PROTECTIVE ROLE OF METHANOLIC EXTRACT OF GOMPHRENA CELOSIODES LEAVES ON ACIDIFIED ETHANOL-INDUCED GASTRIC ULCER IN MALE WISTAR RATS

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ABSTRACT
This study was designed to investigate the effect of the methanol extract of *Gomphrena celosioides* leaves on acidified ethanol-induced gastric ulcer in male Wistar rats. It examined the *in vivo* antioxidant effect and also the antiulcer potentials of *Gomphrena celosioides* leaves. Methanol extract of *Gomphrena celosioides* leaves was administered at 200, 400 and 800 mg/kg body weight by oral gavage and control group received 50mg/kg cimetidine. The treatment lasted for 14 days. *In vivo* antioxidant enzymatic activity, ulcer parameters and histological evaluation of gastric mucosa were assessed. Administration of acidified ethanol decreased the activities of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the untreated ulcerated group with an increase in the levels of malondialdehyde (MDA), and the activity of xanthine oxidase (XO). A decrease in the concentration of reduced glutathione (GSH) was observed in the untreated ulcerated group. However, post treatment with either the extract or cimetidine showed a concentration dependent increase in the activities of SOD, GPx, and the levels of GSH with concomitant decrease in levels of LPO and the activity of XO. The histopathology of the gastric mucosa from rats in the untreated ulcerated group showed sections of leucocyte infiltration and disruption in the epithelial layer, lamina propria, muscularis mucosa and muscular layer which was restored in ulcerated rats post treated with *Gomphrena celosioides* leaves. Our data indicate *Gomphrena celosioides* leaves has profound antiulcer properties and is able to protect the gastric mucosa from ethanol induced gastric lesions. The antiulcer properties of the plant might be mediated through its free radical scavenging activity.
Keywords: Ulcer, Antioxidant, Histopathology, Gastric mucosa.

1. INTRODUCTION

Peptic ulcer occurs in more than 10% of world population (Ishida *et al*., 2010). It is one of the most important ailments of gastrointestinal tract in the world and it becomes a global problem due to its increasing morbidity and mortality (Martins *et al*., 2014). Peptic ulcers are chronic and often single lesions that may occur in any part of the digestive tract (Kumar *et al*., 2017).

The signs and symptoms of peptic ulcer can be constant or sporadic, and the disease cause varies among individuals (Traversa *et al*., 1995). The most common symptoms of peptic ulcer are known collectively as dyspepsia. Dyspepsia may be persistent or recurrent and can lead to a variety of upper abdominal symptoms such as pain, vomiting blood or vomit with the appearance of coffee grounds, black or tar-like stools, unintended and unexplained weight loss weight loss and anemia.

Oxidative stress has been implicated as the major cause of stress ulcers. Evidence supports that psychological stress, in addition to physical stress such as surgical intervention and microbial infection including *Helicobacter pylori* (*H. pylori*) (Ishida *et al*., 2010), leads to oxidative stress in the stomach. Oxidative stress, which is a state of elevated levels of reactive oxygen species (ROS), causes a variety of conditions that stimulate either additional ROS production or a decline in antioxidant defenses. Gastric mucosa is exposed to gastric acid, pepsin, and stimulants among others, while gastroprotective factors maintain the integrity of the gastric mucous layer, microcirculatory system, HCO_3^-, prostaglandins (PGs), epidermal growth factor synthesis, and epithelial cell restitution. Imbalance between the aggressive factors and mucosal defense system envelops the multifactorial process that underlines the disease (Pan *et al*., 2008). Asides stress, factors that may increase the incidence of peptic ulcer disease (PUD) include alcohol consumption, smoking, *H. pylori*, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Vonkeman *et al*., 2007).

The use of drugs such as antibiotics, proton pump inhibitors (omeprazole), prostaglandin analogs, and H2 receptor blockers (cimetidine, ranitidine, and famotidine) have been reported to reduce the mortality of stomach ulcers. However, discovery of new therapeutics for the treatment of this disease is necessary (Massignani *et al*., 2009).

Studies show that numerous natural products including herbs and spices have biological properties alongside gastric ulcer prevention potential (Repetto and Llesuy, 2002). A large number of medicinal plants with gastric antiulcer potential have been reported (Abdulla *et al*., 2010; Mahmood *et al*., 2010; Wasman *et al*., 2010).

Gomphrena celosioides also known as Soft Khaki weed is a shortlived perennial plant that belongs to the Amaranthaceae family (Allison *et al*., 1992). Gomphrena species in different parts of the world are used for various folkloric medicinal purposes (Viera *et al.,* 1994). Earlier research work by Botha and Gerritsma-Van der Vijver (Botha *et al*., 1986) on GC extracts revealed the presence of saponins, steroids, amino acids, nonreducing sugars, phenols, and flavonoids (Viera *et al*., 1994).

This study was undertaken to evaluate the gastroprotective effects of methanolic extracts of this plant against HCl/ethanol-induced gastric ulcer in rats.

2. METHODS AND MATERIALS

1-chloro-2,4-dinitrobenzene, 5,5-dithiobis-(2-nitrobenzoic acid), xanthine, xylenol orange, sulphosalicylic acid, sorbitol, hydrogen peroxide, reduced glutathione, epinephrine, sodium azide, ammonium ferrous sulphate, sodium acetate, potassium sodium tartarate, Tris base, acacia powder (Sigma Aldrich, England); methanol, hydrochloric acid, potassium chloride(BDH, England); tricarboxylic acid, ethanol, diethyl ether (Park, United Kingdom); The chemicals used for this study were all obtained from recognized outlets and were of analytical grade. Cimetidine was obtained from a recognized pharmacy and was prepared freshly before use.

Gomphrena celosioides leaves were obtained from Iwo, Osun state and air dried for a week until it was fully dried. The leaves were blended and then 800g soaked in 6litres of methanol for 72hours after which it was filtered and the filtrate was evaporated using rotary evaporator. The extract was allowed to dry completely before it was administered to the animals.

2.1 Experimental Animals

Male Wistar rats weighing 140-160g were obtained from the department of Veterinary Anatomy, University Of Ibadan, Ibadan and kept in an animal house for 2weeks to acclimatize. Rats were randomly divided into eight groups with five rats within each group. They were fed on a standard rat diet and tap water. The rats were fasted for 24hours but allowed free access to water prior to the oral induction of gastric ulcer.

2.2 Experimental Design

Gastric ulcer was induced by the administration of acidified ethanol (0.15N HCl + 70% v/v absolute ethanol). The animal grouping is shown thus;

Group 1 (normal control): 1ml of 1% gum acacia

Group 2 (ulcer control): 0.5ml of acidified ethanol (0.15N HCl + 70% v/v ethanol)

Group 3: 0.5ml of acidified ethanol + 200mg/kg body weight of the Methanol extract of *Gomphrena celosioides* leaves

Group 4: 0.5ml of acidified ethanol + 400mg/kg body weight of the Methanol extract of *Gomphrena celosioides* leaves

Group 5: 0.5ml of acidified ethanol + 800mg/kg body weight of the Methanol extract of *Gomphrena celosioides* leaves

Group 6: 0.5ml of acidified ethanol + 50mg/kg body weight of cimetidine

Group 7: 50mg/kg body weight of cimetidine alone

Group 8: 400mg/kg body weight of Methanol extract of *Gomphrena celosioides* leaves alone

2.3 Methods

The formation of ulcers and the ulcer lesions were scored according to the methods by Ohara *et al*., 1995. Gross mucosal lesions were recognized as hemorrhage or linear breaks (erosions) with damage to the mucosal surface. The

gastric mucus content of each stomach was determined according to the method by Ueda *et al*., 1992. Protein concentration in the supernatant of the stomach homogenate was determined according to the method of Lowry *et al*., 1951. The levels of SOD activity was determined by the method of Misra and Fridovich 1972. The method of Beutler *et al*., 1963 was followed in estimating the level of reduced glutathione (GSH). Lipid peroxidation was determined by measuring the levels of malondialdehyde produced during lipid peroxidation according to the method described by (Varshney and Kale 1990). Glutathione peroxidase (GPx) activity was determined by the method of Rotruck *et al* 1973 with some modifications, which is based on the reaction between glutathione remaining after the action of GPx. Xanthine oxidase activity was determined by the method of (Prajda and Weber 1975).

2.4 Histopathological Assessment

Stomachs from rats of all groups were fixed in 10% formaldehyde, dehydrated in grade alcohol and embedded in paraffin. Fine sections were obtained, molded on glass slides and stained with hematoxylin-eosin (H&E) for light microscope observations.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1: Effect of methanol extract of *gomphrena celosioides* leaves on ulcer parameters in HCl/EtOH induced gastric ulcer healing in male rats.

PARAMETERS/GROUP	Gastric Volume(ml/4h)	Acid Output(μEq/4h)	Mucus content (μg/gm tissue)
Control	2.36 ± 0.23^{b}	40.03±0.06^{b}	212.96±5.13^{b}
HCl/EtOH alone	3.24 ± 0.22^{a}	80.38±1.18^{a}	58.48±1.22^{a}
200mg/kg GC + HCl/EtOH	2.59 ± 0.16^{b}	60.02±1.00^{ab}	90.51±0.30^{ab}
400mg/kg GC+ HCl/EtOH	2.41 ± 0.45^{b}	50.10±0.88^{b}	87.97±4.87^{ab}
800mg/kg GC + HCl/EtOH	2.11 ± 0.44^{b}	50.07±0.93^{ab}	127.45±4.14^{ab}
50mg/kg cimetidine+ HCl/EtOH	2.23 ± 0.19^{b}	40.75±0.35^{b}	92.62±2.69^{ab}
50mg/kg cimetidine alone	2.41 ± 0.67^{b}	50.25±0.35^{b}	167.79±4.14^{ab}
400mg/kg GC alone	2.48 ± 0.24^{b}	50.45±0.07^{b}	135.38±5.11^{ab}

Table 2: Effect of methanol extract of *gomphrena celosioides* leaves on ulcer parameters in HCl/EtOH induced gastric ulcer healing in male rats.

PARAMETERS/GROUP	Ulcer score	Ulcer index	% Inhibition
Control	-	-	-
HCl/EtOH alone	13.25 ± 2.99[a]	3.31 ± 0.75[a]	-
200mg/kg GC + HCl/EtOH	10.80 ± 2.59	2.16 ± 0.48	34.79%
400mg/kg GC + HCl/EtOH	8.00 ± 3.37	2.00 ± 0.84	39.63%
800mg/kg GC + HCl/EtOH	7.40 ± 1.82[b]	1.53 ± 0.36	53.81%
50mg/kg cimetidine + HCl/EtOH	5.50 ± 1.29[b]	1.37 ± 0.16	58.49%
50mg/kg cimetidine alone	-	-	-
400mg/kg GC alone	-	-	-

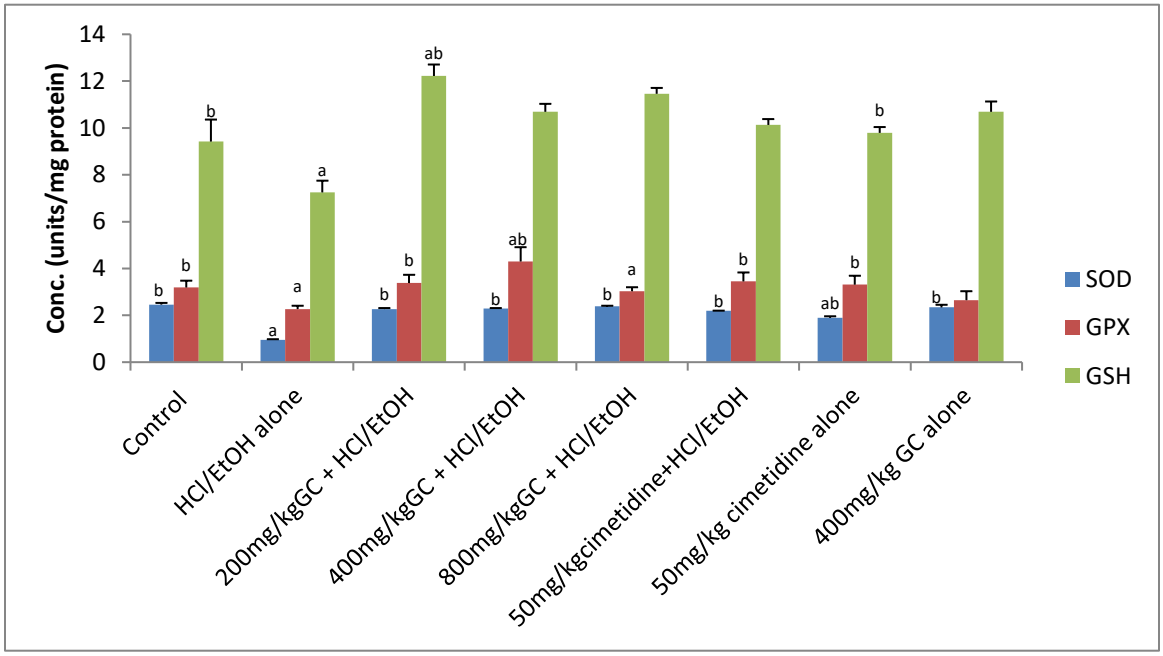

Figure 1: Effect of methanol extract of *gomphrena celosioides* leaves on antioxidant enzymes in HCl/EtOH induced gastric ulcer healing in male rats.

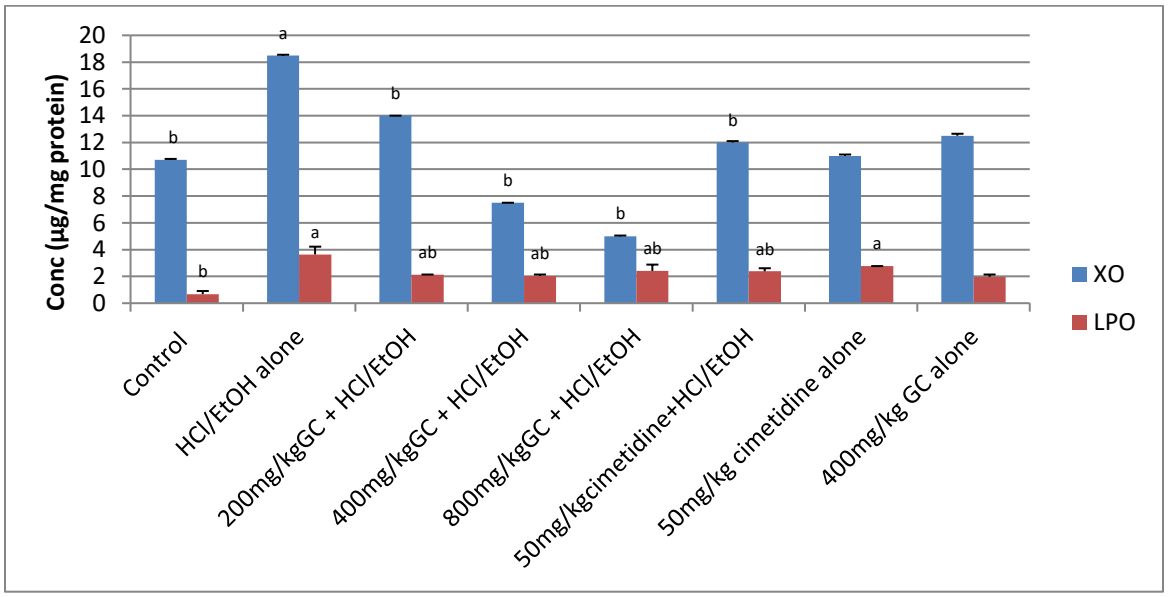

Figure 2: Effect of methanol extract of *gomphrena celosioides* leaves on antioxidant parameters in HCl/EtOH induced gastric ulcer healing in male rats.

Values are expressed as mean ± SD of five rats

[a] significantly different from control group at $p<0.05$

[b] significantly different from HCl/EtOH group at $p<0.05$

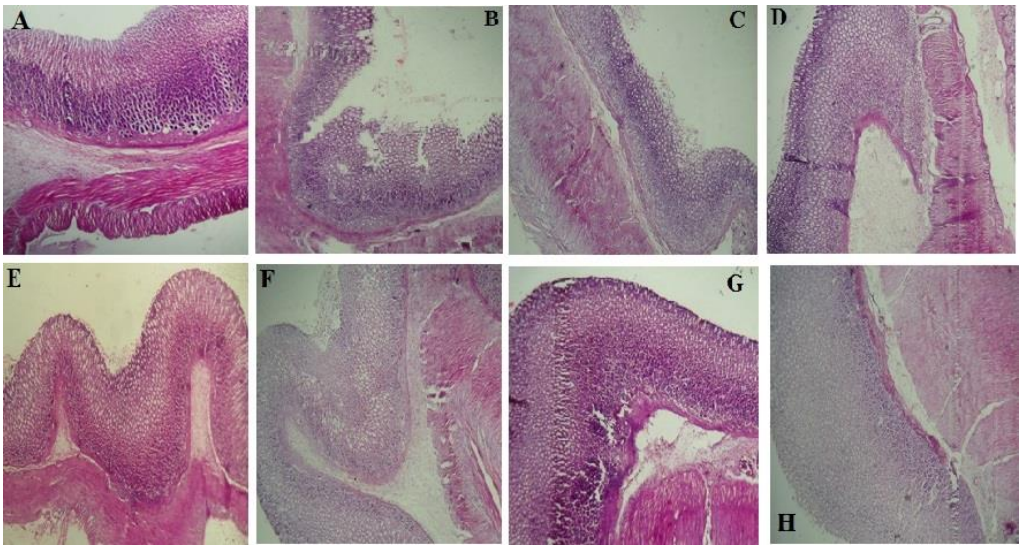

Figure 3: Showing histological slides of the stomach tissue.

A: Control group showing normal histological structure of the epithelial layer, lamina propria, muscularis mucosa, submucosa layer and muscular layer.

B: Rats in the untreated ulcerated group showed sections of leucocyte infiltration and disruption in the epithelial layer, lamina propria, muscularis mucosa and muscular layer.

C: Stomach sections from ulcerated rats post treated with 200mg/kg body weight of *Gomphrena celosioides* leaves showed mild restoration of the epithelial layer, lamina propria and submucosa layer.

D: Stomach sessions from ulcerated rats post treated with 400mg/kg body weight of *Gomphrena celosioides* leaves showed better restoration of the epithelial layer, lamina propria and submucosa layer when compared to those post treated with 200mg/kg body weight of the extract.

E: Stomach of rats post treated with 800mg/kg extract showed almost complete restoration of the epithelial layer, lamina propria, muscularis mucosa, submucosa and muscular propria.

F: Rats post treated with 50mg/kg of the reference drug show almost complete restoration of the epithelial layer.

G: Stomach sections of rats treated with 50mg/kg of the reference drug alone showed no disruption in the epithelial layer, lamina propria and muscularis mucosa.

H: Rats treated with the extract alone showed no disruption in the epithelial layer, lamina propria, muscularis mucosa and submucosa layer.

3.2 Discussion of Result

Gastric ulcer disease is a multi-factorial disease (Khanna *et al*., 2006) and the significant role played by reactive oxygen species and free radicals during its pathogenesis is well experimented in both human and experimental rats (Rao *et al*., 2000).

Some studies have reported that oxygen generated free radicals and lipid peroxidation may play important roles in the pathogenesis of ethanol-induced gastric lesions (Kvietys *et al*., 1990). Ethanol induces a rapid and strong vasoconstriction accompanied by rapid and vigorous arteriolar dilatation. The oxyradicals generated during the ischemia-reperfusion provoke severe changes at the cellular level leading to death (Glavin and Szabo, 1992).

Acidified ethanol as used in this study is a model of mucosal barrier injury (Toker *et al*., 2013). It has been confirmed that exposing gastric mucosa to oxidative stress induced by ethanol administration (Hirokawa *et al*., 1998) leads to the generation of lipid peroxides as expressed by an increase in the gastric tissue level of malondialdehyde (MDA)

accompanied by an impairment of oxidative defense mechanisms such as a reduction in superoxide dismutase (SOD), glutathione transferase (GST) and glutathione peroxidase (GPx) activities (Toker *et al*., 2013).

The efficacies of *Gomphrena celosioides* are often associated with their ability to scavenge stable free radicals (Zheng and Wang, 2001). *Celosioides* extracts have been shown to exhibit potent invitro antioxidant activity in determination of polyphenols, reducing power and lipid peroxidation inhibition in comparison to the known antioxidants, such as vitamin C. Polyphenols have also been shown to be the major plant compounds in the free radical scavenging and antioxidant activity (Maxime *et al*., 2012).

In this study, exposure of the animals to acidified ethanol may have caused severe ulcerogenic effect as ethanol is known to cause corrosion of the gastric mucosal cells resulting in their disruption and disintegration (Brossine, 1979). However, gastric protection was observed by 200, 400 and 800mg/kg dosage of the extract in ethanol induced gastric ulcers. The gastroprotective effect of the plant seems to be related to the reduction in the damage to the mucosa induced by free radicals and this activity may be due to its antioxidant action (Panda and Sonkamble, 2012).

Results from this study indicate that ethanol administration significantly reduced ($P<0.05$) the activities of SOD and GPx when compared with the normal control rats. A decrease in SOD and GPx activities in the gastric mucosa of rats exposed to acidified ethanol leads to the accumulation of reactive oxygen species and consequently to an increase in LPO level and hence, an increased mucosal damage (Vandana and Madhav, 2012). These observations confirmed the findings of several studies which reported alterations in antioxidant enzyme activities in ethanol exposed animals (Ellman, 1959; Sun and Zigman, 1978; Clairborne, 1985; Rotruck *et al*., 1973; Mohandas *et al*., 1984).

The dose related inhibition of acidified ethanol-induced decrease in activity levels of SOD, GSH and GPx when the animals were treated with *Gomphrena celosioides* leaves indicate that the plant contains bioactive substances which can stimulate the activity of the endogenous gastric antioxidant enzyme system (Maitya and Chattopadhyay, 2008). The induced activity of the antioxidant defense system was supported by a decrease in malondialdehyde level (LPO). This is supported by the research of many scientists including (Rony *et al*., 2011 and Meite *et al*., 2014). The effect of the reference drug and plant extract alone was also studied and it was observed that although the results were significantly different from the untreated ulcerated group, they were almost the same with the normal control group. Overall, the present study demonstrates the protective role of the methanol extract of *Gomphrena celosioides* leaves which can be attributed to the phytochemicals present in the leaves as reported by (Onocha *et al*., 2005).

4. CONCLUSION

From this study, it was observed that the ethanol induced gastric lesions in male Wistar rat was mitigated by *Gomphrena celosioides* leaves. The antiulcer properties of the plant might be mediated through its free radical scavenging activity. As observed in this study, all doses of the extract showed healing properties in the experimental animals. These findings suggest the potential therapeutic use of *Gomphrena celosioides* leaves as an effective non-toxic cure for ulcer as it is able to restore the antioxidant state of the gastrointestinal tract.

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