

Anti-Ulcerogenic Potential of Aqueous Extract of *Securinega virosa* Leaf in Indomethacin-Induced Ulcerated Rats

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Abstract

The anti-ulcerogenic activities of *Securinega virosa* aqueous leaf extract on gastric ulcer induced with indomethacin in albino rats were studied. Thirty rats weighing 120-200 g were grouped into six groups of five rats each. All groups except the uninduced-untreated (group A) were starved for 24 hours prior to indomethacin administration. After 4 hours of 30 mg kg⁻¹ b.w. indomethacin administration, the groups (A, B, C, D, E and F) received once daily oral doses of distilled water (5 ml kg⁻¹ b.w.), cimetidine 60 mg kg⁻¹ (b.w.) and the *S. virosa* extract at doses of 35, 70 and 140 mg kg⁻¹ b.w. respectively for 11 days. At the end of the treatment, animals in groups B, C, D, E and F were starved for 18 hours then sacrificed. The extract significantly ($p < 0.05$) decreased gastric secretion volume, mean ulcer index, total acidity, total protein and pepsin secretion relative to the induced-untreated rats. The extract significantly ($p < 0.05$) increased the gastric pH and total carbohydrate content relative to the induced-untreated. These results were similar to those achieved by treatment with cimetidine. Catalase and SOD activities in the 35, 70 and 140 mg kg⁻¹ b.w. *S. virosa* extract-treated groups were increased significantly ($p < 0.05$) over the untreated group. Similarly, the extract reversed the indomethacin-induced decrease in reduced glutathione level (GSSH) and the increase in malondialdehyde concentration in the serum. The histological analysis showed positive effect of the extract on the indomethacin-induced ulceration. It was concluded that the extract has anti-ulcerative and antioxidant activity in indomethacin-induced ulcerative rats.

Keywords: antioxidant enzymes; anti-ulcerogenic activity; cimetidine; gastric ulcer; ulcer induction

Introduction

Gastric ulcer contributes majorly to morbidity and mortality in Nigeria and many other third world countries (Agbakwuru *et al.*, 2006). The aggressive secretion of gastric juice contents (HCl and pepsin) and the inhibition of blood supply, mucus, prostaglandins synthesis in the stomach (Oloyede *et al.*, 2015) contribute to its aetiology. The secretion which is a physiological event, aggravates the ulcer through cell damage and capillary destruction and inhibited wound healing (Tarnawski and Halter, 1995). Excessive intake of non-steroidal anti-inflammatory drugs (NSAIDs), hereditary predisposition to ulcer and *Helicobacter pylori* infection, emotional stress amongst other factors contribute to the development of peptic ulcer diseases.

Most of the commonly used antiulcer drugs like gastric antisecretory drugs-H₂ blockers (such as cimetidine), anticholinergic agents, proton pump inhibitors (such as

Omeprazole), sucralfate and prostaglandin analogues are shown to have side effects and limitations in clinical practice (Eriyamremu and Iorliam, 2018). Plant-derived remedies have been used in the prevention and treatment gastric ulcers in Nigeria (Maria-Ferreira *et al.*, 2014; Rozza *et al.*, 2014). *Carica papaya* seed is widely used in the treatment of gastric ulcerations (Oloyede *et al.*, 2015), as well as *Momodica* species (Vijayakumar *et al.*, 2011) extracts, *Musa paradisiaca* peels (Ghasi, 2014) and *Sesbania* species (Bhalke *et al.*, 2010) stem bark extracts amongst others.

Securinega virosa commonly known as Iranje in Yoruba, Njisi-nta in Igbo, Tsuwaawun karee in Hausa, Shim shim in Kanuri and White Berry Bush in English (Neuwinger, 1996); it is one of the great African medicinal plants described as a true omnipotent cure, of which the root (Neuwinger, 1996) is widely used. The plant is widely distributed throughout tropical Africa, but can also be found in India, Malaysia, China and Australia (Dalziel,

1936). The leaves are used in many parts of Africa in the treatment of fever, body pain, stomach ache, rheumatism, diarrhoea, pneumonia and epilepsy (Neuwinger, 1996).

The objective of this research was to investigate the anti-ulcerogenic potential of aqueous extract of *Securinega virosa* leaf as an ameliorative agent against gastric ulcer induced with indomethacin in rats.

Materials and Methods

Biological material

Securinega virosa plant was identified and authenticated with voucher number UILH/001/166 and samples were deposited at the herbarium of the Plant Biology Department, University of Ilorin, Kwara State Nigeria.

Securinega virosa leaf extract

Fresh leaves of *Securinega virosa* were collected, air-dried under shade for two weeks until crispy and then size-reduced into powder with pestle and mortar. Of the powdered leaves, 300 g was macerated with 3,000 ml distilled water for 5 h with intermittent stirring. The extract was concentrated on water-bath for 10 days at 45 °C. The yield of the extract (aqueous leaves extract of *S. virosa*) was calculated and the extract was stored in the refrigerator at 4 °C prior to use. From this, solution of extract was prepared freshly for each study (Magaji et al., 2008).

Phytochemical screening

Aqueous extract of *Securinega virosa* was subjected to screening for tannin (Makkar, 1994); saponin (Obadoni and Ochuko, 2001); flavonoid (Boham and Kocipal-Abyazam, 1994); alkaloid (Harbone, 1973) and total phenols (Singleton and Rossi, 1965).

Chemicals and assay kits

Indomethacin and Cimetidine were purchased from Alaka-Ola Pharmacy Store, Saki, Oyo State Nigeria. Other chemicals and assay kits used in this research were of analytical grade and they were products of Randox laboratories Co-Artrim, United Kingdom.

Experimental design

Thirty (30) Wistar rats weighing 160-200 g were used for the study. The animals were allowed to acclimatize to the laboratory conditions for a week. The animals had access to tap water and normal rat chow freely until assigned to individual groups. The rats (30) were weighed individually and divided into six (6) groups of five (5) rats per group.

Animal grouping and treatment was done as follows:

Group A (un-induced untreated): This group received 5.0 mg kg⁻¹ body weight of normal saline.

Group B: This group received Indomethacin (30.0 mg kg⁻¹ body weight) + reference drug, Cimetidine (60.0 mg kg⁻¹ body weight, each dissolved in normal saline).

Group C: This group received Indomethacin (30.0 mg kg⁻¹ body weight) + extract (35.0 mg kg⁻¹ body weight, drug and extract dissolved in normal saline).

Group D: This group received Indomethacin (30.0 mg

kg⁻¹ body weight) + extract (70.0 mg kg⁻¹ body weight, drug and extract dissolved in normal saline).

Group E: This group received Indomethacin (30.0 mg kg⁻¹ body weight) + extract (140.0 mg kg⁻¹ body weight, drug and extract dissolved in normal saline).

Group F (INDO-induced untreated): This group received Indomethacin (30.0 mg kg⁻¹ body weight, drug dissolved in normal saline).

The animals except the un-induced untreated group were starved of food for 24 hours, with free access to water only, prior to the administration of indomethacin. After 4 hours of indomethacin administration, the animals were administered daily with the treatments for 11 days consecutively. After the treatments, animal's in-group B, C, D, E and F were starved for 18 hours (with free access to water only) and then sacrificed to check for ulceration.

All the test substances were dissolved in the normal saline solution (Shoaib and Shafiq, 2004).

Evaluation of parameters

Collection of tissue and gastric juice

After two weeks of treatment, the animals were anaesthetized with diethyl ether and sacrificed by stunning method. Their stomachs were excised carefully by keeping the oesophagus closed and opened along the greater curvature and the luminal contents were removed. Gastric contents from each rat were washed with 5 ml distilled water into a centrifuge tube and centrifuged at 1,000 ×g for 10 min; the volume of the supernatant was expressed as ml per 100 gram body weight and the centrifuged samples were decanted and analyzed for gastric volume, gastric pH, carbohydrate level, pepsin level and total acidity.

The cleaned stomachs were examined macroscopically after which some were preserved in 10% formal saline prior to histological studies and others kept in 0.1M phosphate saline buffer before homogenization. Concentration of reduced glutathione, catalase activity, superoxide dismutase activity and lipid peroxidation (measured by malondialdehyde concentration) were determined using the homogenized stomachs.

Preparation of stomach homogenate

Immediately after ulcer scoring, whole stomach was ground in a mortar cooled by liquid nitrogen. The stomach tissues (0.5 g each) after grinding were then homogenized with ice cold 0.1 M phosphate saline buffer (1:4 (w/v), pH 7.4). The homogenates were centrifuged at 2,500 rpm at 4 °C for 10 min. The resulting supernatants were frozen to ensure maximum release of the enzymes located in the tissue before being used for the antioxidant assay.

Determination of mean ulcer index

The excised stomach was viewed under microscope (× 40) to determine the level of ulceration using the method of Raju et al. (2009). The ulcer sores were counted and scored as follows:

| | |
|---------------------------------|-----|
| Grey colored normal stomach: | 0 |
| Pink to Red coloration stomach: | 0.5 |
| Spot ulceration: | 1.0 |
| Hemorrhagic streaks: | 1.5 |
| Number of Ulcer less than 5: | 2.0 |

Number of ulcer more than 5: 3.0
 Ulcer with bleeding: 4.0
 Perforation and edema of gastric wall: 5.0

Mean ulcer score for each animal is expressed as ulcer index:

Ulcer index (UI) = Total ulcer score/Number of animals ulcerated

Gastric acid secretion volume and pH assay

Gastric juice pH and gastric volume were determined as described by (Kurasawa *et al.*, 2005).

Determination of gastric acidity

Gastric acidity was determined as described by (Shay, 1945). One ml of the gastric juice was pipetted and diluted to 10 ml with distilled water. Then, the total acidity of the gastric juice was estimated by titration using 0.01 N sodium hydroxide and phenolphthalein as the indicator. The result was expressed as the free acid output, which was expressed in terms of mEq/L (Prino *et al.*, 1971).

$$\text{Gastric acidity} = \frac{\text{volume of } \frac{0.01 \text{ mol}}{\text{L}} \text{ NaOH} \times 100}{10}$$

Determination of pepsin in gastric secretion

Pepsin was assayed for as described by (Hirohashi *et al.*, 1993). The pepsin activity was determined from a standard curve.

$$\text{Pepsin activity mg/mln} = \frac{\text{unreacted albumin} \times V_t}{\text{incubated time} \times V_e}$$

V_t = Total reaction volume; V_e = Volume of enzyme used; Unreacted albumin = Concentration of unreacted albumin (mg/ml);

Estimation of pepsin activity The centrifuged (5000 g for 10 minutes) gastric juice (0.1 ml) was added to 1 ml bovine albumin (0.5% w/v in 0.01 N HCl; pH 2) and incubated for 20 minutes at 37 °C. A duplicate background control tube (gastric juice blank), in which 1 ml albumin was replaced with 1 ml of 0.01 N HCl, was simultaneously run. Hydrolysis was stopped by adding 2 ml of 10% TCA. All of the tubes were heated in boiling water for 5 minutes, and then cooled. After denaturation of the proteins by heating in a boiling water bath for 5 minutes, the precipitate was removed by centrifugation (9000 g for 10 minutes). A total of 1 ml of the supernatant was mixed with 0.4 ml of 2.5 N NaOH and 0.1 ml of the Folin-Ciocalteu reagent, and then the volume was adjusted to 10 ml using distilled water. Absorbance was measured at 700 nm. The peptic activity was calculated in terms of micrograms of tyrosine liberated per milliliter of gastric juice (Prino *et al.*, 1971)

Determination of reduced GSH

The level of GSH in the homogenate was determined using the procedure described by (Ellman, 1959). Briefly, 1.0 ml of stomach mucosa homogenate was added to 0.1 ml of 25% TCA (trichloroacetic acid) and precipitate was removed by centrifuge at 5,000 × g for 10 min. Supernatant (0.1 ml) was added to 2 ml of 0.6 mmol/L DTNB(5,5'-dithiobis-(2-nitrobenzoic acid) prepared in 0.2 molL⁻¹ sodium phosphate buffer (pH 8.0). The absorbance was read at 412 nm.

Determination of superoxide dismutase activity

The activity of SOD in stomach of rats was determined as described by (Misra and Fridovich, 1972).

Determination of catalase activity

The method of (Beers and Sizer, 1952) was used to determine catalase activity in the stomach homogenates.

Determination of malondialdehyde level

Malondialdehyde concentration was determined using the method described by (Reilly and Aust, 2001).

Histopathological study

After thoroughly washing the stomach with distilled in 10% formaline solution biopsies were collected from these samples. Sections (5 µm) were taken from the biopsies and stained with hematoxylin and eosin prior to visual inspection for damage under a light microscope (160 ×) (Oloyede *et al.*, 2015)

Statistical analysis

Data were expressed as mean ± standard error of mean (n = 6). Comparative analysis was performed between the various groups using analysis of variance (ANOVA) and Duncan multiple range test for the post hoc. Differences were considered at p<0.05.

Results and Discussion

Phytochemical screening

Non-steroidal anti-inflammatory drugs (e.g Indomethacin) is one of the major causative factors of gastric ulcer (Suleyman *et al.*, 2010). To offer remedy to gastric ulcer, many pharmaceutical products including histamine (H2) receptor antagonists, proton pump inhibitors, antacids and anti-cholinergics have been used (Singh *et al.*, 2008). However, majority of these treatment drugs pose varieties of undesirable effects (Devi *et al.*, 2007). As a result of the undesirable effects of the synthetics drugs, there is need to find natural drugs possessing antioxidant and anti-ulcerogenic properties which could serve as a bioactive alternative to the synthetic drugs. Medicinal values of plant are attributed to the present of the phytochemicals that are containing in the plant. Phytochemicals especially alkaloids, phenol, flavonoids and tannin contain in this plant are strongly associated to anti-ulcer benefits of a plant (Parag, 2016). Flavonoids are not only free radical scavengers, but are important increasing mucosal prostaglandin content and can decrease histamine secretion from mast cells by the inhibition of histidine decarboxylase (Borrelli and Izzo, 2000). These effects of flavonoids improve mucus secretion. Tannins prevent ulcer development by improving vaso-constricting effects (Aguwa and Nwako, 1988). It is therefore not surprising when we observed that the plant might be able to ameliorate indomethacin-induced gastric ulcer. The enrichment of phenolic compounds within plant extracts is correlated with their enhanced antioxidant activity. Hence, the presence of phenolics in leaf extracts of *S. virosa* may indicate higher potentials in antioxidant and other medicinal properties.

Recently, it has been reported that the high molecular weight phenolics such as tannins have more ability to reduce or scavenge free radicals (Nagulsamy *et al.*, 2015). Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer (Dharmananda, 2003). Therefore, tannin contents of *S. virosa* may enhance its free radical scavenging, anti-inflammatory, and anti-ulcerogenic potential.

Macroscopic examination of the stomach of rats

Fig. 1 shows morphological visualization of gastric mucosa of rats from each of the experimental group. The gastric mucosa of uninduced-untreated and 60 mg kg⁻¹ BW cimetidine treated group appeared closely the same with no sign of ulceration, an indication of ulcer healing effects of cimetidine. The gastric mucosa of the groups treated with vary dose of the extract appeared somewhat whitish. Similarly, the gastric mucosa of the groups treated with 35 mg kg⁻¹ extract and 70 mg kg⁻¹ extract shows no sign of ulceration, an indication of ulcer healing effects of aqueous extract of *S. virosa* leaf. Gastric mucosa of the group treated with 140 mg kg⁻¹ extract and induced-untreated group appear ulcerated with severe injury on the later.

Acid secretory system

This finding shows anti-ulcerogenic and antioxidant potential of treatment with aqueous extract of *S. virosa* leaf gastric ulcer induced with indomethacin in rats. The production of gastric ulcers in rats treated with indomethacin may be linked to the indomethacin inhibitory effect on prostaglandins formation, where the inhibition increases gastric acid secretion, reduces mucosal blood flow and reduces bicarbonate secretion (Wallace, 2008). The aqueous extract of the leaf of *S. virosa* demonstrated a significant anti-ulcerogenic activity by reducing the gastric volume of animals in the group treated with 70 mg kg⁻¹ BW of *S. virosa* leaf extract and the increase in gastric volume of the untreated indomethacin group is due to increased production of hydrochloric acid as it is evident from the total acidity of the gastric juice

(Sivagnanam *et al.*, 2003) (Table 5). The extract also reduced the gastric acidity in the group treated with 35 mg kg⁻¹ body weight of *S. virosa* leaf and 70 mg kg⁻¹ BW of *S. virosa* leaf (Table 5). Non-steroidal anti-inflammatory drug induced ulcer is mediated through tissue damaging free radicals, which are produced from the conversion of hydroperoxyl to hydroxy fatty acids, which leads to cell destruction. The hydroperoxyl fatty acids are generated from the degeneration of mast cells and generalized lipid peroxidation accompanying cell damage (Prasad *et al.*, 2014).

This study revealed that decrease in the total carbohydrate in the extract-treated groups; suggest significant increase in mucin content on the mucosal cell of the gastric mucosa. The decrease in protein content of the gastric juice of the groups treated with the extract of *S. virosa* leaf suggests the decrease in the leakage of plasma proteins into the gastric juice (Raj *et al.*, 2013). The increased pepsin activity coupled with increase in total carbohydrate content of the gastric juice in the indomethacin-induced ulcerated rats indicated reduced ability of the mucosal membrane to protect against haemorrhagic erosion, and hence damage to mucosal tissue. This suggests that there is reduced gastric mucosal protection against damage caused by indomethacin. Besides antioxidant action that protects the mucus layer and arrests ulcer progression, drugs that increase the synthesis and secretion of gastric mucus would accelerate gastric ulcer healing. Treatment with *S. virosa* however, facilitated ulcer-healing process associated with decreased pepsin activity and elevated mucin level in the gastric mucosa. This in turn has encouraged speedy wound healing of the ulcerated areas of the mucosal epithelia and shielded the gastrointestinal membrane, thus abrogating the catastrophic influence of indomethacin in the ulcerated rats (Sabiu *et al.*, 2015). This study, however, suggested that Cimetidine may be more effective than *S. virosa* in reducing pepsin activity. The possible ulcer treatment effects of the leaf of *S. virosa* in indomethacin-induce ulcer may be due to increased mucus secretion and reduced pepsin activity.

Table 1. Qualitative analysis of aqueous leaf extract of *Securinega virosa*

| Phytochemicals | Qualitative result |
|----------------|--------------------|
| Alkaloid | ++ |
| Tannins | ++ |
| Flavonoid | ++ |
| Terpenoid | ++ |
| Saponin | ++ |
| Anthraquinone | - |
| Steroid | - |
| Phenol | ++ |

Key: (++) indicates the presence of a phytochemical in sufficient amount, while (-) indicates absence of a phytochemical.

Table 2. Experimental survivor of rats in each group

| Group | Censored subject | Deaths/event | Percent survival % |
|---|------------------|--------------|--------------------|
| Uninduced and untreated | 5 | 0 | 100 |
| 60 mg kg ⁻¹ cimetidine | 2 | 3 | 60 |
| 35 mg kg ⁻¹ <i>S. virosa</i> | 3 | 2 | 60 |
| 70 mg kg ⁻¹ <i>S. virosa</i> | 4 | 1 | 80 |
| 140 mg kg ⁻¹ <i>S. virosa</i> | 3 | 2 | 60 |
| 25 mg kg ⁻¹ INDO and untreated | 2 | 3 | 40 |

Table 3. Effect of *S. virosa* leaf extract on gastric volume, gastric pH, total acidity and ulcer index in stomach of rats

| Group | Gastric volume (ml) | pH | TTA% | Ulcer index |
|---|---------------------------|---------------------------|---------------------------|--------------------------|
| Uninduced and untreated | 2.667±0.133 ^a | 3.780±0.116 ^a | 10.578±0.690 ^a | - |
| 60 mg kg ⁻¹ cimetidine | 4.100±0.265 ^{ab} | 4.080±0.224 ^a | 9.566±1.012 ^a | 0.667±0.167 ^a |
| 35 mg kg ⁻¹ <i>S. virosa</i> | 4.267±0.203 ^{ab} | 3.820±0.120 ^a | 10.507±0.164 ^a | 0.667±0.441 ^a |
| 70 mg kg ⁻¹ <i>S. virosa</i> | 4.167±0.033 ^{ab} | 3.600±0.164 ^{ab} | 11.732±0.738 ^a | 1.000±0.500 ^a |
| 140 mg kg ⁻¹ <i>S. virosa</i> | 4.400±0.058 ^b | 2.900±0.110 ^b | 17.526±1.470 ^a | 1.667±0.167 ^a |
| 25 mg kg ⁻¹ INDO and untreated | 4.933±0.120 ^b | 1.980±0.180 ^c | 38.502±5.693 ^b | 4.750±0.750 ^b |

Results are mean ± S.E.M. (n = 3). P<0.05, were considered statistically significant when compared to uninduced untreated (control) group.

Table 4. Effect of *S. virosa* leaf extract on pepsin activity, total protein and total carbohydrate level in stomach of rats

| Group | Pepsin (µg/tyrosine) | Total protein (mg/dl) | Total CHO (mg/l) |
|--|----------------------------|---------------------------|----------------------------|
| Uninduced and untreated | 14.041±0.581 ^a | 14.586±0.049 ^a | 69.385±2.973 ^a |
| 60 mg kg ⁻¹ cimetidine | 16.195±0.553 ^{ab} | 16.001±0.595 ^a | 87.318±1.399 ^b |
| 35 mg kg ⁻¹ <i>S. virosa</i> | 15.527±0.304 ^a | 17.030±0.934 ^a | 90.255±0.669 ^b |
| 70 mg kg ⁻¹ <i>S. virosa</i> | 19.423±0.364 ^b | 14.050±0.056 ^a | 87.570±1.678 ^b |
| 140 mg kg ⁻¹ <i>S. virosa</i> | 23.571±1.869 ^c | 18.551±1.561 ^a | 95.740±7.409 ^b |
| 25 mg kg ⁻¹ INDO & untreated | 31.299±2.021 ^d | 28.628±4.516 ^b | 119.284±0.639 ^c |

Results are mean ± S.E.M. (n = 3). P<0.05, were considered statistically significant when compared to uninduced untreated (control) group.

Table 5. Antioxidant enzymes levels and lipid peroxidation in rat stomach tissues

| Group | SOD(mmol min/mg/tissue) | CAT (nmol/min/mg protein) | GSH-Red (nmol/m mg protein) | LPO/MDA (nmol/m mg protein) |
|---|----------------------------|------------------------------|--------------------------------|--------------------------------|
| Uninduced and untreated | 107.072±2.192 ^a | 4.769±0.142 ^a | 1.239±0.076 ^a | 0.033±0.001 ^a |
| 60 mg kg ⁻¹ cimetidine | 93.104±5.972 ^b | 4.4757±0.077 ^a | 1.088±0.052 ^a | 0.041±0.003 ^a |
| 35 mg kg ⁻¹ <i>S. virosa</i> | 97.148±1.133 ^{ab} | 4.393±0.046 ^a | 1.095±0.511 ^a | 0.040±0.005 ^a |
| 70 mg kg ⁻¹ <i>S. virosa</i> | 79.136±4.214 ^c | 2.979±0.214 ^b | 0.552±0.0426 ^b | 0.064±0.001 ^b |
| 140 mg kg ⁻¹ <i>S. virosa</i> | 85.973±3.342 ^{bc} | 2.553±0.283 ^b | 0.502±0.085 ^b | 0.100±0.007 ^c |
| 25 mg kg ⁻¹ INDO and untreated | 44.713±4.818 ^d | 1.498±0.245 ^c | 0.201±0.018 ^c | 0.129±0.009 ^d |

Results are mean ± S.E.M. (n = 3). P<0.05, were considered statistically significant when compared to uninduced untreated (control) group.

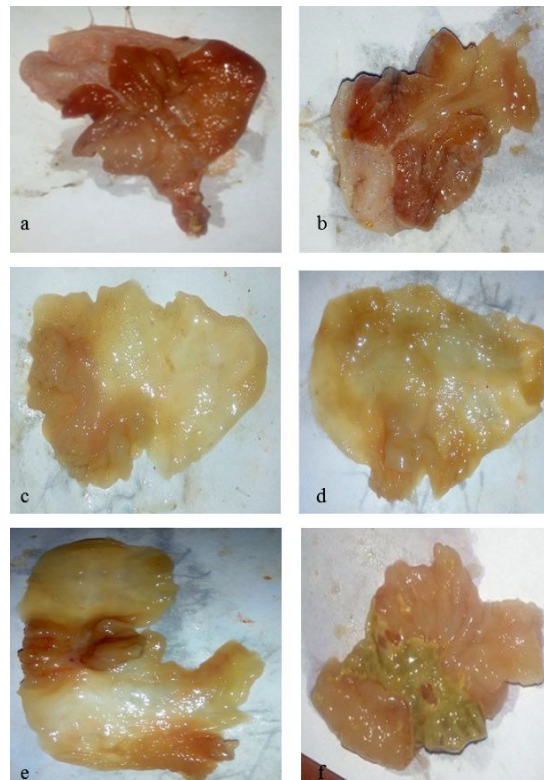


Fig. 1. Morphological visualization of the gastric mucosa of rats: a) uninduced and untreated; b) 60 mg kg⁻¹ cimetidine treated; c) 35 mg kg⁻¹ *S. virosa* extract treated; d) 70 mg kg⁻¹ *S. virosa* extract treated; e) 140 mg kg⁻¹ *S. virosa* extract treated; f) induced & untreated

Anti-oxidants system

Oxidative stress has been shown to be a causative factor in the development and pathogenesis of indomethacin-mediated gastric injury (Utsumi *et al.*, 2006). Indomethacin like any other NAIDS have been reported to cause reduction of activities of SOD, catalase, and glutathione peroxidase as well as reduced glutathione concentration in the stomach thereby leading to ulceration (Halici *et al.*, 2005) in the organ.

This study shows that all treatments with cimetidine and *S. virosa* (35 mg kg⁻¹, 70 mg kg⁻¹ and 140 mg kg⁻¹) increased the GSH content significantly. This affected the antioxidant defence system positively and reduced gastric damage. GSH protects gastrointestinal tissue lipids from oxidative damage. As shown in previous studies, in gastric tissue damaged by indomethacin, GSH level is lowered after treatment (Albayrak *et al.*, 2015). GSH detoxifies hydrogen peroxide and organic acids chemically; hydrogen peroxide accumulates in the absence of GSH (Dalle-Donne *et al.*, 2003). In the presence of transition metals such as Fe and Cu, hydrogen peroxide reacts with superoxide, resulting in the formation of the highly reactive and cytotoxic hydroxyl radical (Malo and Wilson, 2000).

Lipid peroxidation

The significantly high levels of MDA in stomach of the induced-untreated group lend credence to previous reports that suggested that free radicals generated by neutrophils may be an important factor increasing the level of gastric ulcer injury (Olaleye and Akinmoladun, 2013). The significant reduction in MDA levels in the extract treated groups suggests its protective action against free radical damage. The extract decreased the MDA content at 35 mg kg⁻¹ more than the other doses of the extract. An increase in MDA content is correlated to an increase in tissue damage.

MDA is the final product of lipid peroxidation and is used to determine the lipid peroxidation levels (Albayrak *et al.*, 2015). This also suggests that the gastro protective potential of *S. virosa* is connected with the reduction in lipid peroxidation in line with a previous report (Olaleye and Akinmoladun, 2013).

Histopathology

Indomethacin was known for its gastric damage via inhibiting the release of protective factors like cyclooxygenase-1 (COX-1), prostaglandin E2 (PGE2), bicarbonate, and mucus; increasing aggressive factors like acid; and increasing oxidant parameters while decreasing antioxidant parameters (Suleyman *et al.*, 2010). Indomethacin produced gross glandular mucosal damage in rats, an indication of ulcerative lesions, which appeared as eroded mucosa layers in the first 4 hours and as wounds in 11 days (Fig. 2). Treatment with aqueous extract of *Securinega virosa* leaf markedly improved the visible eroded mucosa layers and wounds induced by indomethacin in rat stomachs. The histological studies of indomethacin treated rats showed severe degenerated architecture of the stomach, indicating total ulceration of the submucosa layer (Fig. 6). Treatment of rats with aqueous extract of *S. virosa* leaf at dose of 35 mg kg⁻¹ BW resulted in mucosal layers and gastric cells arrangement similar to the control rats (without ulcer induction) as well as rats receiving cimetidine 60 mg kg⁻¹ BW, these groups showed significant improvement in the treatment of gastric lesions and wound in the submucosa compared to the animals receiving no medicinal intervention after ulcer induction. The observed reduction in the degeneration of the stomach mucosal wall, reduced inflammation and reduced ulceration in the groups treated with the extract may be due to the anti-oxidant and anti-inflammatory activity of the extract.

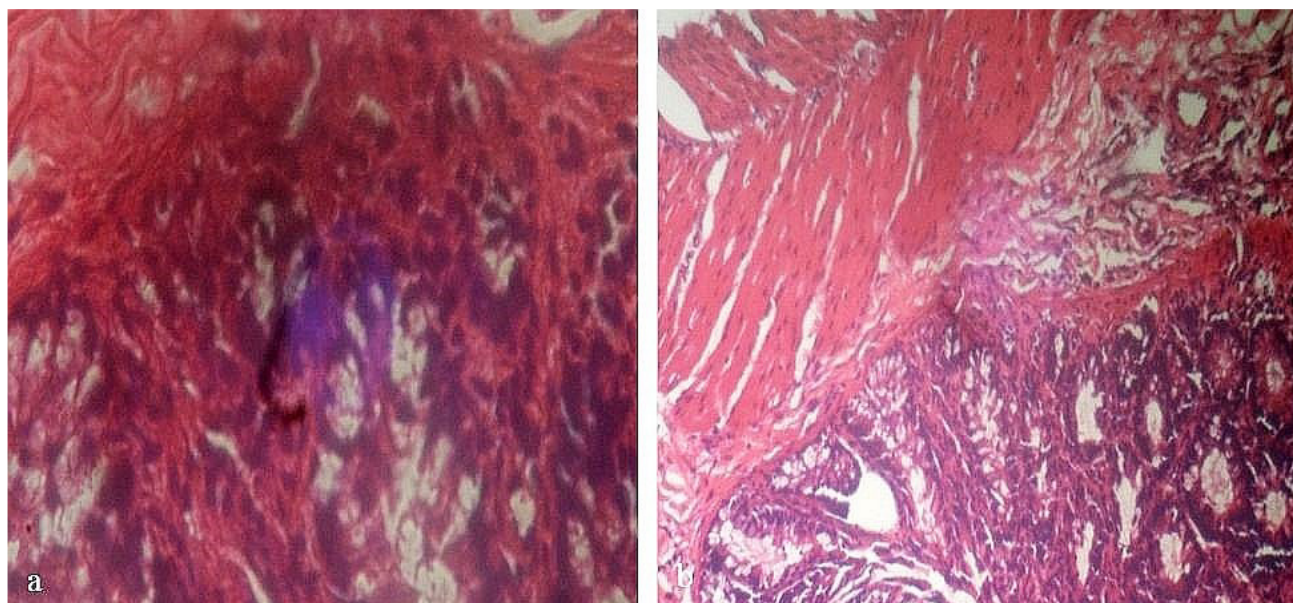


Fig. 2. Induced and treated with 35 mg kg⁻¹ extract. The Photomicrograph shows section of G.I.T. with dense mucosal cells and portion of muscular hyperplasia; a) Mag X 300. The lamina propria is thin lying below extensive area of muscle hyperplasia; b) Mag. X 200

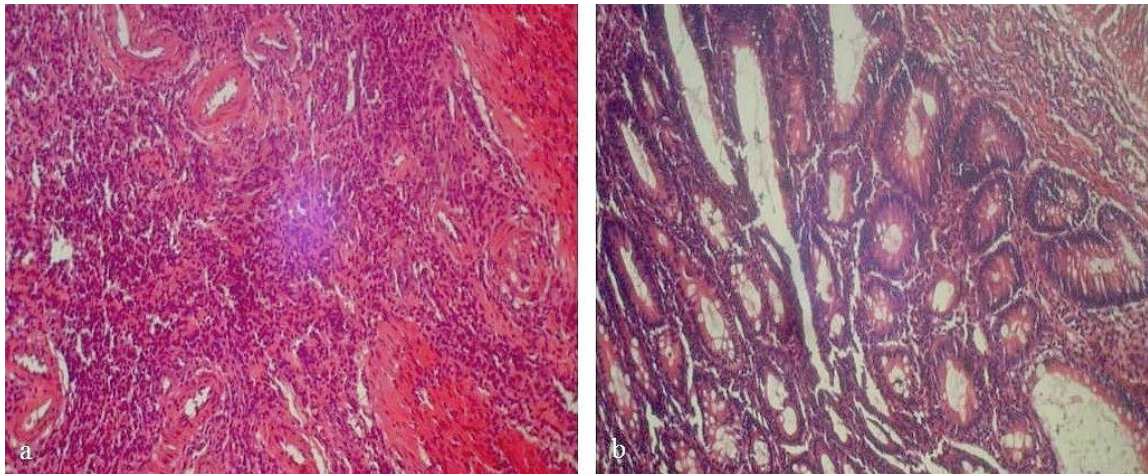


Fig. 3. Induced and treated with 140 mg kg^{-1} extract. The Photomicrograph shows G.I.T. section with dilated blood vessels and extensive infiltration by lymphocytes; a) Mag. X 200. There is mucosal hyperplasia and mild atypical changes; b) Mag. X 200

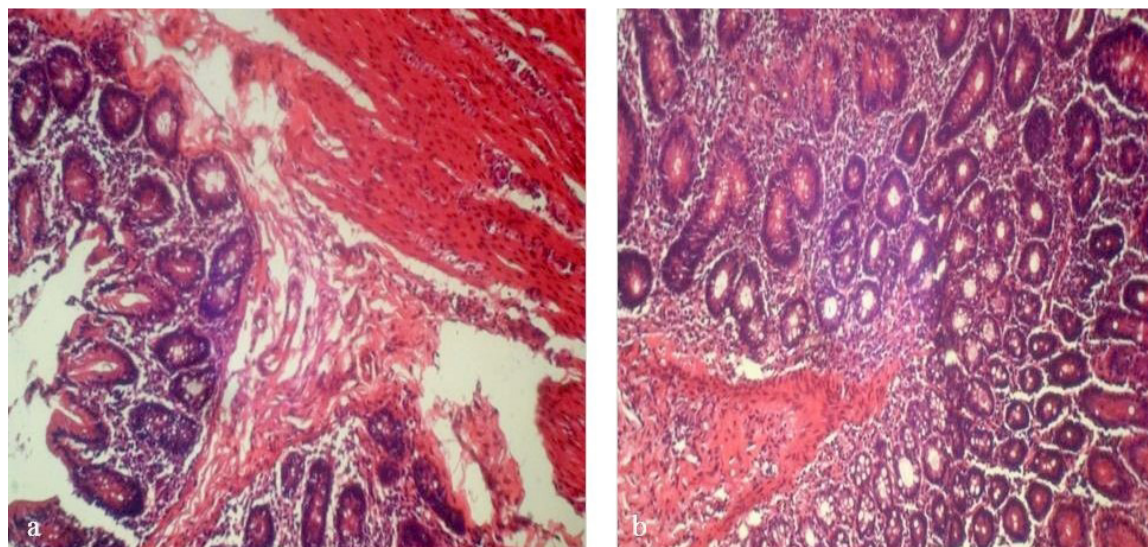


Fig. 4. Induced and treated with 70 mg kg^{-1} extract. The Photomicrograph shows marked muscle hyperplasia and infiltration with neutrophils; a) Mag. X 200. The mucosa shows mild hyperplasia and is lined by tall cuboidal epithelial cells; b) Mag. X 200

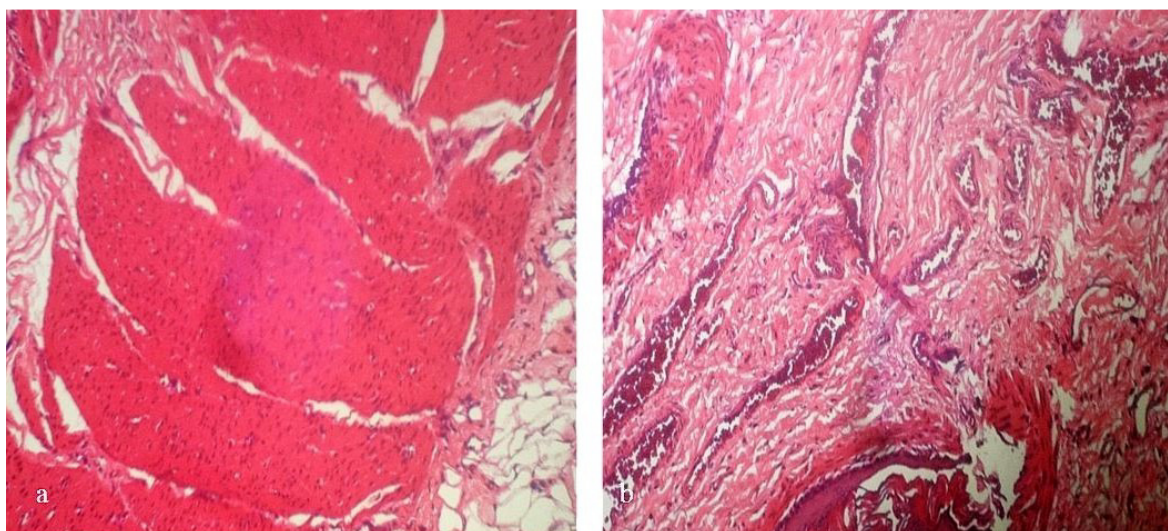


Fig. 5. Induced and treated with 60 mg kg^{-1} cimetidine: Photomicrograph shows G.I.T. section with marked muscle hyperplasia supported by adipocytes; a) Mag. X 200 and numerous dilated blood vessels; b) Mag. X 200

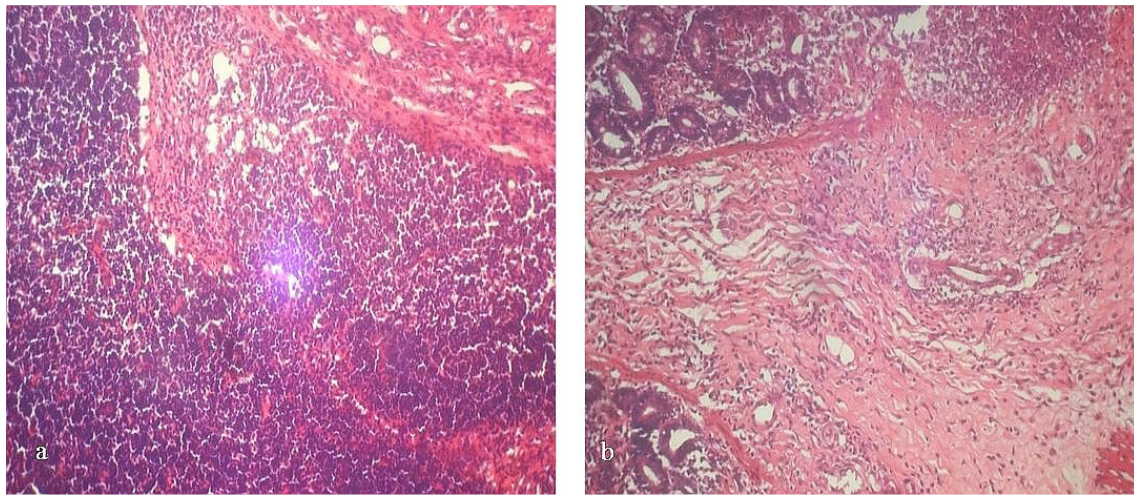


Fig. 6. Induced untreated. The Photomicrograph shows G.I.T. with lymphoid follicular hyperplasia; a) and extensive fibrous muscularis layer populated by chronic inflammatory cells; b) Mag. X 200

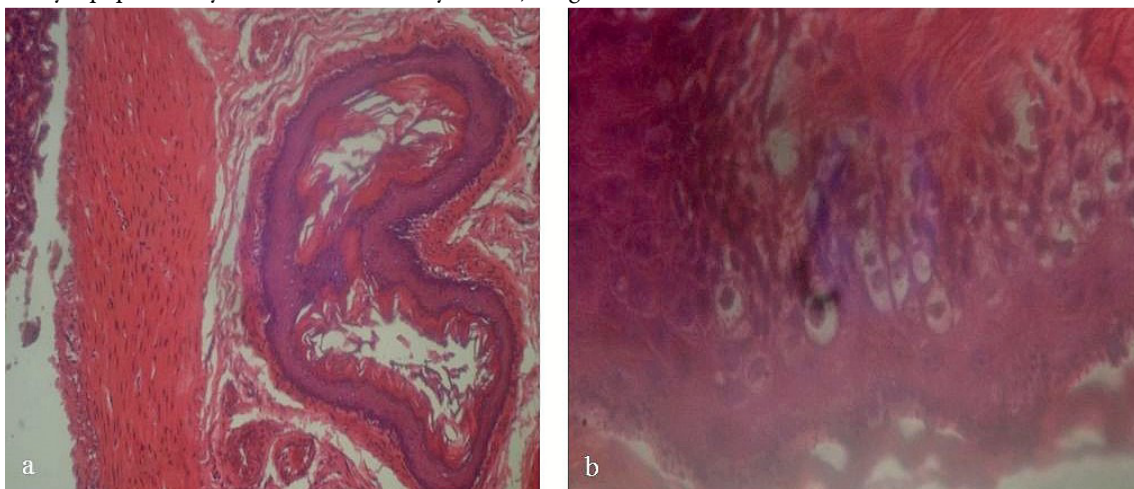


Fig. 7. Uninduced and untreated. The Photomicrograph shows G.I.T. section with longitudinal muscle hyperplasia, keratinized epithelium and with high degree squamation; a) Mag. X 200 and koilocytes with some hyperchromatic cells with dense nuclei; b) Mag. X 300

Conclusions

From this study, we conclude that the extract has anti-ulcerative and antioxidant activity in indomethacin-induced ulcerative rats. The plant extract has the ability to reduce gastric volume, gastric acidity, lipid peroxidation and ameliorates gastric mucosal degeneration caused by ulcer and therefore has the potentials for use as an antiulceration agent.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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