazole may suggest similarity in mechanism of action of the extract and omeprazole. This agrees with Ayada *et al*. (2003), who reported that the extracts may act by blocking H_2-receptor, leading to inhibition of histamine release in the gastric mucosa. Oxalates tend to render calcium unavailable by binding calcium ion to form calcium oxalate complex, which reduces intracellular Ca^{2+} that is necessary for gastric acid secretion (Watcho *et al.*, 2011).

The results (Table 4) also showed that activities of superoxide dismutase (SOD) and catalase were highly statistically significantly increased ($p<0.05$), while malondialdehyde (MDA) was highly statistically significantly decreased ($p<0.05$) in extracts and omeprazole treated rats when compared to those of the

Table 1: Effects of *Tridax procumbens* extracts and omeprazole on gastric ulcer scores, ulcer index and percentage ulcer protection in Wistar rats

Treatment groups (N)	Ulcer scores (units) p = 0.000	Ulcer index p = 0.348	Ulcer protection (%) (Ulcer inhibition (%)) p = 0.000
Control (0.2 mL)	14.70±0.52	10.73±1.99	-
Extracts (100 mg kg^{-1})	14.60±1.11**	13.13±2.00	49.5*
Extracts (250 mg kg^{-1})	10.40±1.01**	12.10±0.62	97.6**
Omeprazole (250 mg kg^{-1})	10.87±0.61**	12.37±1.05	98.7**

Values are expressed as Mean±SD, **Highly significantly different from control, p<0.05, using ANOVA, N: No. of rats per group = 3

Table 2: Effect of *Tridax procumbens* extracts and omeprazole, on gastric juice secretion, gastric pH and titratable acidity in Wistar rats

Treatment groups (N)	Volume of gastric juice (mL) p = 0.000	Gastric pH p = 0.000	Titratable acidity (mmol L^{-1}) p = 0.000
Control (0.2 mL)	4.63±0.35	1.46±0.14	247.23±5.85
Extracts (100 mg kg^{-1})	10.83±0.55**	3.70±0.36	73.57±1.36**
Extracts (250 mg kg^{-1})	14.93±0.42**	4.47±0.15**	44.33±1.15**
Omeprazole (250 mg kg^{-1})	16.83±0.45**	5.53±0.21**	44.23±0.91**

Values are expressed as Mean±SD, **Highly significantly different from control, p<0.05, using ANOVA, N: No. of rats per group = 3

Table 3: Prediction equation for ulcer protection, titratable acidity and malondialdehyde activity of *T. procumbens* extract and omeprazole

Parameter	Prediction equation	P	R	R^2	SE	Remark
UP	29.5-16.7USc+20.1UI	0.39	0.99	99.90	1.52	*
TA	315+3.37Vol. g-64.10pH	0.13	0.96	92.90	22.57	*
MDA	114-0.877SOD+0.089CA	0.10	0.97	94.90	2.92	*

UP: Ulcer protection, TA: Titratable acid, MDA: Malondialdehyde, P: Significance at probability, R: Correlation coefficient, R^2: Power of prediction, SE: Standard error, *Significant

Table 4: Effect of the administration of *Tridax procumbens* extracts on antioxidant enzymes activities in Wistar rats

Treatment groups (N)	Superoxide dismutase activity (μmol mg^{-1} $protein^{-1}$) p = 0.000	Catalase activity (μmol mg^{-1} $protein^{-1}$) p = 0.000	Malondialdehyde activity (μmol mg^{-1} $protein^{-1}$) p = 0.000
Control (0.2 mL)	54.570±3.55	14.43±0.96	66.87±3.33
Extracts (100 mg kg^{-1})	87.400±2.44**	33.07±0.89**	43.60±0.66**
Extracts (250 mg kg^{-1})	91.530±1.43**	52.37±2.54**	33.73±1.17**
Omeprazole (250 mg kg^{-1})	92.030±1.44**	92.03±1.44**	42.63±2.14**

Values are expressed as Mean± SD, **Highly significantly different from control, p<0.05, using ANOVA , N: No. of rats per group = 3

control. The SOD values of the extracts at doses of 100, 200 mg kg^{-1}, omeprazole 250 mg kg^{-1} and the control were 87.4±2.44, 91.53.±1.43, 92.53±1.44 and 54.57±3.55 μmol mg^{-1}, respectively. The superoxide dismutase (SOD) plays an important role in preventing gastric ulceration by catalyzing the breakdown of highly reactive radicals superoxide (0^{2-}) into oxygen and hydrogen peroxide (Zelko *et al*., 2002; Heeba *et al*., 2009). Catalase gastro-protection of the gastric mucosa may be due to its antioxidant activity and decrease in secretion of prostaglandins (Raji *et al.*, 2011). Catalase was 92.03±1.44 μmol mg^{-1} in omeprazole treated group, 52.37±2.54 μmol mg^{-1} in extract (250 mg kg^{-1}) tread group and 14.43±0.9644 μmol mg^{-1} in the control group. The MDA concentration in control rats was 66.87±3.3344, 33.73±1.1744 μmol mg^{-1} in extract (250 mg kg^{-1}) treated rats and 42.03±2.1444 μmol mg^{-1} in omeprazole treated rats. The reduction of the malondialdehyde (MDA) concentration may suggest that the extracts possess anti-ulceration through its Lipid peroxidation activities.

The results of the 250 mg kg^{-1} b.wt. of extract are in consonant with those obtained for 250 mg kg^{-1} b.wt. of omeprazole. The results also showed that doses of the extracts below 500 mg kg^{-1} b.wt. were safe from acute toxicity.

CONCLUSION

The results of this study have shown that the *Tridax procumbens* extracts possess anti-ulcerogenic and antioxidant properties against 80% ethanol-in-0.1 HCl induced gastric ulceration in Wistar rats. The results also showed that doses below 500 mg kg^{-1} b.wt. of the extracts are safe from acute toxicity and may be useful for the treatment of gastropathy such as gastric ulceration and gastritis (gastrointestinal disorders). It is suggested that pharmacokinetics and toxicity studies is necessary before introduction in clinical practice.

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