International Scholars Journals

International Journal of Medicine and Medical Sciences ISSN 2167-0404 Vol. 10 (3), pp. 001-004, March, 2020.
Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Evaluation of Ethanol extract of dietary vegetable, *Gongronema latifolium*, for anti-ulcer activity

Ikoro J.L

Department of Biotechnology, Faculty of Medicine, Delta State University, Abraka, Nigeria.

Accepted 22 August, 2019

Ethanol extract of dietary vegetable, *Gongronema latifolium*, was evaluated for anti-ulcer activity. The extract was obtained from air-dried, pulverized leaves of the plant following its maceration in ethanol, filteration with Whatman No. 1 filter paper and drying at 110°C. Fractionation of the dry crude ethanol extract was stepwisely carried out in with n-hexane, chloroform and ethylacetate, respectively, and their residual ethanol extract washed several times in ethanol. The four fractions were dried at low temperature and stored for use. The anti-ulcer activity of the crude extract was tested on indomethacin-induced and acid/ethanol-induced models of ulcer induction in rats. The activities of the fraction of the crude ethanol extract with respect, to reduction of ulcer index was evaluated only on indomethacin-induced ulcer model. The crude ethanol extract significantly ($p<0.05$) inhibited ulceration dose - dependently in the two ulcer models. This inhibition was higher in acid/ethanol model than in the indomethacin-induced ulcer model. The sub-fractions from the crude extract also inhibited ulcer with the chloroform fraction exhibiting the highest ulcer protection.

Key words: *Gongronema latifolium*, ulcer, protection, indomethacin, acid/ethanol.

INTRODUCTION

The epithelial lining of the gastric and intestinal mucosa is continually exposed to varied changes in chemical substances arising from intake of foods, drugs and drinks (Banks et al., 1976). As a result, tremendous disturbances that may culminate in pathological conditions such as ulcers, cancers could arise (Rang et al., 1995; Piper and Stiel, 1986). Ulcers are open sores or wounds appearing on the skin or mucus membrane caused by destruction of surface tissue (BMA, 2002; Owu et al., 2012). Peptic ulcer is one of the most rampart gastrointestinal (GIT) diseases creating a lot of pain and discomfort (Singh et al., 2008; Owu et al., 2012). Pathogenesis of ulcer has been attributed to effect of acid/ethanol (Goulart et al., 2005), non-steroidal anti-inflammatory drugs (NSAID), such as aspirin and indomethacin, used to inhibit pains, arthritis and inflammation (Vane, 1971) which can be complicated by *Helicobacter pylori* infection (Calam and Baron, 2001). Oxidative disturbances in the digestive system have also been implicated in ulcers especially, that of the activities of reactive oxygen species (ROS) (Repetto and Llesuy, 2002). Mucosal protection has been attributed to endogenous prostaglandin synthesis that stimulates the secretion of mucosa and bicarbonate layer along the GIT (Lanza, 1998).

Pharmacological intervention utilizing histamine H_2 blockers, antacids and anticholinergics have not succeeded to confer immunity from recurrence of disease or total restoration due to a number of limitation (Akhtar et al., 1992; Calam and Baron, 2001; Singh et al., 2008). Many indigenous plants used as food and spices are associated with bioactivities against ulcer and protection

*Corresponding author. E-mail: ikoro.lenin@yahoo.com..

Abbreviations: NSAID, Non-steroidal anti-inflammatory drugs; **ROS,** reactive oxygen species; **GIT,** gastrointestinal.

of gastric mucosa (Ubaka et al*.,* 2009; Ukwe et al., 2010). Plants such as *Rodina rhomifilia* containing c-glycosyl flavones (Montanha et al., 2009 and Akudor et al*.*, 2012) or *Piper nigrum* with antioxidant property (Singh et al., 2008) have been shown to exhibit ulcer protecting as well as ulcer healing properties.

Gongronema latifolium is an edible, perennial climbing vegetable located in many parts of Africa and Nigeria where it is called "Utazi" in Ibo and "Orokeke" in Yoruba. It contains bitter principle and has many folkloric attributes as a healing herb (Dalziel, 1937; Ayodele, 2008). Several studies have revealed that its leaves and stem possess anti-diabetic properties (Ugochukwu and Babbady, 2003; Ezekwe 2005) and intestinal muscle relaxant (Gamaniel and Akah, 1996; Edet et al., 2011) and anti-inflammatory properties (Morebisi et al. 2002; Etim et al*.,* 2008). It is with these characteristics in mind that this work was undertaken to ascertain the efficacy, if any, of the vegetable on drug-induced ulceration.

MATERIALS AND METHODS

Plant

Fresh leaf samples of *G. latifolium* were detached from the stem and dried under shade and pulverized.

Extraction

The pulverized leaves (1 kg) were macerated in 5 L of 96% ethanol for 48 h. The extract was filtered with Whatman No.1 filter paper and dried at 40°C.

Fractionation of crude ethanol extract

Serial fractionation of the crude ethanol extract (75 g) on silica gel using successive volumes of n-hexane (5 L), chloroform (4.6 L) and ethylacetate (2.3 L) was carried out and the residual fraction was washed in ethanol (3.7 l); each fraction was dried and samples stored for further analysis.

Animals

Albino mice (13) weighing between (17 - 30 g) were selected for the acute toxicity study. Wistar albino rats (60) weighing (150 - 250 g) were also used for the anti-ulcer activity. All were housed in metallic cages, fed standard diet and water *ad libitum* and acclimatized for seven days before the study.

Acute toxicity test

The evaluation of toxicity of the crude ethanol extract was determined on mice by the Lorke (1983) method. A two-phase assay involving initial, low dose (10, 100, 1000) mg/kg b.w of crude were administered to three groups of 3 mice each and observation made for 48 h. Then, a second phase using 1600, 2900 and 5000 mg/kg b.w was administered to three groups of one mice each and a fourth group (saline). The mice were also observed for signs of toxicity or fatality for 24 h.

Effect of crude ethanol extract on Indomethacin-induced ulcer

The method of Urishidani et al. (1979) was utilized. Gastric ulceration was induced in four groups of four rats each using oral administration of 20 mg/kg b.w. indomethacin, 30 min after each group had received oral administration of its respective extract, standard drug or saline. After 7 h, the animals were sacrificed in ether chamber and stomachs excised, dissected, washed and fixed in formal saline and mounted on slab. Ulcer craters or wounds were counted, rated from 1-3 and used to compute the ulcer scores. The ulcer indices of a group are summation of ulcer scores (number of ulcer spots × their rating and divided by the magnification). The percent ulcer inhibition was calculated.

The effect of acid/ethanol-induced ulcer (0.3 N HCl/60% ethanol)

The method of Goulart et al. (2005) was employed. An identical set of four groups of four rats each as above, that is, extract (100 /300) mg/kg, control (saline 3 ml) and ranitidine (100 mg/kg) were set up. Each group received its respective dose of extract and control orally 30 min before 25 ml/kg of the acid/ethanol solution was administered orally. After 1 h, the rats were sacrificed and their stomachs prepared using the procedure above. Ulcer indices were calculated.

Effect of crude ethanol extract and its fractions on indomethacin-induced ulcer

The effect of the crude ethanol fractions (400 mg/kg) and crude were tested at a higher level of induction using indomethacin (40 mg/kg). Seven groups of four rats each were administered their respective extracts and the same procedures as above were followed to determine ulcer indices.

Statistical analysis

The results obtained were expressed as mean± standard error of the mean (SEM) for the ulcer indices and also as percentage ulcer inhibition. Differences between means were considered significant at $p<0.05$ using students t-test.

RESULTS AND DISCUSSION

The result obtained from the acute toxicity study showed no fatality so then the crude ethanol extract of *G. latifolium* was safe for consumption up to 5,000 mg/kg b.w. Figure 1 shows that the standard reference drug significantly ($P < 0.05$) inhibited ulcer in rats induced with indomethacin. In the same manner, the crude extracts dose-dependently inhibited ulceration in rats. The highest inhibition of ulcer was 46.5% in the rats administered 300 mg/kg ethanol extract and this was better than the inhibition from ranitidine (42.0%). Figure 2 also shows that in the acid/ethanol-induced ulcer, the reference drug significantly ($P<0.05$) inhibited ulceration in rats. Similarly, the extracts dose-dependently and significantly ($p<0.05$) inhibited ulceration in rats. However, the reference drug had a higher ulcer inhibition (64.12%) than the highest extract dose of 300 mg/kg (62.46%). Table 1 shows the effect of the crude ethanol extract and its fractions had on

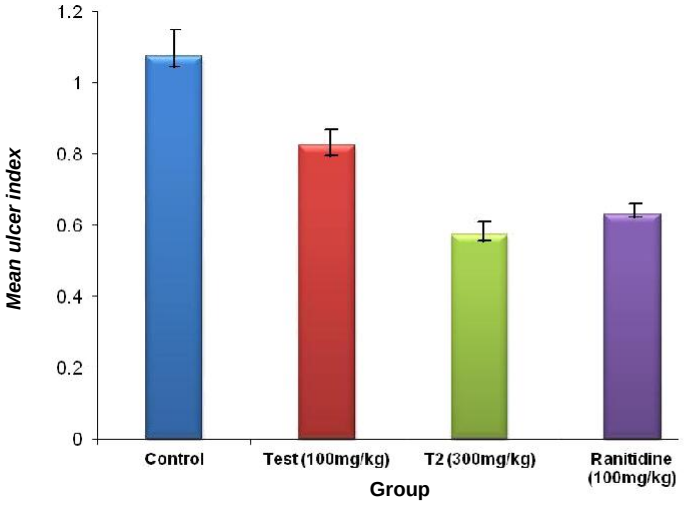

Figure 1. Effect of ethanol extract on *G. latifolium* (100, 300 mg/kg), control (3 ml/kg, 0.9%Nacl) and ranitidine (100 mg/kg) on indomethacin - induced gastric ulcer. ANOVA and students t-test were used to determine significant difference from control.

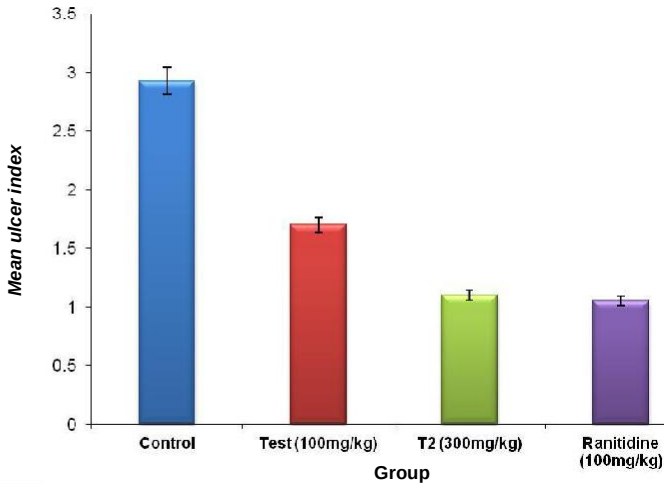

Figure 2. Effect of ethanol extract of *G. latifolium* on (100, 300 mg/kg), control (3 ml 0.9% Nacl), and ranitidine 100 mg/kg on acid/ethanol (25 ml) - induced gastric ulcer. ANOVA and Dunnets test were used to determine significant difference from the control.

severe ulceration induced by 40 mg/kg b.w indomethacin. The highest inhibition of ulcer was given by the chloroform fraction (78.2%) and the least by the residual ethanol fraction (29.02). In comparison, the reference had the highest ulcer inhibition (87.7%) while the crude exhibited only (62.3%).

The outcome of this study revealed that the crude ethanol extract and its fractions inhibited ulceration of the gastric mucosa. Oral administration of these extracts prior to the exposure of the gastric mucosa to necrotizing agents (indomethacin and acid/ethanol) resulted in significant protection from ulceration. The crude extract dose-dependently inhibited ulcer formation in indome0-thacin and for acid/ethanol models. The extract was not hindered by the mode of inducing the ulcer but compared favourably with the reference drug ranitidine for the two models, respectively. The crude extract also showed significant inhibition of ulcer in a more severe ulcer induction using 40 mg/kg indomethacin with ulcer inhibition of 62%. In effect, the ulcer inhibition increased from 23% (100 mg/kg), 46% (300 mg/kg) to 62.3% (400 mg/kg). This showed a case of consistent dose- dependent activity.

The performance of the fractions of the crude in ulcer inhibition at a severe ulcer induction of 40 mg/kg indomethacin was more impressive. The fraction with the highest ulcer protection was the chloroform extract followed by the n-hexane extract. The least was the residual ethanol extract. This trend seems to suggest that the components of the fractions must have a contributory effect on their ulcer inhibition and these are located in the non-polar phase. Phtochemical study by Ezekwe (2005) showed that chloroform fraction contained alkaloid, flavonoid in addition to steroids, terpenoid, fats and oils.

The mechanism of ulcer inhibition by these extracts is not very obvious. The maintenance of mucosal integrity is achieved by a number of mucosal protective devices among which are secretion and action of mucus and bicarbonate (Rang et al., 1995; Shlafer and Marieb, 1989). Prostaglandins stimulate secretion of mucus and bicarbonate (Rang et al., 1995) and especially

prostaglandin E_2 and I_2 are implicated in maintaining gastric integrity (Hogan et al., 1994; Akudor et al., 2012) and also mucosal integrity and regeneration (Lanza, 1998). However NSAID, such as indomethacin are potent inhibitors of prostaglandin synthesis thereby promoting ulceration (Vane, 1971; Shlafer and Marieb, 1989). From this study, it can be seen that by inhibiting indomethacin induced ulceration of the mucosa, the extracts may be preventing the hindering generation of prostaglandin.

Another possible mechanism of ulcer protection is that observed in the inhibition of acid/ethanol induced injury to the mucosa. Ethanol challenge elicits production of oxygen free radicals, that is, reactive oxygen species, which generate lipid peroxidation that cause damage to cell and cell membrane (Cheeseman, 1993; Pihan, 1987; Owu et al., 2012). This result in lesions on the mucosal membrane (Singh et al., 2008) and the gastric mucosal ulceration causes severe damage to the system (Goulart et al., 2005) which can be alleviated by antioxidant especially from natura sources (Cetto and Llesuy, 2002). The crude extract exhibited as much inhibition of ulcer from acid/ethanol as the standard reference drug, ranitidine. This tends to suggest that the crude ethanol extract may be an inhibitor of reactive oxygen species generation and may possess strong antioxidant property. This needs to be investigated.

Table 1. Effect of sub-fractions and crude ethanol extract on indomethacin-induced ulcer.

Group	Dose (ml/kg)	Mean ulcer index	Ulcer inhibition (%)
Control	3	6.10±0.94	0.00
n-Hexane	400	1.83±0.47*	70.00
Chloroform	400	1.33±0.37*	78.20
Ethylacetate	400	3.13±0.54*	48.69
Residual ethanol extract	400	4.33±0.49*	29.02
Ranitidine	100	0.75±0.16	87.70
Crude ethanol extract	400	2.30±0.47*	62.30

Ulcer indices are expressed as mean + SEM. *Statistical significance against control at $P < 0.05$ with ANOVA test, followed by Dunnet posthoc analysis.

REFERENCES

Akudor GC, Mbah CC, Essein AD, Akpan JL, Ezeokpo BC, Iwuanyawu TC, Osunkwo UA (2012). Ulcer-protective and Antidiarrhoeal effects of the aqueous Stem bark of *Bridelia ferruginea* in Rodents.Pharmacolgia, 3:591-597.

BMA (2002). The British Medical Association Illustrated Medical Dictionary Darling Kimberly Limited, London.

Calam J, Baron JH (2001). ABC of the upper gastrointestinal tract: Pathophysiology of duodenal and gastric ulcer and gastric cancer. Brit. Med. J. 323:980-982.

Cheeseman KH (1993). Lipid peroxidation in biological systems, London: Ellis Cytoprotol, by alcohol, HCl, NaOH, Hypertonic Nacl and thermal injury. Gastroenterol. 17:433-43.

Dalziel JM (1937). The useful plants of West Tropical Africa. Crown Agents for the Colonies, London.

Edet EE, Akpanabiatu MI, Uboh FE, Edet TE, Eno AE, Itam EH (2011).*Gongronema latifolium* Crude leaf extract reverses alterations in haematological Indices and weight loss in diabetic rats. J. Pharmacol. Toxicol. 6:81-174.

Etim OE, Akpan EJ, Usoh IF (2008). Hepatotoxicity of carbon tetrachloride;Protective Effects on *Gongronema latifolium.* Pak. J. Pharm. Sci. 21:74-268.

Ezekwe CI (2005). The effect of *Gongronema latifolium Benth*(*Asclepiadaceae*) on some oxidative parameters of alloxan-induced diabetes mellitus being M.Sc thesis presented in the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria,Nsukka

Gamaniel KS, Akah PA (1996). Analysis of the gastrointestinal relaxing effect of the stem extract of *Gongronema latifolium*. Phytomed. 2(4):293-296.

Goulart YCF, Sela VR, Obici S, Martins JVC, Otobone F, Cortez DA, Audi EA (2005). Evaluation of gastric anti-ulcer activity in a hydroethanol extract from *Kielmeyera coriacea.* Brazillian Archives Biol. Technol. 46(1):211-216.

Hogan DL, Ainsworth Ma, Ibensberg JI (1994). Gastroduodenal bicarbonate secretion eliminate. Pharmacol. Therap. 8:475-479

Lanza FL (1998). A guide for the treatment and prevention of NSAID-induced ulcers. Amer. J. Gastroenterol. 90:2037-2045.

Lorke D (1983).A new approach to practical acute toxicity testing. Archiv. Tox. 54:251-281.

Montanha JA, Schenkel EP, Cardoso - Taketa AT, Dresch AP, Langeloh A, Dallegrave E. (2009). Chemical and anti-ulcer evaluation of *Jodina rhombifolia* (Hook and Arn.) Reissek extracts. Braz. J. Pharmacog, 19(IA):29-32.

Morebisi O, Fafunso MA, Makinde JM, Olajide OA, Awe EO (2002).Anti inflammatory property of the leaves *Gongronema latifolium.* Phytotherapy Res. 16 (51): 75-77.

Owu DU, Nwokocha CR, Essien AD, Ikpi DE, Osim EE (2012). Effect of *Gongronema latifolium* ethanol leaf extract on gastric acud secretion and cytoprotection in streptozotocin-induced diabetic rats. West Ind. Med. J. 61:1-27.

Pihan G, Regillo C, Scrabo S (1987). Free radicals and lipid peroxidation in ethanol or aspirin induced gastric mucosal injury. Digest. Dis. Sci. 32:1395-1401.

Piper DW, Stiel DD (1986). Pathogenesis of chronic ulcer, current thinking and clinical implications. Med. Progression 2:7-10.

Rang HP, Dale MM, Ritter JM, Gardener P (1995). The gastrointestinal tract, in Pharmacology, 3rd ed., Churchill Livingstone, New York, USA, 386-402.

Repetto MG, llesuy SF (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcer. Brazilian J. Med. Biol. Res. 35:523-534.

Singh R, Madan J, Rao HS (2008). Pharmacognosy Magazine 4(15):232 235.

Ugochukwu NH, Babbady NE (2003). Anti-hyperglycaemic effect of aqueous and ethanolic-extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetes. J. Biosci. 28(1):1-5.

Ukwe CV, Ubaka CM, Madusque UJ (2010). Evaluation of the antiulcer activity of *Olax Subscorpioidea* Oliv roots in rats. Asian Pacific J. Trop. Med. 3:1-5.

Urishidani TU, Kasanya OS (1979). The mechanism of aggravation of indomethacin-induced gastric ulcer by adrenalectomy in rats. Japan J. Pharmacol. 89:775-779.

Vane JR (1971). Inhibition of prostaglandins as mechanism of action for aspirin like drugs. Nature 231:232-235.