



Evaluation of the Urease Inhibitory, Antiulcer and Acute Toxicity Effects of Ethanolic Seed Extracts of *Garcinia Kola* against Chemically Induced Ulcers

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Abstract	Article History
There is growing interest in medicinal plants due to their perceived safety, affordability, and effectiveness compared to synthetic drugs. This study evaluated the <i>in vitro</i> urease inhibitory activity, antiulcer effects, and acute toxicity profile of ethanolic seed extract of <i>Garcinia kola</i> against chemically induced ulcers. Quantitative phytochemical analysis using standard techniques revealed saponins as the most abundant component (6.471 ± 0.02 mgQAE/g), followed by glycosides (3.421 ± 0.01 mgDE/g), phenolics (2.471 ± 0.01 mgGAE/g), flavonoids (2.041 ± 0.05 mgQCE/g), alkaloids (0.647 ± 0.03 mgAE/g), and tannins (0.342 ± 0.02 mgTAE/g). Anti-urease activity was assessed using commercial urea and urease enzymes at concentrations of 25 – 400 mg/mL, with omeprazole as control. Results showed a significant ($P < 0.05$) dose-dependent inhibition of urease activity, highest at 400 mg/mL. Acute toxicity was evaluated via oral administration in albino mice monitored for 72 h and no toxicity was observed at doses up to 400 mg/mL. Antiulcer activity was investigated using three ulcer-induction models: aspirin (30 mg/mL), acetic acid (1 mL of 95%), and indomethacin (30 mg/mL). The extract (400 mg/mL) significantly reduced ulcer formation across all models ($P < 0.05$). Potency was greatest against aspirin-induced ulceration, with an inhibition rate of 79.69 %, exceeding that observed in the acetic acid and indomethacin models. Notably, the effect of the 400 mg/mL extract was not significantly different from that of omeprazole, a standard proton pump inhibitor. These findings demonstrate that <i>Garcinia kola</i> ethanolic seed extract possesses urease inhibitory and gastroprotective properties while being safe at tested doses. The results provide scientific validation for its ethnomedicinal use in managing inflammatory states, wound healing (ulcers), and other infectious diseases.	Received: 25 Sept 2025 Accepted: 04 Oct 2025 Published: 06 Oct 2025
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Introduction

Urease is a big enzyme that is within amidohydrolase family. Commonly found in bacteria, fungi, algae, plants, some invertebrates and as soil enzyme (Olebunne *et al.*, 2022). They possess nickel-ions as a metalloenzyme of great molecular weight. Urease catalyzes the hydrolysis of urea to form ammonia and carbon dioxide, hence automatically alters the PH of the surroundings to basicity level as a result of high concentration of ammonia produced. This resultant pH elevation is of diverse clinical and agronomic significance (Olebunne *et al.*, 2022). Although mammalian cell does not produce urease, it enters the mammalian body through different bacteria, especially within the intestine (Stepankov *et al.* 2017). Urease is a virulence factor for

Helicobacter pylori, *Proteus mirabilis*, *S. saprophyticus* and *Mycobacterium tuberculosis* among others that are associated with certain diseases in human and animals. Its activity adds up to kidney stones formation, observed in urolithiasis that contribute to acute pyelonephritis and other urinary tract infections. It is also implicated in arthritis, gastric intestinal infections and finally the urease disparity that enhances peptic ulcer development (Olebunne *et al.*, 2022).

Peptic ulcer is one of the most prevalent diseases around the world affecting four million people each year. Peptic ulcer is the term which refers to acid peptic injury of the digestive tract, and it results in mucosal break reaching the submucosa. The disease

involves an imbalance between offensive and defensive factors such as pepsin, acid and *Helicobacter pylori*; and bicarbonates, prostaglandins, mucin, nitric oxide and growth factors, respectively (Tripathi *et al.*, 2021). Peptic ulcer commonly develops along the gastrointestinal tract, especially the stomach and duodenum, where gastric acid and pepsin are secreted. Commonly observed symptoms of peptic ulcer include bloating, burning, pressure or feeling of fullness, occasional bloody or dark stools, pain in the chest, fatigue, vomiting, changes in appetite, indigestion, and weight loss (Irfan *et al.*, 2024). Numerous drugs, including proton pump inhibitors and H₂ receptor antagonists, are included in the treatment regimen of peptic ulcer (Irfan *et al.*, 2024). Nevertheless, these drugs lead to the occurrence of adverse effects and relapses and interact with other drugs. As an effort to reduce adverse effects stemming from these drugs and prevent relapses, the relentless search for potent herbal medication is still relevant and crucial (Ayaz *et al.*, 2014).

Medicinal plants possess numerous active phytoconstituents that are responsible for several biological activities (Tripathi *et al.*, 2021). The herbal drugs are less toxic than synthetic drugs; however, the toxicity evaluation is required to determine the safety profile of herbal drug. In this concern, the drugs of natural origin can be used for the management of gastric ulcers as a better alternative to synthetic drugs (Tripathi *et al.*, 2021). *Garcinia kola* (angiospermae) is a dicotyledonous plant from the Clusiaceae/Guttiferae family. It is commonly known in English as bitter kola, male kola and false kola, Orobo in Yoruba, Cidagoro in Hausa, Akuilu or Ugugolu in Igbo, Efiari (Efik), and Igoligo (Idoma), and it is found in the tropical forests of Sierra Leone, Angola and Nigeria (Dalziel, 2017). *G. kola* is a miracle plant that grows in the southern Nigerian rainforest and has been proven to have medicinal properties in all of its parts (Chukwudi – Emenike *et al.*, 2022). The seeds of *G. kola* are currently used in traditional medicine in many herbal formulations and have potential therapeutic benefits such as antibacterial properties due largely to the activity of their flavonoids and other bioactive compounds (Dah-Nouvlessounon *et al.*, 2015). Other phytochemical constituents of *Garcinia kola* seeds include phenols, alkaloids, tannins, and saponins, which have a variety of beneficial effects in humans and animals. They are used to prevent or treat colic in babies, as well as to treat upper respiratory tract infections, when chewed fresh. They've been used for centuries in several West African countries to treat head and chest colds, dysentery, diarrhea, and urinary infections, as well as poison antidote. The seeds are said to be used as a stimulant in the treatment of liver and diarrhea, diabetes, bronchitis, and throat infections. It is a highly desired product due to its widely perceived medicinal properties and the fact that when consumed in large quantities, it does not cause indigestion (Chukwudi – Emenike *et al.*, 2022). These species are sources of caffeine in processing and pharmaceutical industries and often chewed by some ethnic's group settings as stimulants (Okoli *et al.*, 2012). The presence of other chemicals in kola nuts such as kolanin and theobromine also makes them suitable for use in drug preparation (Dewole *et al.*, 2013).

Although studies on the effects of *G. kola* on the blood system, neurological system, and physiological system have been published, there is a scarcity of research on the effects of *G. kola* on the gastrointestinal system. There is also need to research into natural agents with anti-urease activity to alter the ability of *Helicobacter pylori* implicated in gastro intestinal ulcer to create a favourable environment for its activities. As a result, it's critical

to explore the underlying *In Vitro* urease inhibitory activity, antiulcer activity and acute toxicity profile of ethanolic seed extracts of *G. kola* against chemically induced ulcers. Also, the phytochemical constituents were evaluated as well.

Materials and Methods

2.1 Study Site

The fresh seeds of *Garcinia kola* were collected from Eke-Awka Market, Awka, in Awka South Local Government Area, Anambra State.

2.2 Preparation of Seeds for Extraction

The seeds were cut into smaller portions and dried under shade at room temperature for 14 days. The dried seeds were grounded using electric grinder model to powdery form, weighed and kept ready for extraction of active ingredients as in (Umeh *et al.*; 2021; Ubani *et al.* 2024; Mundi *et al.*, 2014).

2.3 Extraction Procedure

A 20g portion each of the seed powder was extracted by maceration in 200 mL each of ethanol and water for 24hours. The resulting extracts were subsequently filtered using Whatman No. 1 filter paper. The ethanolic extracts was evaporated to dryness at room temperature in a steady air current (Okoye *et al.*; 2014; Uba *et al.*, 2016; Uba *et al.*, 2024; Dokubo and Uba, 2023). The aqueous extract was evaporated in a water bath at 100°C to dryness. The standard extracts obtained were stored in a refrigerator at 4°C until required for use (Uba and Obiefuna, 2023; Okafor *et al.*, 2023).

2.4 Phytochemical Studies

The phytochemical constituents of the seed's extracts were investigated using the methods used by Sofowora, (1993), Ezeonu, and Ejikeme, (2016), Anameze *et al.* (2023), Ele *et al.* (2025), Okoye *et al.* (2020a); Okoye *et al.* (2020b) and Okoye *et al.* (2020c). The phytochemical tests are of paramount importance in order to identify the various constituents in the extracts respectively responsible for the various pharmacological actions elicited by the extracts as follows: tannins; phlobatannins, saponin, steroid, terpenoids, flavonoids, alkaloids and glycoside, respectively.

2.5 Preparation of Test Sample

In this study, concentration of 400 mg/mL of each extract was used for the screening of antibacterial activity. This was done by dissolving 4.0 g of the extract in 10 mL of dimethyl sulphoxide solvent (NCCLS, 2000).

2.6 Effect of Aqueous and Ethanolic Extracts of *Garcinia kola* on Urease Activity

This was carried out using the modified method of Olebunne *et al.*, (2022). Commercially produced urea and urease enzyme were used for the assay. Equal mixture of urea and urease solutions were mixed in different test tubes labeled A, B, C, D, E, F and G. Different concentrations of the extracts; 400, 200, 100, 50 and 25mg/mL were added to test tubes A, B, C, D and E respectively. Test tube F contained the mixture and omeprazole while test tube G contained only the mixture (urea and urease). The test tubes were incubated at 37°C for 30minutes. Then, the absorbance was measured using spectrophotometer at 625nm. Then the activity of the mixture in each test tube was calculated from the absorbance and the percentage of urease inhibition was calculated using the formula:

$$\% \text{ Inhibition of urease} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

2.7 Anti-Ulcer Activity

This was carried out using the modified method of Irfan *et al.*, (2024). Three models of experimental ulcer induction were used to determine the anti-ulcer activity of the extract. These include indomethacin (30 mg/mL), acetic acid (1 mL of 95 %) and Aspirin (30 mg/mL). Fifteen (15) mice each were used for each of the models making up to 45 mice while six (6) mice each were used for the positive (Omeprazole) and negative (distilled water) controls for each of the three models. Thus, twenty-one (21) mice were put into seven groups, five groups were administered with various concentrations (25, 50, 100, 200, and 400 mg/mL) of the extract respectively while the remaining two groups received water and omeprazole, respectively. Thirty (30) minutes later, ulcers were induced with indomethacin (30 mg/mL); all administrations were by oral route. The same process was repeated for acetic acid (1 mL of 95%) and aspirin (30 mg/mL). After 8 hours, the animals were sacrificed and the stomachs were ligated, the content was washed out with normal saline. Subsequently the guts were slit open, the inner surface was washed thoroughly with normal saline and ulcer damage was assessed with the naked eyes. The length of the ulcer was measured using Vernier Calliper, and the ulcer severity was estimated by means of scoring. Ulcers were scored according to the ulcer severity score used by Abd El Hady *et al.* (2013) where 0 = normal, 0.5 = punctuated hemorrhagic ulcer, 1.0 = ulcers less than 3 mm in diameter, 2.0 = ulcers greater than 3 mm in diameter and 3.0 = severe ulcers. From the above score, the mean ulcer index and ulcer inhibition were calculated.

The ulcer index is defined as mean ulcer severity score for each animal. The ulcer index was calculated using the following formula (Irfan *et al.*, 2024):

$$UI = UN + US + UP \times 10 - 1$$

where UI = ulcer index, UN = average number of ulcers per animal, US = average severity score, and UP = percentage of animal with ulcer

$$\text{Inhibition of ulceration (\%)} = \frac{MUI_{\text{control}} - MUI_{\text{test}}}{MUI_{\text{control}}} \times 100$$

Where, MUI_{control} = Mean ulcer index of control; MUI_{test} = Mean ulcer index of test

2.8 Acute Toxicity Test

Preliminary toxicity test was carried out using three modified method of Tripathi *et al.* (2021). This was done to determine the degree to which the extract could be harmful to living organism. Albino mice were used. The animals were put into six (6) groups of three each (A, B, C, D, E and control). The average weight (in grams) of each group was taken using digital weighing balance (Sartorius ED153-N). Groups A, B, C, D and E were administered orally with different concentrations of the extracts (25, 50, 100, 200 and 400 mg/mL) respectively while the control group was given ordinary distilled water. The toxicity was monitored after 24, 48 and 72 h.

2.9 Statistical Analysis

The results were expressed as mean $\pm$ standard deviation (mean $\pm$ s. d.) of three different measurements. Statistical analysis was performed on data generated from the study using Microsoft excel and SPSS softwares. Statistical significance was determined using

one way analysis of variance (ANOVA) and student's t-test. Values with $P < 0.05$ compared with the control groups were considered as being significantly different (Okoye *et al.* 2016a; Okoye *et al.* 2016b; Uba, 2018; Uba, 2019; Uba *et al.* 2019; Ibo, *et al.*, 2020).

Results and Discussion

The result the quantitative phytochemical analysis of *Garcinia kola* seed extract is shown in Table 1. Quantitatively for ethanolic extract, the highest component was saponins (6.471 $\pm$ 0.02 mgQAE/g) followed by glycosides (3.421 $\pm$ 0.01 mgDE/g), phenolics (2.471 $\pm$ 0.01 mgGAE/g), flavonoids (2.041 $\pm$ 0.05 mgQCE/g), alkaloids (0.647 $\pm$ 0.03 mgAE/g) and tannins (0.342 $\pm$ 0.02 mgTAE/g). Extraction of bioactive agents from medicinal plants permits the demonstration of their physiological activity. These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. The presence of these phytochemicals in cola species, most especially saponins and flavonoids explains the reason why these medicinal plants are used to treat hypertension because alkaloids and saponins prevent the excessive intestinal absorption of cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension (Omwirhiren *et al.*, 2017) This observation is in concordance with the research works done by Bolarinwa *et al* (2017).

The result of the effect of ethanolic extract of *G. kola* on urease activity is reported in Table 2. In the modified method, inhibitions of urease activity ranging from (72.30 $\pm$ 0.79 - 31.90 $\pm$ 0.52) was seen at a dose concentration of 25 mg/mL and continued and became highest at 400 mg/mL. The control containing omeprazole had very high inhibition (20.89 $\pm$ 0.56) and control without omeprazole had the lowest inhibition (82.73 $\pm$ 0.45) of urease activity. Since urease of *H. pylori* is an essential enzyme for its colonization, we focused on natural source that could inhibit the activity of this enzyme. *G. kola* seeds are chewed by many people and ranked well among the medicinal plants used routinely among many tribes in Nigeria and some parts of Africa for the treatment of infections caused by microorganisms. The results obtained from this study revealed that the aq extract of *G. kola* seed at 25, 50, 100, 200, and 400 mg/mL produced a significant ($p < 0.05$) anti-urease activity when compared with urea + urease (untreated) and omeprazole (treated) control albino mice (Table 2). This suggests that if the dose of *G. kola* ethanol extract is increased beyond 400 mg/ml used in this study, it will have a higher inhibition of urease activity i.e. it is dose dependent. Omeprazole inhibits proton pump and it could be possible that *G. kola* may also use the same mechanism of action since from the results there is no significant difference in the group treated with omeprazole and 400 mg/ml of *G. kola* extract. Olebunne *et al.* (2022) reported that the acetone extract was the most active of the three with a mean % urease inhibition of 51.6 % though significantly ($p < 0.05$) lower compared to the at the same 1 mg/mL test concentration. Of reference thiourea (see Table 2). However, the trend in its urease inhibition activity appears to reduce with fractionation as its daughter fractions AOP (43.3 %) and AAP (12.0 %) were significantly ($p < 0.05$) lower. This could probably be due to a synergistic effect among the constituents of the acetone extract. Our study also implicated high tannin presence in *G. kola* extract which is a polyphenol which could contribute to urease activity inhibition. Tannins are used in medicine primarily because of their astringent properties; they react with the proteins of the tissue layers and precipitate as micro proteins at the site of the peptic ulcer, forming a protective pellicle that prevents absorption to toxic substances, and promote

resistance to the action of proteolytic enzymes, an associated activity against *H. pylori* (Vasconcelos *et al.*, 2008).

The results of the effect of ethanolic seed extracts of *G. kola* on aspirin, acetic acid and indomethacin - induced ulcer mice model is reported in Figures 1, 2 and 3. It was observed that protection of gastric mucosa against aspirin, acetic acid and indomethacin was more significantly ($P < 0.05$) prominent in albino mice pre-treated with 400 mg/mL of *G. kola* ethanolic seed extracts. However, the potency of the seed extract on aspirin-induced ulcerogenesis at increasing dose was found to be higher (79.69 %) than the other experimental gastric ulcer models (Figure 1). The reason for these differences could be that these gastro-protective effects followed their prostaglandins – mediated cytoprotective mechanism of action. This finding was similar to that obtained with unripe banana and plantain (*Musa spp.*) and *Synclisia cabrida* extracts on aspirin - and indomethacin - induced ulcerogenesis in rats and albino mice, respectively (Onwudiwe, 2012). The significant inhibition of gastric ulcer in pre-treatment with extracts were compared with omeprazole which is a standard drug used for curing gastric ulcer with 400 mg/mL treated animals and their actions was found not to be the same. It also suggests that if dose is increased beyond 400 mg/mL (the highest concentration used in this study), it would have a higher antiulcer effect than omeprazole since it is dose dependent. The results further showed that gastro-protective ability of the extracts increased with the increase in their various concentrations and highest with omeprazole (standard proton pump inhibitor) and least with water (without protection). In other words, ulceration was highest with water and decreased with increase in concentration of the extract and least with Omeprazole. The possible reason for these differences could be attributed to high presence of bioactive compounds such as flavonoids, saponins,

tannins contained in *G. kola* ethanolic extract thereby conferring and demonstrating the anti-ulcer benefits on them (Vasconcelos *et al.*, 2008; Tabin *et al.*, 2016). Tripathi *et al.* (2021) in their study on antiulcer activity of ethanolic leaf extract of *Capparis zeylanica* against chemically induced ulcers reported that the area of ulcer was significantly reduced in both acute [naproxen-induced ulcer model (3.62 mm²), histamine-induced ulcer model (3.2 mm²) and ethanol-induced ulcer model (106.4 mm²) and chronic [chronic naproxen-induced ulcer model (2.14 mm²), chronic histamine-induced ulcer model (0.16 mm²). Irfan *et al.* (2024) in their study on anti-ulcer activity of aqueous ethanol extract of *Rheum spiciforme* and its fractions in animal model reported that a significant decrease in ulcerative lesions, ulcer index, ulcer severity score, volume, free acidity, and total acidity of gastric juice as well as a marked increase of gastric pH in the groups treated with plant extract at doses of 250 and 500 mg/kg. The findings of these authors uphold the observations obtained in the present study.

Table 3 showed the acute toxicity test of *G. kola* ethanol and aqueous extracts. The animals (albino mice) were treated with the extracts at doses varying between 25 and 400 mg/mL and were kept under observation for 72 hr. All the animals remain alive and did not manifest any significant visible signs of toxicity at these doses of the extract under study. The results could be considered favourable judging from the absence of adverse clinical manifestations in experimental animals (albino mice) after 72 hours of observation. It is concluded that the extract has no acute toxicity. Similar observation was documented by Iniaighe and Onyemaonyeoru (2015) who reported that the oral LD₅₀ was estimated to be more than 5000 mg/kg showing that the extract is relatively safe for use.

Table 1: Quantitative phytochemical analysis of *Garcinia kola* ethanolic seed extracts

Phytochemical	Ethanolic extract
Alkaloids (mgAE/g)	0.647±0.03
Tannins (mgTAE/g)	0.342±0.02
Saponins (mgQAE/g)	6.471±0.02
Flavonoids (mgQCE/g)	2.041±0.05
Phenolics (mgGAE/g)	2.471±0.01
Glycosides (mgDE/g)	3.421±0.01

Key: mgGAE/g: Milligrams of Gallic Acid Equivalent per gram; mgTAE/g: Milligrams of Tannic Acid Equivalent per gram; mgQE/g: Milligrams of Quercetin Equivalent per gram; mgRE/g: Milligrams of Rutin Equivalent per gram; mgAE/g: Milligrams of Atropine Equivalent per gram; mgDE/g: Milligrams of Digoxin Equivalent per gram; mgQAE/g: Milligrams of Quillaic Acid Equivalent per gram; Values are mean of triplicate measurements.

Table 2: Effect of ethanolic extract of *Garcinia kola* on urease activity

Test (Extract)	Urease activity (%)
Urease + urea = 400mg/ml	31.90 ± 0.52
Urease + urea = 200mg/ml	36.20 ± 0.23
Urease + urea = 100mg/ml	46.27 ± 0.14
Urease + urea = 50mg/ml	52.70 ± 0.31
Urease + urea = 25mg/ml	72.30 ± 0.79
Urease + urea + Omeprazole	20.89 ± 0.56
Urease + urea	82.73 ± 0.45

Values are mean ±S.E.M. of triplicate measurements. Unit of activity is in µ/ml

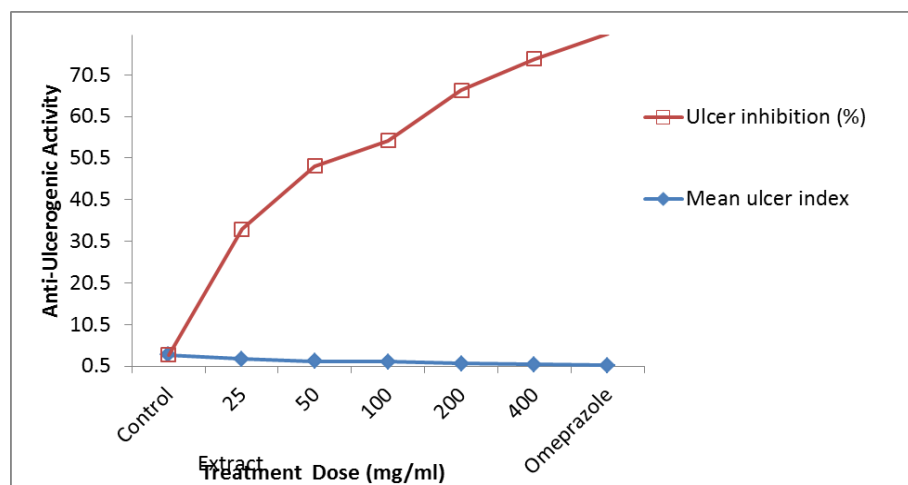
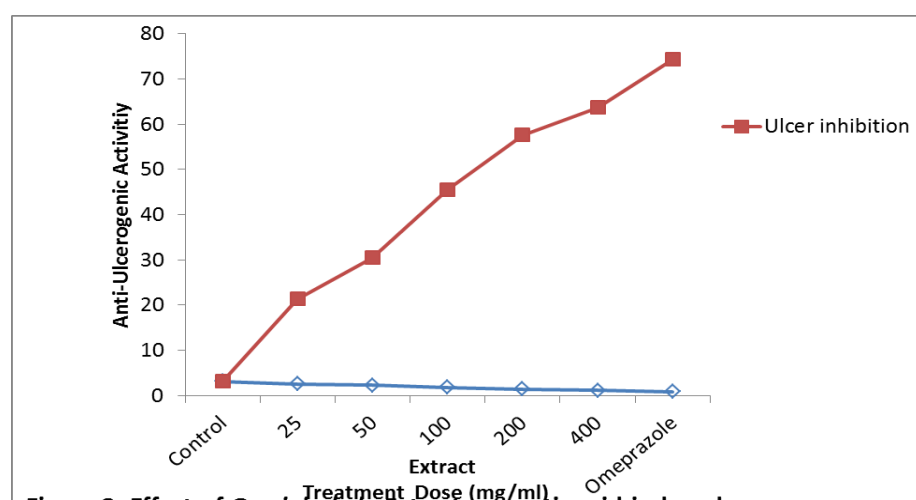
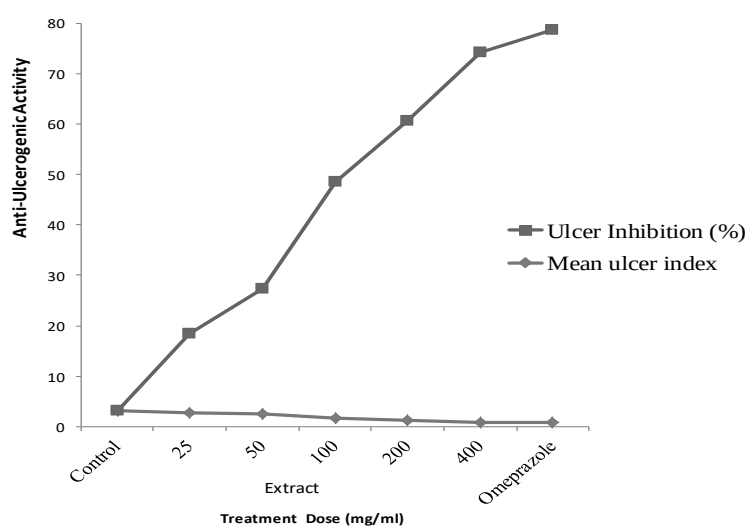
**Figure 1:** Effect of ethanolic extract of *Garcinia kola* on aspirin-induced ulcer**Figure 2:** Effect of ethanolic extract of *Garcinia kola* on acetic acid-induced ulcer**Figure 3:** Effect of ethanolic extract of *Garcinia kola* on indomethacin – induced ulcer

Table 3: Acute toxicity test of *Garcinia kola* extract

Extract	No. of mice	Average weight (g)	Dose (mg/ml)	Death after			No. of survived animals
				24 h	48 h	72 h	
Ethanolic	3	26.50	25	-	-	-	3
	3	26.00	50	-	-	-	3
	3	27.00	100	-	-	-	3
	3	27.80	200	-	-	-	3
	3	29.50	400	-	-	-	3
Aqueous	3	26.40	25	-	-	-	3
	3	27.60	50	-	-	-	3
	3	27.00	100	-	-	-	3
	3	28.80	200	-	-	-	3
	3	28.50	400	-	-	-	3

Key: - = No death recorded

Conclusion

The present work has revealed that ethanolic seed extracts of *G. kola* possesses both anti-ulcer and anti-*H. pylori* effects by significantly protecting the gastric mucosa against aspirin-, acetic acid and indomethacin – induced gastric ulcers and significantly inhibiting the function or production of the enzyme urease. The activities could be attributed to the presence of the phyto-constituents such as flavonoids, saponins and tannins present. There were statistical significances between the dose concentrations but no much difference between omeprazole treated control group with 400 mg/mL extract-treated group. Thus, these extracts may therefore, be of value in the development of novel agents in the treatment of various forms of gastrointestinal disorders.

Conflict of interests: The authors declare that they have no conflict of interests.

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