AMELIORATIVE EFFECTS OF ETHANOL EXTRACT OF *SYZYGIUM AROMATICUM* (CLOVE) ON INDOMETHACIN-INDUCED GASTRIC ULCER IN ALBINO RATS

[1]UHUO, Emmanuel Nnaemeka, [1]MBA, Joseph Obinna, [1]UROKO, Robert Ikechukwu and [2]CHIME, Mirabel Ogochukwu

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

[2]Department of Chemical Sciences, Godfrey Okoye University, Enugu, Enugu State, Nigeria.

Corresponding Author: Uhuo, E. N. Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. **Email:** uhuo.emmanuel@mouau.edu.ng **Phone:** +234 8067346144

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ABSTRACT

Impaired gastroprotection and increased gastric acid secretion has been attributed to decrease prostaglandin level which are important events in the etiology of mucosal ulceration. The study evaluated the ameliorative effect of ethanol extract of Syzygium aromaticum on indomethacin-induced gastric ulcer in rats. Forty five male albino rats of mean weight (105 ± 0.71 g) were used in this study. Rats were grouped into five groups having six rats per group. Gastric ulcer was induced with indomethacin (40 mg/kg body weight).Group 1 received feed and water only. Group 2 was the negative control induced with indomethacin without treatment. Group 3 received standard drug (omeprazole 20 mg/kg body weight). Group 4 and 5 were administered 100 and 500 mg/kg body weight of the extract respectively. Treatment was carried out once daily for 21 days prior to ulcer induction. Gastric secretions, antioxidant parameters and histopathological defects were evaluated. There was significant increase (p<0.05) in ulcer indices; gastric volume, acidity, malondialdehyde and nitric oxide levels in the negative control against the test group that received 100 mg kg of the S. aromaticum ethanol extract. Also, significant decrease (p<0.05) of these parameters were observed between the negative control and the positive control. Decrease in the ulcer indices were recorded in the group 3 compared with Groups 4 and 5. The protective effect of the S. aromaticum extract was buttressed by degree of percentage inhibition against ulceration. It is therefore; evident that the ethanol extracts of the plant can ameliorate indomethacin induced gastric ulceration.

Keywords: Gastric ulcer, Indomethacin, *Syzygium aromaticum*, Antioxidants, Omeprazole

INTRODUCTION

Ulceration is a non-malignant injury on the mucosal epithelium upon exposure of the stomach to surplus acid and belligerent pepsin activity (Khazaei and Salehi, 2006). It is the most predominant gastrointestinal ailment ever known, summing up approximately 15 deaths out of every 15,000 diseases annually in the world (Sonnenberg, 1985; Shristi *et al.,* 2012). Despite the increasingly changing notion of gastric ulcer treatment from conventional vagotomy, prostaglandin analogs, H_2 receptor antagonists and antacids to proton pump inhibitors, gastrointestinal toxicity remains impairment to their usage in clinical practice. Explicitly, gastrointestinal toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) occurrence

might be as high as 4 – 8 % per annum and the impairments are even greater for those with extra risk factors such as previous history of ulcer disease (Griffin and Scheiman, 2001). Several synthetic antiulcer drugs are currently obtainable and some of these like cimetidine, misoprostol, ranitidine, omeprazole and esomeprazole are used to manage and treat NSAID-induced gastric ulcer. Moreover, each of these drugs confers harmful side effects, resulting to a search for non-toxic, easily available and cheap antiulcer medication (Akah *et al.,* 1998). Examination of the phytotherapy of medicinal plants that are greatly valued and extensively employed in the traditional systems of medicine might offer effective formulation for improved management of ulcer (Hawkins and Hanks, 2000).

Syzygium aromaticum Merrill and Perry (Myrtales: Myrtaceae) commonly called clove is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes. Clove represents one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid and possesses great potential for pharmaceutical, cosmetic, food and agricultural applications. The antioxidant and antimicrobial activity of clove is higher than many fruits, vegetables and other spices and should deserve special attention (Cortés-Rojas *et al.,* 2014).

NSAIDs are broadly employed for their analgesic, anti-inflammatory antipyretic potentials. Nevertheless, their administration is often related with peptic ulcer induction (Lee *et al*., 2016). Indomethacin is a significant member of the NSAIDs family. It is widely used in joint stiffness, arthritis and obstetrics to delay uterine contractions. In neonatal unit, it is equally used to facilitate patent ductus arteriosus closure. However, it provokes aggressive ulcerogenic potential in both animals and humans (Simon, 1993). Several earlier studies have attempted to reduce damaging indomethacin effect on gastrointestinal mucosa (Cheng *et al.,* 2017; Soliman *et al*., 2017; Ibrahim *et al.,* 2017).

Indomethacin induces gastrointestinal toxicity through numerous ways by increasing gastric acid secretion; interferes with mucosal cell regeneration through inhibition of prostaglandin E2 (PGE2) synthesis, production of free radicals, a decline in gastric nitric oxide concentration and invasion of activated neutrophils as well as stimulation of gastric cells apoptosis (Matsui *et al*., 2011). The present study was undertaken to study the ameliorative effect of ethanol extract of *S. aromaticum* on indomethacin induced gastric ulcer in albino rats.

MATERIALS AND METHODS

Plant Material: The plant was obtained from Eziagu in Enugu State, Nigeria. Identification (Fayaz, 2011) and authentication was done by a plant taxonomist in the Department of Plant Biology and Biotechnology Herbarium Unit, Faculty of Life Sciences, University of Benin, Benin City, Edo State in collaboration with Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, where voucher specimen (UBH-S385) was deposited in the Department's herbarium.

Experimental Animals: Forty five (45) male Wistar rats of mean weight 105 ± 0.71 g purchased from the Animal Breeding and Genetics Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka were used for this research. They were housed in metallic cages of six rats per cage and were fed *ad libitum* with pellet growers feed (19 % protein and 3.3 ME/Kg metabolizable energy) (Vital, Nigeria) with free access to water. Rats were acclimatized for two weeks in 12 hour light/dark daily cycle in line with the recommendation of the guide for the care and use of experimental animal (NRC, 2010).

Chemicals and Drugs: Indomethacin and omeprazole were respectively procured from Kapit Pharmaceutical Limited, Nigeria and Ranbaxy Laboratories, India. Trichloroacetic acid (TCA), dimethylaminobenzaldehyde, epinephrine, acetyl acetone, gallic acid, aluminium chloride, quercetin and thiobarbituric acid (TBA) were obtained from Sigma Chemical Company, St. Louis, MO, USA. Kits used were obtained from

Randox Laboratory Limited, United Kingdom. Other chemicals were of analytical grade.

Preparation of Extract: The leaves of *S. aromaticum* were air dried at room temperature for 10 days to constant weight. The dried samples were then pulverized with an electric blender (MS-223; Blender/Miller III Taiwan, China), weighed and kept air tight prior to extraction. 500 g of milled sample was extracted in 1.5 litres of analytical grade ethanol for 72 hours with continuous shaking by orbital shaker maintained at 300 rpm. The solutions obtained were then filtered with Whatman Number 4 filter paper and the filtrate was concentrate using rotary evaporator at 40^{o}C.

Experimental Design: The experiment was laid done using a complete randomized design of five treatments replicated thrice, with each replicate having three rats. Group 1 (Vital Feed and water only - positive control). Group 2 (Indomethacin-induced gastric ulcer untreated - negative control). Group 3 (Indomethacin-induced gastric ulcer treated with 20 mg/Kg b.wt of omeprazole - standard control). Group 4 (Indomethacin-induced gastric ulcer treated with 100 mg/Kg b.wt of *S. aromaticum* ethanolic extract and Group 5 (Indomethacin-induced gastric ulcer treated with 500 mg/Kg b.wt of *S. aromaticum* ethanolic extract. Treatment was carried out once daily for 21 days prior to ulcer induction.

Induction of Ulcer: The procedure described by Bhattacharya *et al*. (2007) was used for the induction of the ulcer. Briefly, the rats were administered with a single oral dose of indomethacin (40 mg/kg body weight). They were deprived of food but had free access to water 24 hours prior to ulcer induction. Various degrees of ulceration (one or two lesions, severe lesions, very severe lesions, mucosa full of lesions) manifested four hours after indomethacin administration.

Isolation of Stomach and Collection of Gastric Juice: One rat per replicate was sacrificed under anaesthesia by cervical dislocation on the twenty-third day. The stomach was excised, thereafter opened along greater curvature and gastric content was drained into a centrifuge tube. Five (5) ml of distilled water was added and resultant solution centrifuged at 3000 rpm for 10 minutes. The supernatant obtained thereafter was used for biochemical analyses. The stomachs samples were preserved in 0.1 M phosphate saline buffer (1:4 w/v, pH 7.4) prior to homogenization and microscopic examination.

Determination of Gastric Secretion Parameters: Gastric acid output (volume) was determined in the supernatant (2 ml) by titration with 0.0025 N NaOH using Toepfer's reagent as indicator (Shay *et al*., 1945). Gastric juice pH was determined with a pH meter.

Quantification of Ulceration: Degrees of ulceration in the indomethacin-induced rats were quantified by the procedure outlined by Szabo and Hollander (1989).

Cleaned stomachs were pinned on a corkboard and ulcers were scored using dissecting microscope with square-grid eyepiece based on grading on a 0 – 5 scale (showing severity of vascular congestions and lesions/haemorrhagic erosions). Mucosal damaged areas were expressed as a percentage of the total surface area of the glandular stomach in square millimetres. Mean ulcer score for each animal was expressed as ulcer index (U.I) and the percentage of inhibition against ulceration was determined using the expressions: U.I. = [Ulcerated area/total stomach area] ×100 % Ulcer inhibition = [U.I. in control - U.I. in test] × 100/U.I. in control.

Superoxide Dismutase: Superoxide dismutase (SOD) activity was determined using the method of Xin *et al*. (1991). Briefly, 0.9 ml of distilled water and 0.1 ml of sample was pipetted into test tubes. Afterwards, 0.1 ml of this mixture was mixed with 0.9 ml of carbonate buffer, and 75 µl of xanthine oxidase added. The absorbance was read at 500 nm for 3 minutes at 20 seconds interval. The change in rate of absorbance was used to determine the superoxide dismutase activity.

Lipid Peroxidation: The determination of malondialdehyde (MDA) concentration was by the method of Wallin *et al*. *(*1993). Briefly, 0.1 ml of sample, 0.9 ml of distilled water, 0.5 ml of 25 % trichloroacetic acid (TCA) and 0.5 ml of 17 % TBA in 0.3 % NaOH were pipetted into test tubes. The mixture was incubated at 95°C for 40 minutes and cooled in water after incubation. Afterwards, 0.1 ml of 20 % sodium dodecyl sulphate was added to the mixture. The absorbance of the mixture was determined at 532 and 600 nm against a blank.

Determination of Reduced Glutathione (GSH): Reduced glutathione (GSH) was determined by the method described by Ellman (1959). This method was based on the development of yellow colour when 5, 5'- dithio-bis-2-nitrobenzoic (DTNB) is added to compound containing sulphydryl groups. The colour developed was read at 412 nm in spectrophotometer. A volume, 0.2 ml of sample was mixed with 1.8 ml of EDTA solution. To this 3.0 ml of precipitating reagent was added, mixed thoroughly and kept for 5 minutes before centrifugation. To 2 ml of the filtrate, 4 ml of 0.3 M disodium hydrogen phosphate solution and 1 ml of DTNB reagent were added and the colour developed was read at 412 nm in spectrophotometer. A set of standard solutions containing 20 – 100 µg of reduced glutathione was treated similarly. The values were expressed as mg/dl for plasma.

Histopathological Evaluation: Gastric tissues were fixed in buffered 10 % formalin and processed for histopathological examination as described by Abdel-Raheem (2010). Briefly, four micrometer-thick paraffin sections were prepared and stained with Haematoxylin and Eosin for light microscope examination by a pathologist.

Statistical Analysis: Data collected were analysed using one-way analysis of variance (ANOVA). Level of significance was used to assess significant difference between the control and treated group at $p<0.05$. Results were expressed as mean ± SEM.

RESULTS

In group1, pH recorded the highest mean value among the ulceration essay, while the mucosa weight had the least mean value. The values were not statistically significant ($p>0.05$) with a probability value of $p = 0.29$. The ulcer index and % ulcer inhibition had no record for the mean value hence ignoring its values since zero was irrelevant (Table 1).

For Group 2, ulcer index recorded the highest mean value; while mucosa weight recorded the least means value (Table 1). The values were not statistically significant ($p>0.05$) with a probability value of $p = 0.40$).

For Group 3, % ulcer inhibition had the highest mean value, while mucosa weight had the least mean value (Table 1). The values were not statistically significant ($p>0.05$) with a probability value of $p = 0.08$).

For Group 4, ulcer index recorded the highest mean value; while mucosa weight recorded the least mean value. The values were not statistically significant ($p>0.05$) with a probability value of $p = 0.43$.

For Group, 5 % ulcer inhibition had the highest mean value, while mucosa weight had the least mean value (Table 1). The values were statistically significant ($p<0.05$) with a probability value of $p = 0.04$.

In Group 1, reduced glutathione recorded the highest mean value among the antioxidants, while MDA had the least mean value. The values were statistically significant ($p<0.05$) with a probability value of $p<0.00$ (Table 2).

For Group 2, reduced glutathione had the highest mean value, while nitric oxide had the least mean value. The values were statistically significant ($p<0.05$) with a probability value of $p<0.00$ (Table 2).

In Group 3, reduced glutathione had the highest mean value, while MDA recorded the least mean value. The values were statistically significant ($p<0.05$) with a probability value of $p<0.01$ (Table 2).

For Group 4, reduced glutathione recorded the highest mean value, while nitric oxide recorded the least mean value.

Table 1: Indices of indomethacin-induced gastric ulcer in albino rats treated with ethanol extract of S*yzygium aromaticum* (clove)

Group ID	Ulcer index	% ulcer inhibition	Mucosa weight (g)	pH	Gastric juice weight (g)	Gastric juice volume (ml)	Acidity
Group 1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.02 ± 0.00^{c}	5.95 ± 0.46^{e}	0.45 ± 0.05^{c}	0.47 ± 0.04^{c}	5.29 ± 0.12^{d}
Group 2	68.00 ± 2.83^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.92 ± 0.63^{a}	0.16 ± 0.13^{a}	0.26 ± 0.11^{a}	2.60 ± 0.28^{a}
Group 3	21.50 ± 3.54^{c}	77.67 ± 4.61^{d}	0.00 ± 0.00^{a}	5.14 ± 0.23^{d}	0.30 ± 0.12^{b}	0.40 ± 0.03^{b}	4.70 ± 2.40^{c}
Group 4	64.50 ± 3.54^{d}	38.26 ± 6.09^{b}	0.00 ± 0.00^{a}	3.02 ± 0.21^{b}	0.36 ± 0.07^{b}	0.43 ± 0.10^{bc}	2.75 ± 1.06^{a}
Group 5	12.00 ± 4.24^{b}	57.09 ± 2.80^{c}	0.01 ± 0.00^{b}	4.60 ± 0.18^{c}	0.51 ± 0.10^{d}	0.55 ± 0.07^{d}	3.60 ± 0.85^{b}

Superscripts indicate comparison along columns. Values in same column with different letter superscript are significantly different (p<0.05)

Table 2: Antioxidant profiles of indomethacin-induced gastric ulcer albino rats treated with ethanol extract of S*yzygium aromaticum* (clove)

Group ID	MDA (mg/dl)	SOD (U/mg)	Reduced glutathione (mg/dl)
Group 1	0.03 ±0.01^{a}	9.07 ± 0.68^{a}	16.74 ± 1.30^{a}
Group 2	3.71 ± 0.42^{e}	22.98 ± 3.17^{e}	32.90 ± 3.36^{d}
Group 3	0.08 ± 0.02^{b}	10.78 ± 0.35^{b}	16.11 ± 2.59^{a}
Group 4	2.38 ± 0.30^{d}	21.03 ± 2.95^{d}	30.68 ± 1.11^{c}
Group 5	0.95 ± 0.09^{c}	15.01 ± 1.72^{c}	17.51 ± 1.68^{b}

Superscripts indicate comparison along columns. Values in same column with different letter superscript are significantly different (p<0.05), MDA = Malondialdehyde, SOD = Superoxide dismutase

The values were statistically significant (p<0.05) with a probability value of p<0.00.

Reduced glutathione in Group 5 recorded the highest mean value, while nitric oxide recorded the least mean value. The values were statistically significant with a probability value of p = 0.00 (Table 2).

Histopathology of the ulcerated stomachs of rats indicated normal stomach with muscularis external (ME) in control rats (Figure 1).

Moderate to severe effect on the lining of the stomach with severe ulceration, moderate aggregate of inflammatory cell within the ulcerated area was observed in indomethacin-induced gastric ulcer untreated rats - negative control (Figure 2). The indomethacin-induced gastric ulcer treated rats with 20 mg/Kg b.wt of omeprazole - standard control (Group 3) demonstrated moderate healing with mild ulceration within the submucosa (Figure 3). Figures 4 and 5 arising from rats treated with 100 and 500 mg/kg b.wt with *S. aromaticum* ethanolic extract respectively had moderate healing with mild ulceration and moderate aggregate of inflammatory cell within the submucosa. Mild distortion and clumping within the mucosa was also recorded.

DICUSSION

Inhibitory activity of indomethacin on prostaglandin synthesis coupled with free radicals formation has been suggested as critical biochemical events in the pathogenesis of gastric ulceration (Lichtenberger, 1995; Inas *et al.*, 2011; Ajani *et al.*, 2015). These events might be of utmost relevance in designing new antiulcer drugs. The inherent adverse effects and affordability of synthetic drugs, exploiting natural products of plant source which are believed to be less toxic, efficacious and affordable will be most appropriate in the management of gastric ulcer. Phytotherapy is rapidly gaining grounds in sustaining human health and in the prevention of certain diseases like gastric ulcer resulting from drug toxicity (Raji *et al.*, 2011).

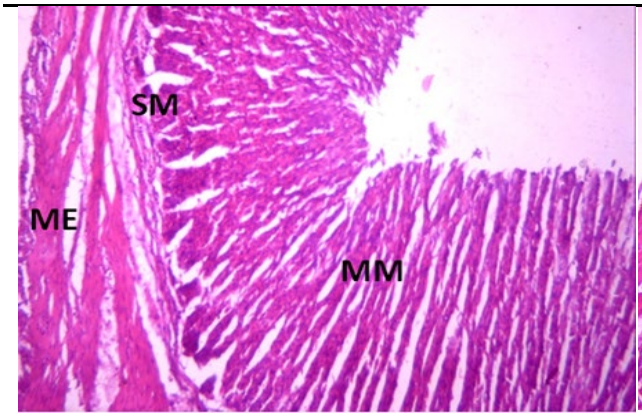

Figure 1: Photomicrograph of Group 1 (control) section of rat stomach showing normal stomach with muscularis externa (ME), the muscularis mucosa (MM) and submucosa (SM), H&E, x150

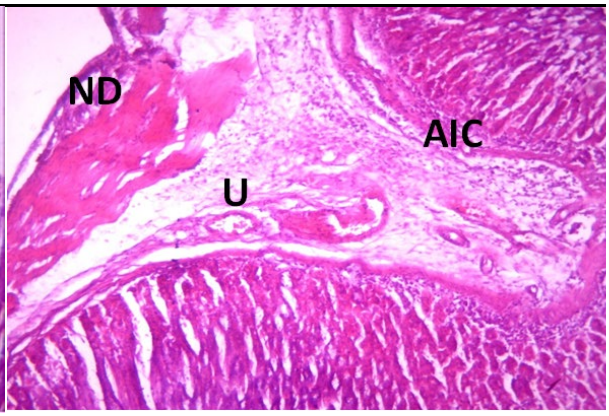

Figure 2: Photomicrograph of Group 2 rats induced with 40 mg/kg of indomethacin only showing moderate to severe effect on the lining of the stomach with severe ulceration (U) and moderate aggregate of inflammatory cell (AIC) within the ulcerated area at submucosa layer and mild necrotic debris (ND) within the muscularis externa, H&E, x150

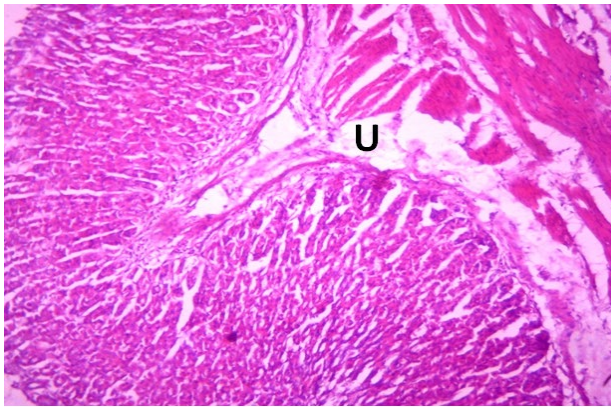

Figure 3: Photomicrograph of Group 3 rats induced with 40 mg/kg of indomethacin and treated with 20 mg/kg of omeprazole showing moderate healing with mild ulceration (U) within the submucosa, H&E, x150

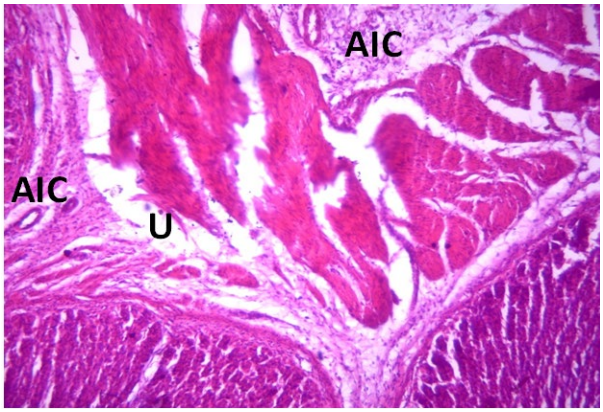

Figure 4: Photomicrograph of Group 4 rats induced with 40 mg/kg of indomethacin and treated with 100 mg/kg of aqueous extract of cloves showing mild to moderate healing with mild ulceration (U) and moderate aggregate of inflammatory cell (AIC) within the submucosa, H&E, x150

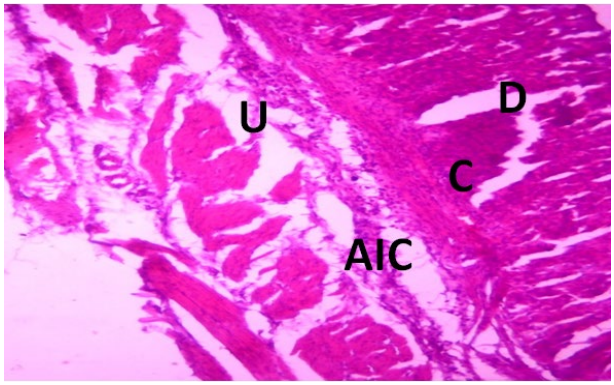

Figure 5: Photomicrograph of Group 5 rats induced with 40 mg/kg of indomethacin and treated with 500 mg/kg of aqueous extract of cloves showing moderate healing with mild ulceration (U) and moderate aggregate of inflammatory cell (AIC) within the submucosa and mild distortion (D) and clumping (C) within the mucosa, H&E, x150

This has been ascribed to possession of phytonutrients with excellent antioxidant properties that play significant roles in managing toxicity related disorders. Studies have revealed the presence of some bioactive principles in ethanol extract of *S. aromaticum* and as well have been reported to promote good health (Batiha *et al.,* 2020; Mahmoud *et al*., 2021). Biochemical evaluation of gastric secretions and mucosal integrity of stomach is usually employed to ascertain its status immediately after the exposure to pharmacological agents (Adhikary *et al*., 2011). The pH gives an insight of the degree of acidity and volume of gastric secretions. Low pH value signifies decreased hydrogen ion concentration in gastric juice. It has been attributed to pathogenesis of ulcer and gastric damage in experimental animals (Lüllmann *et al*., 2000). Inas *et al.* (2011) also correlated gastrointestinal injury to eroded mucin content. The onslaughts of both internal (pepsin and oxidants produced in the gastric lumen) and external (drugs and chemicals) aggressive agents on mucosal epithelia has facilitated this erosion.

In this research, the significant increase in ulcer indices and gastric volume following oral administration of indomethacin to the experimental animals may be attributed to either free radicals formation as well as the inhibition of prostaglandin synthesis. Decreased prostaglandin synthesis has been linked to impaired gastroprotection and increased gastric acid secretion, the important events in the etiology of mucosal ulceration. This agreed with the reports of Beck*et al*. (2000), Adhikary *et al*. (2011) and Ashraf *et al*. (2012) who demonstrated that indomethacin caused alterations in gastric secretions of rats. Conversely, administration of the extracts significantly reduced the ulcer indices. In fact, the result of pH compared with both normal control and standard drug used in this study suggested possible gastroprotective attributes.

Release of preformed mucus, wound retraction and re-epithelialization were involved in ulcer-healing process after toxicological injury (Naito *et al*., 1995). Besides providing significant buffering capacity for the neutralization of luminal acid, the mucus also offers protection against both endogenous aggressors and exogenous gastro toxic agents such as indomethacin, thereby enhancing the rate of local healing process (Alanko *et al*., 1999).

In this study, the increase in acidity coupled with decrease in mucin secretion in the experimental rats indicated changes in hydrophobicity and impaired protective ability of the mucosal membrane against haemorrhagic erosion, thus, resulting in tissue damage. This may be attributed to inability of the gastric mucosa to withstand the offensive onslaught of indomethacin. For the facts that antioxidant protects the mucus layer and inhibits ulcer progression, agents that increase the synthesis and secretion of gastric mucus would facilitates gastric ulcer healing. Administration of the plant extract extracts however, improved the healing process. This mechanism has encouraged accelerated wound healing of the affected areas of the mucosal epithelia and shielded the gastrointestinal membrane, thus abrogating the catastrophic influence of indomethacin in the ulcerated rats (Naito *et al.,* 1995). This may be inferred to mucus secretory potential of the extracts and suggestive of their significant role in the mechanism of healing ulcer. The extract demonstrated its ability to heal mucosa epithelia cell damage at 500 mg/kg body weight, indicating a better ulcer healing capacity as against the reference drug used.

Cells or tissues are in a stable state if the rates of free radical formation and scavenging capacity are essentially constant and in equilibrium. However, disequilibrium between free radical and oxidants further deregulates cellular functions leading to different pathological conditions (Sabiu *et al*., 2014). In this research, increased concentration of MDA and reduced activity of SOD in the test rats was an indication of lipid peroxidation and over production of free radicals resulting in mucosal damage. Free radicals hinder antioxidant enzymes activities and trigger lipid peroxidation which is an essential event in the toxicity mechanism of indomethacin (Halici *et al.,* 2005). Indomethacin was reported to decrease antioxidant enzymes activities in rat thereby inducing gastric ulceration (Odabasoglu *et al.,*

2006). This occurred because cellular antioxidant defense systems were overwhelmed by free radicals leading to oxidative injury. However, the significantly reduction in concentrations of MDA coupled with marked increase in the activity of SOD in rats treated with ethanol leaves extract of *S. aromaticum* was an obvious indication of antiperoxidative potential and thus its anti-oxidative ability.

Generally, the protection offered by the ethanol extract of *S. aromaticum* against indomethacin-induced gastric ulcer may be linked with their beneficial medicinal attributes occasioned by its phytometabolite constituents. The ability to scavenge free radicals and regulate mucosal membrane permeability makes it better option to manage ulcer induced by indomethacin. This was in agreement with the findings of Inas *et al.* (2011), Ashraf *et al.* (2012) and Gege-Adebayo *et al.* (2013) where gastroprotective potentials of plant extracts against indomethacin-ulcerated rats were attributed to polyphenolic compounds and other various bioactive constituents. Since omeprazole is a proton pump inhibitor, then the effect produced by the ethanol extract of *S. aromaticum* might have perhaps mimic its mechanism of action by modulating cells in the mucosal lining of the stomach against excess acid secretion (Tulassay *et al.*, 2008; Fornai *et al.*, 2011). Evidence of necrotic tissue was clearly observed in the test group with reference to treated group, especially those that received high dose of the extract. It is, therefore, in agreement with results of other parameters so far discussed.

Conclusion: The research indicates that ethanol extract of *S. aromaticum* exhibits gastro ameliorative and antioxidative potentials in rats and could be used in the management of ulcers and diseases implicated by free radicals.

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