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IN THE STOMACH OF RATS.**

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KOLAVIRON ATTENUATES ISCHEMIA-REPERFUSION INJURY IN THE STOMACH OF RATS.

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Abstract

Kolaviron (KV), an active complex of at least three compounds in *Garcinia kola* seed known for its antioxidant and anti-inflammatory activity was investigated for its gastro-protective effect in the stomach of rats subjected to ischemia-reperfusion-induced gastric ulceration. Male adult Wistar rats (180 – 210 g) were used, randomized into six groups (n = 15) as follows; 1: control, 2: ulcerated untreated (UU), 3: KV alone (KVA), 4: KV + ulcer (KVU), 5: Ulcer + KV (UKV) and 6: Ulcer + omeprazole (20 mg/kg) (UOme). Ulcer was induced through ischemia-reperfusion method after two weeks of daily oral KV (100 mg/kg). Rats were weighed daily, gastric acid secretion, ulcer scores, hematological, biochemical and histological variables were assessed 1 h after induction, 3 and 7 days post ulceration. Body weight decreased in KVA (179.1 ± 1.6 g), and KVU (170.1 ± 2.2 g) compared with UU (199.0 ± 1.4 g). Gastric acid secretion decreased significantly in KVU after 1 h and 3 day post ulceration (0.27 ± 0.03 mEq/L; 0.49 ± 0.02 mEq/L) compared with UU (0.60 ± 0.06 mEq/L; 0.85 ± 0.29 mEq/L), respectively. There was significant reduction in neutrophil/lymphocyte ratio of KVA (0.29 ± 0.06) and KVU (0.35 ± 0.02) compared with UU (0.54 ± 0.04). Malondialdehyde level decreased significantly with concomitant increase in anti-oxidative activities and nitric oxide level in the KV treated groups (KVA, KVU, UKV) compared with UU. In conclusion, treatment with KV protects the stomach by reducing gastric acid secretion, promoting antioxidant activity and suppressing action of reactive oxygen species.

Key Words: Gastric ulcer, Kolaviron, Gastro-protection, Ischemia-reperfusion, Rat

Introduction

The gastrointestinal (GI) tract is protected from various endogenous injuries by a series of physical and chemical barriers which prevent the auto-digestion and erosion of the gut (Copeman et al. 1994). The major physical barrier regions of the GI tract are predominantly the epithelial tight junctions, while the main chemical barriers include mucus secreted by the goblet cells found throughout the gut length as well as gastric acid neutralizing bicarbonates (Turner 2009). An imbalance between these barriers and factors causing erosion of the gut results in the formation of gastric ulcer.

Ischemic injury occurs when the blood supply to an area of tissue is cut off. Experimental studies using variety of animal models and clinical studies involving patients undergoing surgery show that damage to the mucosa is done during reperfusion (Flaherty 1999). Reperfusion of ischemic tissues is often associated with microvascular dysfunction (Carden and Granger 2000). Activated endothelial cells in all segments of the microcirculation produce more oxygen radicals but less nitric oxide, thus leading to the production and release of inflammatory mediators. This enhances the biosynthesis of adhesion molecules which mediate leukocyte-endothelial cell adhesion (Carden and Granger 2000).

Gastric ischemia causes formation of xanthine oxidase which breaks down excess hypoxanthine produced during reperfusion, resulting in the formation of toxic Reactive Oxygen Species (ROS) (Collard and Gelman 2001). This surge in ROS levels causes a breakage in the cell membrane integrity, thereby resulting in ulcer formation. Superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are biological products of ROS capable of damaging molecules of biological classes. Most of the ROS target the cell membrane thus leading to its damage with increased induction of

lipid peroxidation (Marisa et al. 2012). Antioxidants control mechanisms in living tissues that keep ROS in check. Their significance is dependent on the type of ROS, the site and process of generation of ROS, as well as prevents or delay oxidative damage to a target substrate (Halliwell and Gutteridge 2007).

Ulcer healing is a process of reconstructing the gastric mucosa through the formation of granulation tissues via a series of processes. These processes include formation of an ulcer base and blood vessels (angiogenesis) as well as reestablishment of glandular architecture (Syam et al. 2009). This process entails the stimulation of adequate blood flow needed to sustain gastric acid secretion, thus providing mechanisms for the clearance of harmful agents.

There is increased use of medicinal foods or herbs rich in antioxidants such as *Garcinia kola* for the treatment of gastrointestinal disorders or diseases (Iwu et al. 1993). *Garcinia kola* (Species authority: Heckel, *Guttiferae* family) is a specie of flowering plants native to several West African countries. The tree is about 14 m tall and produces reddish, yellowish or orange coloured fruits containing 1 to 4 seeds (Olabanji et al. 1996). Kolaviron has been identified as the active compound of *Garcinia kola* seed and has been studied extensively for its protection on the stomach of rats with experimentally induced gastric injury (Olaleye 2005; Olaleye and Farombi 2006). However, there is dearth of information on the role of kolaviron during I/R induced-gastric injury. This study was conducted to investigate the activities of kolaviron during I/R gastric injury in Wistar rats.

Materials and methods

Plant materials

Seeds of *Garcinia kola* were obtained from Oje market in Ibadan, Nigeria in August 2015 and identified by Prof. Egunyomi in the Department of Botany, University of Ibadan. A voucher specimen is available in the herbarium of the University. The seeds were peeled, sliced, dried at room temperature, ($25 \pm 2^\circ\text{C}$) and pulverised with a blender. Kolaviron was isolated according to the method described by Iwu et al (1993) as modified by Ijomone and Obi (2013). Briefly, 2 kg of the powdered seeds were defatted with n-hexane (temperature at $40\text{--}60^\circ\text{C}$) in a Soxhlet for 24 h. The defatted, dried marc was repacked and extracted with methanol. The concentrated methanol extract was diluted to twice its volume with distilled water and further subjected to chloroform extraction. The concentrated chloroform fraction yielded about 20 g yellow solid known as kolaviron which is about 1% of the starting material. The purity and identity of kolaviron were assessed by presenting it to thin-layer chromatography (TLC) using silica gel GF 254-coated plates and solvent mixture of methanol and chloroform in the ratio 1:4 v/v (Adaramoye et al. 2005a)

Experimental Animals and Treatment Protocol

Adult male Wistar rats (180 – 210 g), aged 12 weeks and housed in the Central Animal House, College of Medicine, University of Ibadan were used for this study. They were acclimatized under standard laboratory conditions and had free access to Ladokun[®] feeds and water. The rats were divided into six experimental groups of 15 animals each, 5 animals were sacrificed on each of the three assigned experimental days (1, 3 and 7 days post ulceration). Group 1- Control (no ulcer); 2- Ulcerated untreated (UU); 3- Kolaviron alone (KVA); 4- Kolaviron Pre-treated + ulcer

(KVU); 5- Ulcer + Kolaviron treatment (UKV); 6- Ulcer + Omeprazole (UOme). Kolaviron (100 mg/kg) was given orally through an oral cannula to all kolaviron groups daily for 2 weeks (Farombi and Nwaokeafor 2005), except UKV group that was given kolaviron for 1 week after ulcer induction. The UOme group received omeprazole (20 mg/kg) orally in 1 mL solution once daily for 1 week through an oral cannula after induction of injury. The study was approved by the Gastrointestinal Research Group, University of Ibadan, Nigeria and conformed to the Guidelines of the National Institute of Health - Guide for the Care and Use of Laboratory Animals (National Institute of Health 1985).

Ischemia-reperfusion method of gastric ulcer

Ischemia-reperfusion was carried out as described by Ueda et al (1989) and modified by Wada et al (1996). The rats were fasted for 24 h after which they were anaesthetized by a cocktail intramuscular injection of ketamine (0.015 mL/kg b.w) and xylazine (0.0005 mL/kg b.w). The left side of the abdomen of the rat was shaved and an incision was made below the thoracic cage. The stomach was located, brought out and freed of fat and adjoining muscles. The left gastric artery was clamped for 30 min using a bulldog clip (ischemic stage). About 30 min after clamping of the gastric artery, the bulldog clip was removed to permit re-oxygenation of the gastric tissue for another 20 min (reperfusion stage) (Wada et al. 1996). Thereafter, the abdomen were sutured back and reopened after 1 h (for day 1), 3 and 7 days post ulceration for excision of stomach in order to assess gastric ulcer healing.

Haematological Analysis

Blood was obtained from all animals through retro-orbital plexuses for the determination of full blood count (Dacie and Lewis 1994)

Gastric acid Secretion

Gastric acid secretion was measured using the continuous perfusion method of Ghosh and Schild (1958) as modified by Amure and Ginsburg (1964). The abdomen of each rat was opened by a transverse midline longitudinal incision. A cannula was passed down the oesophagus until the tip just lay in the luminal portion of the stomach. The stomach was then mobilized from its bed and delivered through the abdominal wound, with minimal stretching. A cannula was passed from the duodenal sphincter to collect 10 mL effluent into a beaker. The cavity of the stomach was perfused with introduction of 0.9% saline, at a temperature kept at 35-37 °C through an oesophageal tube at a constant rate of 1 mL/min for 10 min each. In order to determine acidity, 10 mL of the stomach perfusate was titrated against 0.0025M NaOH solution with 2-3 drops of phenolphthalein as indicator. The acidity was expressed in mmol/10 min after calculation in each sample by applying $C_A = (C_B \times V_B)/V_A$. (C_A : acid concentration of gastric secretion, C_B : concentration of NaOH, V_B : volume of sodium hydroxide, V_A : volume of gastric effluent). pH was obtained from the equation, $pH = -\log [C_A]_{10}$.

Ulcer Assessment and Scoring

The degree of ulceration was assessed using macroscopic grading (with 2x magnification hand lens) and then microscopic analysis (histology). Scoring of the gastric ulcerated area was done as follows: the stomach was opened along the greater curvature, bathed in cold phosphate saline and spread out on a filter paper and scored by three blind observers before it was photographed.

The ulcerated area was calculated according to the method described by Kulkarni (2002). Normal stomach score = 0, Red coloration score = 0.5, Spot ulcer score = 1, Hemorrhagic streak score = 1.5, Ulcer greater than 3 mm and less than 5 mm score = 2, Ulcer greater than 5 mm score = 3. The % ulcer healing was obtained according to the method described by Onasanwo et al (2010).

$$\% \text{ ulcer healing} = \left\{ (U_c - U_x) / U_c \right\} \times 100$$

(where, U_c is the control mean ulcer index, U_x is the test mean ulcer index).

Biochemical analysis

Protein Quantification

Protein concentration of stomach samples were measured according to the method of Gornal et al. (1949), with slight modification in which potassium was added to the Biuret reagent to prevent precipitation of Cu^{2+} as cuprous oxide.

Determination of Lipid peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1990) while the MDA level was calculated by method described by Adam and Seregi (1982).

Determination of Superoxide Dismutase (SOD) activity

The activity profile of SOD in the gastric homogenates was determined by the method of Misra and Fridovich (1972).

Determination of Catalase activity

Catalase activity was determined by Sinha method (Sinha 1972).

Determination of Nitric Oxide (NO)

Tissue levels of NO were quantified indirectly as total nitrite (NO₂) using Griess reagent in which the reaction relies on diazotization with sulfanilic acid and N-1-naphthyl-ethyle diamine to give a colored product that can be read at 548 nm (Ignarro et al. 1987).

Histological Analysis

Small sections of the stomach corpus in each group were collected in 10% neutral phosphate buffered formalin for proper fixation. These tissues were subsequently processed and embedded in paraffin wax for histological analysis.

Statistical Analysis

Results were expressed as Mean $\pm$ SEM and analysed using one-way ANOVA with Bonferroni comparison *post hoc* test on GraphPad Prism version 5.0 for Windows (GraphPad software Inc., San Diego, CA), $p < 0.05$ was considered statistically significant.

Results

Effect of kolaviron treatment on body weight change during ischemia/reperfusion gastric ulcer healing in experimental rats

The effect of kolaviron on weight change of the stomach is shown in figure 1. There was significant decrease in the body weight of kolaviron pre-treatment groups (KVA, KVU) when compared with control group on day 1.

Effect of kolaviron on hematological indices during ischemia/reperfusion induced ulcer healing in experimental rats

Table 1, Figures 2, 3 and 4 show the effects of kolaviron on hematological variables, Red Blood Cell (RBC) counts, White Blood Cell (WBC) count, Packed Cell Volume (PCV), and hemoglobin (Hb) concentration of ischemia/reperfusion induced rats on 1, 3 and 7 days post-ulceration.

There were no significant differences in the hematological variables measured 1 h post ulceration in all experimental groups. There was significant increase in the platelet count of the ulcerated untreated group compared with the control. On days 3 and 7 post ulceration, a significant reduction in platelet count of KVA, KVU, UKV and omeprazole treated groups was observed when compared with UU group. The hemoglobin concentration and packed cell volume (PCV) values in the UU group were not significant compared with control throughout the days examined.

Figure 3 show the effects of kolaviron on neutrophil lymphocyte ratio (N/L) of ischemia/reperfusion induced rats on days 1, 3 and 7. There was no significant difference in the

N/L after 1 h post ischemia/reperfusion ulceration between the groups. On day 3, the N/L of the kolaviron treated ulcerated group reduced significantly compared with ulcerated untreated while a significant increase was observed in the UKV or omeprazole treated animals compared with KVA animals. The N/L ratio of the ulcerated (kolaviron or omeprazole) treated groups decreased significantly compared with UU animals.

Effect of kolaviron on basal gastric acid secretion, acidity and pH during ischemia/reperfusion gastric ulcer healing

Table 2 shows the effect of kolaviron on the basal gastric acid secretion. There was significant decrease in the basal acid secretion on days 1 and 3 post ulceration in kolaviron and omeprazole treated groups as well as an increase in pH levels when compared with ulcerated untreated group.

Effect of kolaviron treatment on ulcer scores during ischemia/reperfusion gastric ulcer healing in experimental rats.

Figure 5 shows the percentage healing of the stomach from ulcerated kolaviron treated group when compared with ulcerated untreated group. There was a significant increase in the percentage healing rate of all the treated (kolaviron and omeprazole) ulcerated groups compared with the ulcerated untreated group all through experimental days of the study.

The effects of kolaviron on lipid peroxidation (MDA) during ischemia/reperfusion gastric ulcer healing in experimental rats

Figure 6 depicts the levels of lipid peroxidation in all the treatment groups during (ischemia/reperfusion) gastric ulceration healing. There was significant increase in the MDA level 1 h post

ulceration of the ulcerated untreated, UKV or UOme groups when compared with control and KVV groups. The lipid peroxidation levels (MDA estimation) in the KVV, UKV treated groups reduced significantly when compared with ulcerated untreated group by day 3 post ulceration. A significant increase in the MDA level was observed in the ulcerated untreated and UOme treated groups when compared with control group. By 7th day post ulceration, the MDA levels reduced significantly in KVV, UKV and UOme groups compared with ulcerated untreated group.

Effect of kolaviron on Superoxide Dismutase (SOD) and Catalase activity during ischemia/reperfusion gastric ulcer healing in experimental rats

Figure 7 shows significant decrease in the activity of Superoxide Dismutase on day 1 of I/R in the UU, UKV or UOme treated groups compared with the control. There was significant increase in KVA group on day 3 when compared with the UU, KVV, UKV or UOme group. However, there was significant reduction in the SOD level of the UU group on day 7 compared with all other experimental groups.

Figure 8 shows significant increase in catalase activity of control compared with UU, KVV, UKV or UOme groups' 1 h post ulceration. A significant decrease in the SOD level of UU group was observed compared with all experimental groups 3 days post ulceration. The kolaviron pretreated ulcerated group had a significantly increased SOD level on day 3 post ulceration compared with all experimental groups. There was significant decrease in the SOD level of the ulcerated untreated group compared with all experimental groups on day 7 post ulceration.

Effect of kolaviron on nitric oxide (NO) level during ischemia/reperfusion gastric ulcer healing in experimental rats

Figure 9 revealed a significant increase in nitric oxide level of ulcerated untreated (UU), KVU, UKV and UOme groups, 1 h post ulceration compared with control and KVA groups. On the third day post-ulceration, the NO level in the ulcerated untreated increased significantly compared with control, KVA, KVU and UOme treated groups. A significant increase was also observed in the KVU, UKV or UOme groups compared with control and KVA groups. The ulcerated untreated group had a significantly increased NO level compared with all other experimental groups on day 7 post ulceration.

Effect of kolaviron on gross and microarchitecture of stomach during ischemia/reperfusion gastric ulcer healing in experimental rats

Figure 10 and Table 3 show the effect of kolaviron on ischemia/reperfusion ulcer on gross and microscopic scoring of stomach ulcers after 1 h post-ulcer induction and by days 3 and 7. There was significant reduction in ulcer score of all ulcerated treated groups compared with ulcerated untreated group.

Discussion

Ischemia-reperfusion in the gastrointestinal system is known to cause alteration in the tissue due to prevention of oxygen supply which inhibits aerobic metabolism and promotes tissue injury (Stefanutti 2005). The re-introduction of oxygen makes the injury caused by ischemia more severe with the release of pro-inflammatory substances and formation of oxygen-derived free radicals (ROS) (Cuzzocrea 2002). Meanwhile, antioxidants have been documented to inhibit the production of ROS by: direct scavenging; reducing the amount of oxidants in and around the cells; preventing ROS from reaching their biological targets; limiting the propagation of oxidants such as the ones occurring during lipid peroxidation hence thwarting oxidative stress (Ozougwu 2016). Kolaviron has been suggested as a good source of antioxidants (Jonathan et al. 2012) in the biological system which is partly responsible for its therapeutic potentials during deleterious or disease state (Ijomone and Obi 2013; Adaramoye et al. 2005a; Adaramoye and Adeyemi 2006; Obi and Nwoha 2014).

Reduction in the body weight during pre-treatment with kolaviron is in accordance with the study conducted by Obi and Nwoha (2014). This loss in weight could be attributed to the decreased intestinal absorption of food following ingestion of *Garcinia kola* components (Braide 1990), and through inhibition of oxidative processes of low density lipoprotein by kolaviron (Adaramoye et al. 2005a).

Rapid mesenteric ischemia can result into systemic inflammatory syndrome with disturbed homeostasis which can precipitate damage to distant organs (Bartels et al. 2013).

This disturbed biological homeostatic milieu may stimulate the response of both the haematological and immune systems for restoration of normalcy. In this study, kolaviron aided

in ameliorating these complications resulting from ischemia/reperfusion gastric ulceration and healing. This was observed in the kolaviron treated groups unlike in the ulcerated untreated group with increased WBC counts. The neutrophil lymphocyte ratio has been found to be a useful tool in the assessment of systemic inflammation during disease and deleterious conditions (Imtiaz et al. 2012). Neutrophils have also been observed to produce the ROS – superoxide anion during disease or inflammatory conditions via increased neutrophil infiltration in tissues (Kwiecień et al. 2002). There was a significant reduction in the neutrophil lymphocyte ratio of the kolaviron treated animals throughout the experimental days. This observed decrease in N/L responses may be attributable to the anti-inflammatory properties of the biflavonoid kolaviron (Olaleye et al. 2010) which the ulcerated animals were treated with. It could also be that the kolaviron helped in modulating deleterious activities caused by reducing radicals such as, superoxide anion supposedly generated by the released neutrophils during the ulceration process while accelerating healing.

Platelets are known for their role in prevention of breakage, repair and restoration of blood vessels after injury (Nurden et al. 2008; Gawaz and Vogel 2013). A general increase was reported in the platelet count of all the ischemia/reperfusion induced gastric ulcerated animals on day 3. However, the platelet count of the animals treated with kolaviron reverted to values comparable with that of the ulcerated untreated animals by day 7. This observed biological response is suggestive of kolaviron stimulating effects on the factors responsible for enhanced healing in the treated groups.

Gastric ulceration has been documented to result from an imbalance between the protective (or defensive) and aggressive factors within the gastric mucosa (Kumar et al. 2012). Increased gastric acidity (low pH) and increased gastric acid secretion are major aggressive factors causing

ulceration in various experimental models (Nakamoto et al. 1998) in which ischemia/reperfusion is not left out (Brzozowski 2000). Most treatments of gastric ulceration target a reduction in the gastric acid secretion and reduced gastric acidity (high pH) in order to favour healing (Tasman – Jones 1986).

In this study, kolaviron treated groups showed reduced gastric acid secretion compared with the ulcerated untreated animals. Kolaviron also caused a significant reduction in ulcers formed with a corresponding increase in percentage of healing. These observations further corroborate previous experiments which used other gastric ulcer models (Olaleye and Farombi 2006).

The gastric ulcer produced during ischemia/reperfusion has been documented to be the result of generation of superoxide oxygen species as well as depletion of energy (Zimmerman and Granger 1994). These formed superoxide anions attack the membranes of the cells by causing peroxidation of the lipid layer (McCord 2008); a radical chain reaction (Cuzzocrea et al. 2002). It has been well documented that SOD is a scavenger of superoxide anion while catalase is a scavenger of hydrogen peroxide formed during the dismutase of superoxide (Yoshikawa et al. 1993). Researchers have observed that once these radicals have been significantly reduced, the lesions produced on the gastric mucosa during ischemia/reperfusion also diminish (Yoshikawa et al. 1989). Several studies (Olaleye and Farombi 2006; Ayepola et al. 2013) have documented the ability of kolaviron to act as an antioxidant, hence ameliorating varied diseased conditions. In this study, it was observed that treatments with kolaviron prevented lipid peroxidation during ischemia/reperfusion gastric ulceration and healing. Pre- and post-treatment with kolaviron led to a significant increase in the varied antioxidant enzymes (Superoxide Dismutase and Catalase) measured. These increased activities of antioxidant markers might possibly be a way by which

kolaviron has facilitated and enhanced healing of the gastric ulcerations produced during ischemia/reperfusion.

Under normal physiological conditions, NO production plays an important role in mediating many aspects of inflammation (Fiorucci et al. 2007). However, in abnormal situations, it is regarded as a pro-inflammatory mediator that induces inflammation due to its excess production by inducible Nitric Oxide Synthase (iNOS) (Cross and Wilson 2003). In this study, the level of nitric oxide increased significantly in the ischemia-reperfusion induced ulcer of the untreated control group, and a gradual reduction in the treated groups on days 3 and 7. The increased concentration of NO (in ulcerated untreated rats as observed in this work) could lead to its reaction with superoxide anion to form a poisonous nitrite anion which might damage the colon mucosa. Treatments with kolaviron significantly reduced nitric oxide levels which further corroborate earlier reports (Martín et al. 2001; Khattab et al. 2011). It may well be that the varied healing properties conferred on the gastric mucosa by kolaviron in the treated experimental rats might have been as a result of the modulatory activities of nitric oxide. Histological analysis shows that the epithelial layer of rat stomach were protected in kolaviron treated groups against infiltration, unlike in the untreated groups that suffered serious epithelial damage. This meant ingesting kolaviron has no proven adverse effect on the stomach mucosa.

Conclusion

In conclusion, pre- and post-treatments of ischemia-reperfusion induced ulcer with kolaviron offered appreciable cyto-protection to the gastric mucosa by reducing ulcer formation and promoting ulcer healing via reduction of gastric acid secretion, increased antioxidant activities and NO levels. Other possible mechanisms of its healing properties could be investigated to further strengthen the research focus in line with the findings in this study. This study will

benefit from further work in the areas of real-time blood flow assessment and inflammatory cytokines determination.

Declaration of Interest

Authors declared no conflict of interest

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Draft

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Table 1: The effect of kolaviron on the platelet, red blood cell and white blood cell counts during ischemia/reperfusion gastric ulcer healing in experimental rats.

GROUPS	PLATELET (millions/cu mm)			RBC (millions/ cu mm)			WBC (millions/cu mm)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
C	140000±23	140000±23	140000±23	6.48±0.01	6.48±0.01	6.48±0.01	3983±66.7	3983±66.7	3983±66.7
UU	119000±99	235300±29 ^a	95330±80 ^a	6.99±0.30	6.62±0.14	6.95±0.14	5667±441.0	12600±115.5 ^a	5800±351.2 ^a
KVA	142000±25	148700±30 ^b	60670±38 ^b	6.47±0.04	6.49±0.03	7.59±0.04	3633±583.3	3953±1.7 ^b	4053±1.7
KVU	157000±11	177300±82 ^b	67000±35 ^b	7.38±0.14	6.47±0.02	6.65±0.07	8900±652.6	5533±318.0 ^{ab}	4850±50.0
UKV	119000±99	164700±23 ^b	60670±29 ^b	6.99±0.29	6.99±0.15	6.64±0.06	5667±441.0	5900±450.9 ^b	4600±264.6 ^b
UOme	119000±98	179700±18 ^b	64000±44 ^b	7.39±0.31	6.89±0.17	6.56±0.18	5667±441.0	8100±862.2 ^{ab}	4467±169.1 ^b

C= Control, UU= Ulcerated untreated, KVA= *Kolaviron* alone, KVU= *Kolaviron* Pre-Treated + Ulcer, UKV= Ulcer + *Kolaviron* and UOme= Ulcer + Omeprazole.

Values are expressed as Mean± SEM and considered significant when $p < 0.05$ except on day 0. Keys of significance; ^a- compared with control, ^b- compared with Ulcerated untreated, ^c- compare with *Kolaviron* Pre-treated + Ulcer.

Table 2: Effect of kolaviron on basal gastric acid secretion, acidity and pH during ischemia-reperfusion gastric ulcer healing in experimental rats.

	GASTRIC ACID SECRETION (cm ³ /10mins)			ACIDITY (10 ⁻⁴ mmol)			pH		
Groups	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
C	0.33±0.03	0.34±0.03	0.23±0.01	0.8±0.1	1.0±0.8	0.57±0.03	4.08±0.04	4.08±0.04	4.25±0.03
UU	0.60±0.06 ^a	0.85±0.29 ^a	0.38±0.03 ^a	1.5±0.14 ^a	2.0±2.1 ^a	0.94±0.07 ^a	3.83±0.04 ^a	3.67±0.15 ^a	4.03±0.03 ^a
KVA	0.31±0.02 ^b	0.45±0.05 ^b	0.36±0.03	0.8±0.04 ^b	1.4±1.0 ^b	0.9±0.07	4.10±0.02 ^b	3.95±0.049 ^b	4.05±0.04
KVU	0.27±0.03 ^b	0.49±0.02 ^{ab}	0.25±0.02	0.7±0.08 ^b	1.2±1.3 ^{ab}	0.63±0.06	4.18±0.06 ^b	3.91±0.016 ^{ab}	4.20±0.06
UKV	0.60±0.07 ^{ac}	0.58±0.20 ^{ab}	0.25±0.04	1.5±0.14 ^{ac}	1.5±1.4 ^{ab}	0.62±0.09	3.83±0.04 ^{ac}	3.84±0.02 ^{ab}	4.22±0.06
UOme	0.60±0.05 ^{ac}	0.39±0.01 ^b	0.26±0.02	1.5±0.14 ^{ac}	0.9±1.0	0.65±0.05	3.83±0.04 ^{ac}	4.02±0.02	4.19±0.04

C= Control, UU= Ulcerated untreated, KVA= Kolaviron alone, KVU= Kolaviron Pre-treated + Ulcer, UKV= Ulcer + Kolaviron and UOme= Ulcer + Omeprazole. Values are expressed as Mean ± SEM and considered significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with Ulcer untreated, ^c-compared to Kolaviron alone group.

Table 3: Effect of kolaviron on ulcer scores during ischemia-reperfusion gastric ulcer in rats.

GROUPS	DAY 1	DAY 3	DAY 7
C	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
UU	15.50 ± 4.16	12.00 ± 0.76	10.83 ± 0.73
KVA	0.00 ± 0.00	1.01 ± 0.06 ^{b,e,f}	0.00 ± 0.00 ^b
KVU	4.31 ± 0.52 ^{b,d,c}	1.50 ± 0.29 ^{b,e,f}	0.00 ± 0.00 ^b
UKV	15.30 ± 4.13	6.00 ± 1.53	1.31 ± 0.90 ^b
UOme	15.52 ± 4.10	2.00 ± 0.58 ^{b,e}	1.0 ± 0.61 ^b

C= Control, UU= Ulcerated untreated, KVA= Kolaviron alone, KVU= Kolaviron Pre-treated + Ulcer, UKV= Ulcer + Kolaviron and UOme= Ulcer + Omeprazole. Values are expressed as Mean ± SEM and considered significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with Ulcer untreated, ^c-compared to Kolaviron alone group, ^d- compared with Kolaviron Pre-treated + ulcer, ^e- compared with Ulcer + kolaviron treated.

LIST OF FIGURE CAPTIONS

Figure 1: Effect of kolaviron on body weight changes during ischemia/reperfusion ulcer healing in experimental rats.

C= Control (no ulcer), UU= Ulcerated untreated, KVA= Kolaviron Alone, KVU = Kolaviron Pre-Treated +Ulcer, UKV= Ulcer + Kolaviron and Uome= Ulcer + Omeprazole.

Values are expressed as Mean $\pm$ SEM and considered significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with ulcer untreated.

Figure 2: Effect of kolaviron on packed cell volume during ischemia-reperfusion gastric ulcer healing in experimental rats.

C= Control, UU= Ulcerated Untreated, KVA= Kolaviron Alone, KVU= Kolaviron pretreated + Ulcer, UKV= Ulcer + Kolaviron and Uome= Ulcer + Omeprazole. Values are not significantly different ($p>0.05$).

Figure 3: The effect of kolaviron on haemoglobin concentration during ischemia/reperfusion gastric ulcer healing in experimental rats.

C= Control, UU= Ulcerated untreated, KVA= Kolaviron alone, KVU= Kolaviron Pre-treated + Ulcer, UKV= Ulcer + Kolaviron and UOme= Ulcer + Omeprazole. Values are not significantly different ($p>0.05$).

Figure 4: Effect of kolaviron on neutrophil-lymphocyte ratio (N/L) during ischemia/reperfusion induced gastric ulcer healing in rats.

Control, UU= Ulcerated untreated, KVA= Kolaviron alone, KVU= Kolaviron Pre-treated + Ulcer, UKV= Ulcer + Kolaviron and UOme= Ulcer + Omeprazole. Values are expressed as Mean $\pm$ SEM and considered significant when $p < 0.05$ except on day 0. Keys of significance; ^a – compared with Control, ^b- compared with Ulcerated Untreated, ^c- compare with Kolaviron Pre-treated ulcer.

Figure 5: Effects of kolaviron on percentage healing of ischemia/reperfusion induced ulcer in experimental rats.

C= Control, UU= Ulcerated Untreated, KVA= Kolaviron Alone, KVU= Kolaviron Pre-treated + Ulcer, UKV= Ulcer + Kolaviron and UOme= Ulcer + Omeprazole. Values are expressed as Mean $\pm$ SEM and significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with Ulcer Untreated, ^c- compared with Kolaviron alone, ^d- compared with Kolaviron pre-treated group.

Figure 6: Effects of kolaviron on lipid peroxidation during ischemia/reperfusion gastric ulcer healing in experimental rats.

C= Control, UU= Ulcerated untreated, KVA= Kolaviron alone, KVU= Kolaviron Pre-treated + Ulcer, UKV= Ulcer + Kolaviron and UOme= Ulcer + Omeprazole. Values are expressed as Mean $\pm$ SEM and considered significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with Ulcer untreated.

Figure 7: Effect of kolaviron on superoxide dismutase activities during ischemia/reperfusion gastric ulcer healing in experimental rats.

C= Control, UU= Ulcerated Untreated, KVA= Kolaviron Alone, KVU= Kolaviron Pre-Treated Ulcerated, UKV= Ulcerated treated with Kolaviron and UOme= Ulcerated treated with Omeprazole. Values are expressed as Mean $\pm$ SEM and considered significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with Ulcer Untreated, ^c- compared with Kolaviron alone, ^d- compared with Kolaviron pre-treated + Ulcer group

Figure 8: Effect of kolaviron on catalase activities during ischemia/reperfusion gastric ulcer healing in experimental rats.

C= Control, UU= Ulcerated untreated, KVA= Kolaviron alone, KVU= Kolaviron Pre-treated+ Ulcer, UKV= Ulcer + Kolaviron and Uome= Ulcer + Omeprazole. Values are expressed as Mean $\pm$ SEM and considered significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with Ulcer untreated, ^c-compared with kolaviron alone, ^d- compared with kolaviron pre-treated + Ulcer group.

Figure 9: Effect of kolaviron on nitric oxide level during ischemia-reperfusion gastric ulcer healing in experimental rats.

C= Control, UU= Ulcerated untreated, KVA= Kolaviron alone, KVU= Kolaviron Pre-treated + Ulcer, UKV= Ulcer + Kolaviron and UOme= Ulcer + Omeprazole. All values are expressed as Mean $\pm$ SEM and considered significant when $p < 0.05$. Keys of significance; ^a- compared with Control, ^b- compared with Ulcer untreated, ^c-compared with Kolaviron alone, ^d- compared with Kolaviron pre-treated + Ulcer group, ^e- compared with Ulcer +Kolaviron, ^f- compared with Ulcer + Omeprazole .

Figure 10: Effect of kolaviron on gross and microscopic structure of the stomach following ischemia-reperfusion ulcer. U- ulcers, HLUS- healing ulcer scar, PU- pin point ulcer, H- haemorrhagic streak. C = Control, UU= Ulcerated Untreated, KVA=Kolaviron alone, KVU= Kolaviron Pre-Treated + Ulcer, UKV= Ulcer + Kolaviron treatment and UOme = Ulcer + Omeprazole. White arrows shows changes in mucosa integrity, blue arrows shows submucosa oedema and cellular aggregation. Magnification x100, Stain – Haematoxylin & Eosin.

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