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Protective Role of Methanolic Extract of *Gomphrena Celosioides* Leaves on Acidified Ethanol-Induced Gastric Ulcer in Male Wistar Rats

Abisoye Fafioye[1] & Olakunle Omotayo[2]
Department of Science Laboratory Technology, Federal Polytechnic Ilaro, Ogun State, Nigeria.
abisoyefafioye@gmail.com[1]; olakunleomotayos@gmail.com[2]

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Abstract

This study investigated the effect of the methanol extract of *Gomphrena celosioides* leaves on acidified ethanol-induced gastric ulcer in male Wistar rats. Methanol extract of *G.celosioides* leaves was administered at 200, 400 and 800 mg/kg body weight by oral gavage and control group received 50mg/kg cimetidine 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 previously treated with *G.celosioides* leaves. The results indicate *G. celosioides* leaves have profound antiulcer properties and can 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: Antioxidant, gastric mucosa, histopathology, ulcer, wistar rat

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1. Introduction

Peptic ulcer occurs in more than one-tenth of the world population (Ishida *et al*., 2010). It is one of the most prevailing diseases of the gastrointestinal tract in the world and has become a global concern due to its increasing morbidity and mortality rates (Martins *et al*., 2014). Peptic ulcers are long-lasting and often single lesions that may occur in any part of the digestive tract (Kumar *et al*., 2017).

The etiology of peptic ulcer can be constant or sporadic, and the disease cause varies among individuals (Traversa *et al*., 1995). Dyspepsia refers to the common symptoms of peptic ulcer. It may be long-lasting and can lead to a lot of upper abdominal symptoms such as pain, nausea, vomiting blood, black or tar-like stools, unintended and unexplained emaciation and anemia.

Oxidative stress has been implicated as the major cause of stress ulcers. Research shows that psychological and physical stress such as surgical intervention and infection with microbes including *Helicobacter pylori* (Ishida *et al*.,

2010), lead to oxidative stress in the stomach. Oxidative stress, a state of elevated levels of reactive oxygen species (ROS), is the underlying cause of a lot of disease conditions that stimulate either increased ROS production or a decline in the antioxidant defence system. The mucosa lining of the stomach is exposed to stimulants like gastric acid and pepsin while gastro protective factors maintain the integrity of the gastric mucous layer, microcirculatory system, HCO_3^-, prostaglandins (PGs), epidermal growth factor synthesis amongst other factors. An imbalance between aggressive factors and mucosal defence system envelops the comprehensive process that underlines peptic ulcer disease (Pan *et al*., 2008). Asides stress, other factors that may aggravate the occurrence of peptic ulcer disease (PUD) include smoking, alcohol consumption, *H. pylori*, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Vonkeman *et al*., 2007).

Administration of drugs such as the proton pump inhibitors, antibiotics, prostaglandin analogues, and histamine receptor blockers (cimetidine and famotidine) have been reported to reduce the adverse effects of stomach ulcers. However, the discovery of novel 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 & Llesuy, 2002). An array of medicinal plants with ulcer-preventing ability has been reported by Abdulla *et al*., 2010; Mahmood*et al*., 2010; Wasman*et al*., 2010, amongst others.

Gomphrenacelosioides also is known as Soft Khaki weed is a short-lived 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 such as treatment of gastrointestinal and respiratory problems, skin infections, and so on. (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 phytochemicals such as saponins, steroids, non-reducing sugars, phenols, amino acids, and flavonoids (Viera*et al*., 1994).

This study evaluated the gastro protective effects of methanolic extracts of *G. celosioides* against HCl/ethanol-induced gastric ulcer in rats.

2. Materials and Methods

1-chloro-2,4-dinitrobenzene (CDNB), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), 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 were obtained from Sigma Aldrich(England); methanol, hydrochloric acid, potassium chloridewere purchased from BDH (England); tricarboxylic acid, ethanol, diethyl ether (Park, United Kingdom); All the chemicals and reagents used in this study were of analytical grade. Cimetidine obtained from a recognized pharmacy was prepared freshly before use.

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

2.1 Experimental Animals

Forty (40) male Wistar rats of weights 140-160g were obtained from the department of Veterinary Anatomy, University of Ibadan, Ibadan and kept in an animal house for 2weeks to acclimatize. The rats were randomly divided

into eight groups with five rats in each group. They were fed on standard pellet 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 in the rats 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 methanolic extract of *G.celosioides* leaves

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

Group 5: 0.5ml of acidified ethanol + 800mg/kg body weight of methanolic extract of *G. 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 methanolic extract of *G. 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 by the method of Lowry *et al*., 1951. SOD activity were determined by the method of Misra and Fridovich 1972. The method of Beutler*et al*., (1963) was used to assess the level of reduced glutathione (GSH). Lipid peroxidation was assayed by measuring the levels of malondialdehyde (MDA) produced during lipid peroxidation according to the method of Varshney & Kale, (1990). Glutathione peroxidase (GPx) activity was determined by the method of Rotruck *et al* 1973 with slight modifications, which is based on the reaction between glutathione remaining after the action of glutathione peroxidase GPx. The activity of xanthine oxide was determined by the method of Prajda & Weber, 1975.

2.4 Histopathological Assessment

Stomachs from rats of all groups were fixed in 10% formaldehyde, dehydrated in histological 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 methanolic extract of *celosioides* leaves on ulcer parameters in Cl/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]

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 $p<0.05$

Table 2: Effect of methanolic extract of *G.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	-	-	-

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 $p<0.05$

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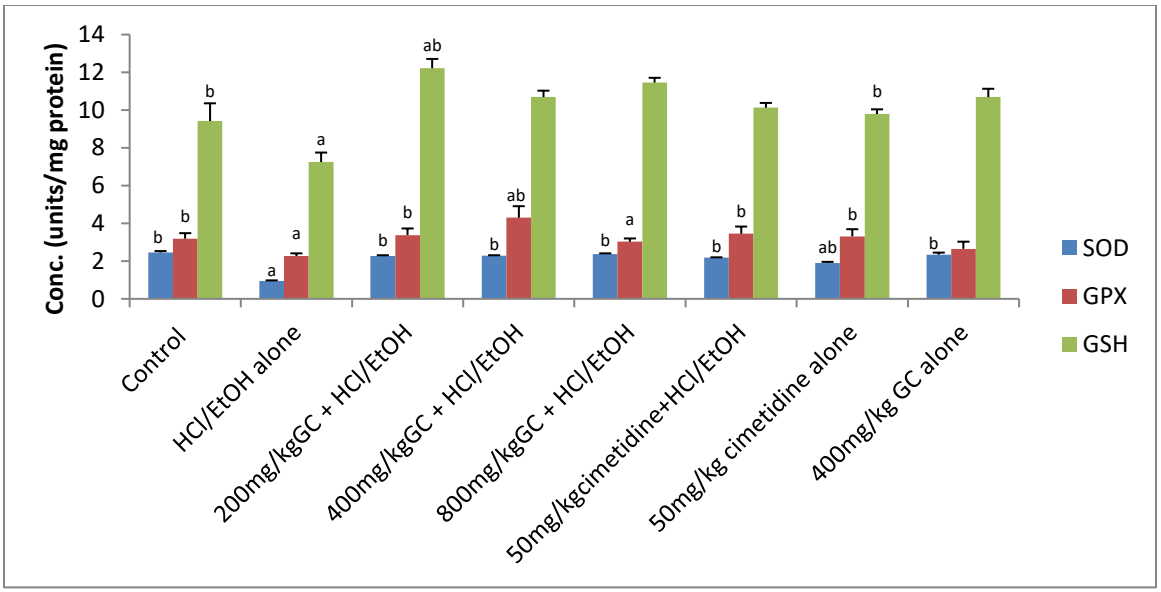

Figure 1: Effect of methanolic extract of *G.celosioides* leaves on antioxidant enzymes in HCl/EtOH induced gastric ulcer healing in male rats.

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 $p<0.05$

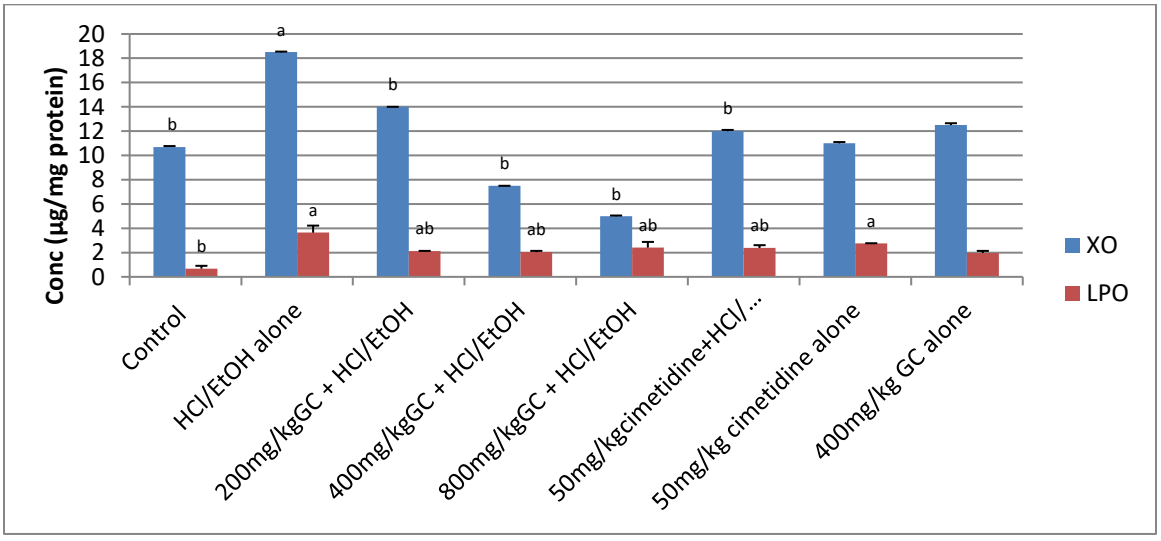

Figure 2: Effect of methanolic extract of G. *celosioides* leaves on antioxidant parameters in HCl/EtOH induced gastric ulcer healing in male rats.

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 $p<0.05$

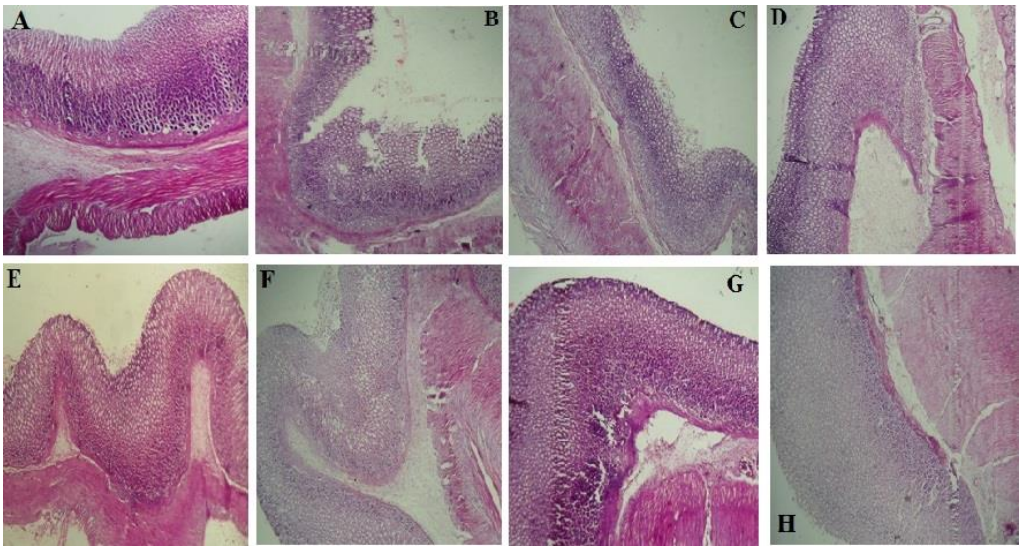

Figure 3: Histological slides of the stomach tissue.

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 lamina propria, muscularis mucosa, epithelial layer and muscular layer.

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

D: Stomach sessions from ulcerated rats post treated with 400mg/kg body weight of *G.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 methanol 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

As used in this study, acidified ethanol is an accepted model of mucosal barrier injury (Toker*et al*., 2013). It has been shown that exposing the gastric lining to oxidative stress caused by administration of ethanol (Hirokawa*et al*., 1998) leads to the production of lipid peroxides as expressed by an increase in the malondialdehyde (MDA) level of gastric tissues which is supported by the impairment of oxidative defense mechanisms including a reduction in the activities of superoxide dismutase (SOD), glutathione transferase (GST) and glutathione peroxidase (GPx) (Toker*et al*., 2013).

The efficacies of *G.celosioides* are often associated with their ability to scavenge stable free radicals (Zheng & Wang, 2001). Previous studies have shown *Gomphrena celosioides* extracts to exhibit potent in vitro antioxidant activity in determination of polyphenols, reducing power and lipid peroxidation inhibition in comparison to the known antioxidants. Polyphenols has been shown to be the major plant compounds in the scavenging of free radicals and antioxidative activity (Meite*et al*., 2014).

In this study, prior exposure of the rats to acidified ethanol may have caused severe erosion and ulcerative effects 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 acidified ethanol induced gastric ulcers. The gastroprotective effect of the plant might be related to the reduction decline in the damage to the mucosa caused by free radicals which may be due to its antioxidant action. This is supported by the findings of Panda & Sonkamble (2012).

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From this study, it can be deduced that ethanol administration brought about a significant reduction ($P<0.05$) in the activities of SOD and GPx when compared with the rats in the normal control. A decline in the activities of SOD and GPx in the stomach of rats exposed to acidified-ethanol leads to the generation of reactive oxygen species and a concomitant increase in the MDA level, thus leading to an increased mucosal damage. This confirmed the reports of several studies that reported alterations in antioxidant enzyme activities in animals exposed to acidified ethanol (Ellman, 1959; Rotruck*et al*., 1973; Sun & Zigman, 1978 ;Mohandas *et al*., 1984 Clairborne, 1985; Vandana & Madhav, 2012).

The dose related inhibition of acidified ethanol-induced decrease in activity levels of SOD, GSH and GPx when the animals were treated with *G.celosioides* leaves indicate that the plant contains biologically active compounds which can stimulate the activity of the endogenous antioxidant enzyme of the gastric system. Induction of the activity of the antioxidant defense system was supported by a decrease in malondialdehyde level (LPO). This is supported by the findings from the work of Meite*et al*., (2014). 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, this study demonstrates the protective role of the methanolic extract of *G.celosioides* leaves which can be attributed to a lot of factors including the phytochemicals present in the leaves.

4. Conclusion

This study shows the mitigating ability of the methanolic extract of *G.celosioides*leaves in the treatment of acidified ethanol induced gastric lesions in male Wistar rat. The ulcer-protecting 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 results suggest the latent therapeutic use of *G.celosioides* leaves as a potent non-toxic cure for ulcer as it is able to restore the antioxidant state of the gastrointestinal tract.

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