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Investigation of Polyisoprenyl Benzophenone for Anti-ulcer Potentials in Ethanol-HCl-Induced Gastric Ulcerations in Albino Rats

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ABSTRACT

Acute upper gastrointestinal bleeding resulting from peptic ulcers are increasingly a common medical emergency worldwide. Endoscopic treatment and acid suppression with proton-pump inhibitors are major milestones in the management of the disease, even though these treatments options have shown promising results; therapeutic management of gastric ulcer remains a challenge. In a quest to further new drug discovery, this study was carried out to evaluate the effect of polyisoprenyl bezophenone (kolanone), an isolate from the seeds of *Garcinia kola* on ethanol-induced gastric ulcer the effect was compared against a known anti-ulcer drug omeprazole.

Kolanone was isolated from dried seeds of *Garcinia kola* via a series of chromatographic separations techniques involving the use of analytical solvents in different ratio combinations. Animals were fasted for 18 h before treatment with different doses of Kolanone (25, 50 and 75 mg /kg) or omeprazole (20 mg/kg). Gastric ulcer was induced by oral administration of ethanol-acid (25 mL/kg of 0.3 M HCl in 60% ethanol). One hour later, animals were humanely euthanized and gastric mucus was analyzed for antioxidants.

Kolanone showed a significant gastro-protective effect against ethanol-induced stomach ulcers at 50 and 75 mg/kg compared to omeprazole (20 mg/kg) and distilled water-treated rats. It also prevented the activation of lipid peroxidation induced by ethanol presumably by enhancing antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) potential and lowering lipid peroxidation (TBARS production) of the gastric mucosa thereby lowering mucosal injury. This effect may find beneficial applications in the therapy for ulcer patients and in wound healing.

Keywords: Polyisoprenyl bezophenone (kolanone), Gastric ulcer, Omeprazole, Oxidative stress

Introduction

Peptic ulcer is a chronic, non-malignant inflammatory bowel disease with symptoms of ulceration in the stomach and duodenum.[1] The cause of gastric ulceration is not clearly defined although multifactorial, but some predisposing factors including duration of starvation, nature of food ingested, bile reflux,[1] lessened mucosal resistance[2] and alteration of gastric mucosal blood flow. Also implicated as causative factors of gastric ulcers include disruption of the gastric mucosal barrier by stress[3,4] leading to decrease in alkaline mucosal bicarbonate and mucus secretion,[5] overdosage and or prolonged administration of non-steroidal anti-inflammatory drugs have been implicated.[6] *Helicobacter pylori* is a gram negative, spiral, flagellated bacterium with a capability for abundant urease production which has also been implicated in several upper gastrointestinal diseases that present as dyspepsia,[7] is a major aetiological agent in chronic gastritis, peptic ulcer disease and gastric carcinoma. Persistent infection with *Helicobacter pylori*,[8] *Zollinger-Ellison* syndrome[9] and genetic factors are suggested to be of a higher incidence of duodenal ulcers in patients with positive family history of this disorder or blood type O.[10] Most anti-ulcer drugs require a prolonged period of intake, yet ulcer relapse is a common occurrence[11] and most of them have various adverse side effects.[12] No drug proves solely effective in treating peptic ulcer. Nutritional supplements in the form of antioxidants come from plants sources. *Garcinia kola* Heckel (family *Guittiferae*) is one of such plants. It is a plant commonly grown in Nigeria and the seeds possess a unique astringent bitter and resinous taste. This plant has been called a "wonder plant" since every part of it has been found to be of medicinal importance. *Garcinia kola* seeds is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhoea, laryngitis, bronchitis, gonorrhoea and stomach upset. The seed is masticatory and also used to prevent and relieve colic, chest colds, and cough and can as well be used to treat headache.[8] Studies have also reported the use of this plant for the treatment of jaundice, high fever, as purgative and as chewing stick.[13] The plant has also found usefulness in the treatment of stomach ache and gastritis.[5, 6] The phytochemical compounds isolated from *G. kola* include oleoresin, tannins, saponins, alkaloids, cardiac glycosides. Other phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybiflavonols. *Garcinia kola* seed is also used in folklore remedies for the treatment of various infections caused by pathogens.[8] The diversity presented in the biological and therapeutic activities and the pharmacological significance of *Garcinia kola* has provided a renewed interest in the use of this plant for further search for novel products of pharmaceutical benefit.[9] More so, their role as a basis for new drug discovery and development; advances in the field of natural product chemistry provide valuable information on Garcinia fruits which revealed the presence of biologically important secondary metabolites known as polyisoprenylated

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benzophenones.[10] They are mainly present in the genus Garcinia (Guttiferae) which occupies a prominent position in the history of natural products and when compared to the long history of medicinal uses and widespread research on Garcinia, the study of polyisoprenylated benzophenones (PIBs) has been relatively limited.[11] During recent years, these PIBs have been recognized as interesting and valuable biologically active secondary metabolites as many of the isolated polyisoprenylated benzophenones exhibited significant cytotoxic activity in *in vitro* and *in vivo*.[12] During the past decades, some promising advances had been achieved in understanding the chemistry and pharmacology of polyisoprenylated benzophenones.[11,12]

Kolanone (Figure 1) is a natural polyisoprenyl benzophenone with reported antioxidant property extracted from the seeds of *Garcinia kola* (Guttiferae) common to West Africa, phytochemical investigations of *G. kola* resulted in the isolation of cycloartenol, 24-methylene-cycloartenol and kolanone[14] from the light petroleum extract and C-3/8''-linked hydroxybiflavanonols from the ethyl acetate extract of the seeds.[15] The bioflavonoid has been shown to improve the negative effects of oxidative stress in lipids, proteins and DNA in rats.[16] This study investigated the possible ameliorative effects of kolanone on ethanol-induced gastric ulcers in albino rats.

Materials and Methods

General Instrumental Procedure

UV spectrum was determined on Shimadzu U-2000 spectrophotometer (Shimazu Scientific Instruments, Japan). Infrared spectrum was obtained using KCl discs in a Shimadzu/IR-408 spectrophotometer (Shimazu Scientific Instruments, Japan); ^{1}H- and ^{13}C-NMR spectrum (in pyridine-*d5*) was run on a PicoSpin spectrometer (Thermo Fisher Scientific, UK) equipped with a 5 mL ^{1}H and ^{13}C probe operating at 90 MHz and 360 MHz, respectively, with tetramethylsilane (TMS) as internal standard. Mass spectrum was obtained from a gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS QP5050A equipment connected to an ion trap detector operating in Electron Impact mode at 70 eV, with a sampling rate of 0.50 scans/s and scanning speed of 1000. Melting point was determined on Mettler melting point apparatus FP 80 HT. Optical rotation was carried out on Perkin-Elmer-241 spectrophotometer.

Solutions, reagents, drugs and chemical

Methanol (Sigma-Aldrich St. Louis USA), ethanol, light petroleum, ethyl acetate and chloroform were obtained from Zayo-Sigma, Jos, Nigeria; Omeprazole capsules (XL laboratories, Rajasthan, India). All solvents and reagents used were of analytical grade and standard.

Plant Material

Fresh seeds of *Garcinia kola* were purchased from Nsukka market in Enugu State corresponding to the coordinates 6°51'24"N, 7°23'45"E. The seeds were identified by a taxonomist with Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria, Voucher No.: BDCP19/05172. The seeds were dried at room temperature (25 - 27^{o}C) and pulverized into coarse powder using hammer mill.

Extraction and Isolation

A 1500 g of the powdered seeds was extracted using a Soxhlet (Thermo Fisher Scientific), with light petroleum ether (boiling point 40 – 60°C). The light petroleum ether extract was concentrated under reduced pressure and, on standing, gave a yellow-brown precipitate.

Isolation of Kolanone

About 10 g of the above precipitate was chromatographed on a silica gel (Silica gel-G, 230-400 mesh) column (8 x 100 cm) eluted with crescent polarity mixtures of *n*-hexane/ethyl-acetate and ethyl acetate/methanol to give 10 mL aliquots that were grouped by similarity in TLC (thin layer chromatography) Hussain *et al*.[17] The aliquots were monitored by thin layer chromatography (TLC) (GF_{254} silica gel) (Sigma-Aldrich, USA) with solvents systems of ethyl acetate: chloroform: methanol 1:9:1 and then 1:4:1. A total of 5 fractions were obtained with the following R_f values: A (R_f 0.92), B (R_f 0.85), C (R_f 0.80), D (R_f 0.45), E (R_f 0.35). Preparative thin layer chromatography of A, B and C using the solvent systems - ethyl acetate: chloroform:methanol 1:9:1 and then 1:4:1 gradient elution was done followed by elution of the bands with methanol. Methanol gave only compound B in appreciable amounts of crystalline material.

Acute toxicity test

Thirty (30) adult albino rats of both sexes weighing 136 ± 2.5 g were randomly separated into 5 groups (A–E) of 6 rats per group. Groups A–D were dosed orally with varying doses (50, 100, 250, and 700 mg/kg) of kolanone while group E was given an equivalent volume of distilled water (10 mL/kg). The rats were allowed access to feed and water *ad libitum* for 48 h and observed for signs of toxicity and death. The experiment was approved and conducted according to the permission and prescribed guidelines of the Institutional Animal Ethics Committee, University of Calabar, Nigeria.

Experimental animals

Twenty-five (25) adult albino rats of both sexes weighing between 138 ± 2.65 g were used for the study. They were kept in well-ventilated metal cages at the animal house of Pharmacology Department, University of Calabar, Nigeria. They were fed with pelleted growers mash feed (Vital®, Jos) and water *ad libitum*. The rats were allowed 7 days to acclimatize before the experiments were conducted according to the permission and prescribed guidelines of the University of Calabar, Nigeria, Animal Ethics Committee (Approval Number: 019C20351). All animals were humanely handled and their welfare respected throughout this study as stipulated in the 1964 Helsinki Declaration, as amended (WMA, 2019).[18]

Effect of Kolanone on ethanol-induced gastric ulcers in rats

Ethanol-induced ulcers were evaluated in rats as previously described by Suleyman *et al*.[19] Animals were fasted for 18 h but allowed access to only water prior to the experiment and divided into 5 groups (n = 5). Groups I, II and III received 25, 50 and 75 mg/kg body weight of kolanone, respectively while groups IV and V received omeprazole (20 mg /kg b.w) and distilled water, respectively. Treatments were by oral gavage. Thirty minutes after oral administration of kolanone and Omeprazole, gastric ulcer was induced with oral administration of chilled absolute ethanol (90%) at 1 mL/200 g body weight. One hour later, the animals were sacrificed and their stomachs removed and cut along the greater curvature and rinsed under a stream of water. Lesions on the gastric mucosa were observed with a hand lens (x10)[20] and scored 0-4 using an arbitrary scale where 0 = no lesions; 0.5 = hyperemia; 1 = one or two lesions; 2 = severe lesions; 3 = very severe lesions and 4 = mucosa full of lesions.[21] The mean ulcer index for each group was subjected to Mann-Whitney test and the effectiveness of the extract and drug was calculated using the equation below.

$$PI = \frac{UI_c - UI_t}{UI_c} X\ 100$$

Where: *PI* = Preventive index (%), UI_c = Ulcer index of control and UI_t = Ulcer index of treated.

Analysis of gastric mucus for antioxidant activity

The gastric mucus was analyzed for antioxidants. The glandular segments from the stomach were removed and immediately homogenized in 3 mL of 20 mM phosphate buffer (pH 7.4) by using Heidolph homogenizers with a Teflon pestle. A 10 µL 0.5M BHT in acetonitrile was added to 1 mL of tissue homogenate to prevent sample oxidation. The precipitate was removed by centrifugation (2000 rpm). An aliquot was collected and frozen immediately at -20°C prior to testing. 0.2 mL of the homogenate was used for the assay. Aliquots were taken from this preparation for quantitative analysis of the activities of glutathione peroxidase (GPx).[22] Catalase (CAT) was assayed colourimetrically using dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The intensity was measured at 620 nm and the amount of hydrogen peroxide hydrolysed was calculated for the catalase activity.[23] Superoxide dismutase (SOD) activity was determined by the modified method of Kakkar *et al*.[24] A single unit of enzyme was expressed as 50% inhibition of NBT (nitroblue tetrazolium) reduction 1 min/mg protein. Lipid peroxidation was determined by quantifying the thiobarbituric acid reactive substances (TBARS).[25] In brief, 0.1 mL of homogenate was treated with 2 mL of (1:1:1) ratio (TBA)

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thiobarbituric acid - (TCA) trichloroacetic acid - (HCl) reagent (TBA 0.37%, 0.25 N HCl and 15% TCA) and placed in a water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank.

Statistical Analysis

The data were subjected to ANOVA using GraphPad prism version 5.0. for windows. Mean Ulcer scores of different groups were compared using Kruskal-Wallis followed by Dunn's post hoc tests. $P < 0.05$ was considered significant. While percentage protection for each group was calculated from the mean ulcer score.

Results and Discussion

Extraction and characterization of Kolanone

Kolanone (397.1 mg) was isolated as pale-yellow needles in a crystalline form. Kolanone was recrystallised from 80% ethanol/water as pale-yellow needles, melting point: 107 - 109°C. UV λ_{max}: 243, 357 nm; unchanged on addition of $AlCl_3$ or NaOH. IR ν_{max} 3550 - 3250 (broad, OH), 1660, 1650 (C=O), 1590, 1230, 1180 cm^{-1}. ^{1}H-NMR (90 MHz) δ: 7.40 - 7.55 (5H, m, H-9 to H-13), 4.80 - 5.30 (4H, m, =CH-CH_2), 3.10 - 3.30 (2H, dd, CH_2CH=). 2.50 - 2.80 (4H, m, CH_2CH=), 2.10 (4H, s, H-5", H-6"), 1.60 - 1.80 (21H, m, =C-Me). ^{13}C-NMR (360 MHz) δ: 195.12, 192.93, 189.17 (C-3, C-7, C-1, 174.12 (s, C-5), 141.8 (s, C-3"), 135.90, 135.17, 131.17 (s, C-3', C-3'", C-8"), 131.71 (d, C-11), 128.80 (s, C-8), 128.14 (d, C-9, C-13), 127.95 (d, C-b, C-12), 123.77, 121.10, 118.79, 118.07 (d, C-2', C-2", C-7", C-2"'), 109.87, 108.17 (s, C-2, C-4), 57.39 (s, C-6), 39.90, 37.49, 36.34 (t, C-1', C-1", C-6"), 26.27,25.97, 25.78 (q, C-4', C-9", C-4'"), 21.66, 21.23 (t, C-1'", C-7"), 18.08, 17.96, 17.72 (q, C-5', C-5'", C-10"), 16.26 (q, C-4").

MS found: M^+ 502.3087; $C_{33}H_{42}O_4$ needs 502.3083; m/z, %): 502 (38, M^+), 434 (27, M^+-C_5H_8), 433 (41, $M^+C_5H_9$), 377 (31, M^+-C_9H_{17}), 365 (8, M^+-$C_{10}H_{17}$), 349 (32, M+ $C_{11}H_{21}$), 311(23, M^+-$C_{14}H_{23}$), 309(49, M^+-$C_{14}H_{25}$), 297 (35, M^+ -$C_{15}H_{25}$), 255 (12, M^+-$C_{18}H_{31}$), 229 (2, M^+-$C_{20}H_{33}$), 105 (100, C_7H_5O), 77 (35, $C_6H_5^+$).

Acute toxicity

No death was recorded in the rats treated orally with kolanone up to the highest dose of 700 mg/kg used. Kolanone was well tolerated by the rats without any overt signs of toxicity.

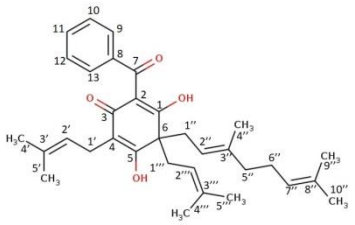

Figure 1: Chemical structure of polyisoprenyl bezophenone (kolanone)

Effect of Kolanone on ethanol-induced gastric ulcers in rats

Kolanone at all doses administered significantly protected the development of ulcers induced by ethanol acid as shown by the Mean Ulcer Score (Figure 2A) and percentage protections (Figure 2B). These activities were comparable to Omeprazole at 20 mg/kg although kolanone at 75 mg/kg gave a better protection (88.02%) than Omeprazole at 20 mg/kg (86.6%) and distilled water (0 %).

Effect of Kolanone on antioxidant systems in ethanol-induced gastric ulcers in rats

Table 1 gives the extent of oxidative stress and lipid peroxidation as seen by the formation of TBARS and antioxidants in gastric mucus of animals in each group. Significantly lower *($P < 0.05$)* level of TBARS was observed in the kolanone and omeprazole treated rats as compared to rats treated with distilled water. The activities of SOD, CAT and GPx concentration were significantly lower in the ulcerated rats *($P < 0.05$)* as compared to treated rats. The activities of these enzymes in Kolanone-treated rats were comparable to those in the rats that were treated with omeprazole the reference drug.

Ethanol-induced gastric ulcers have been widely used for the experimental evaluation of antiulcer agents.[25-27] Alterations in gastric secretion, damage to gastric mucosa, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol consumption especially on empty stomach.[26]

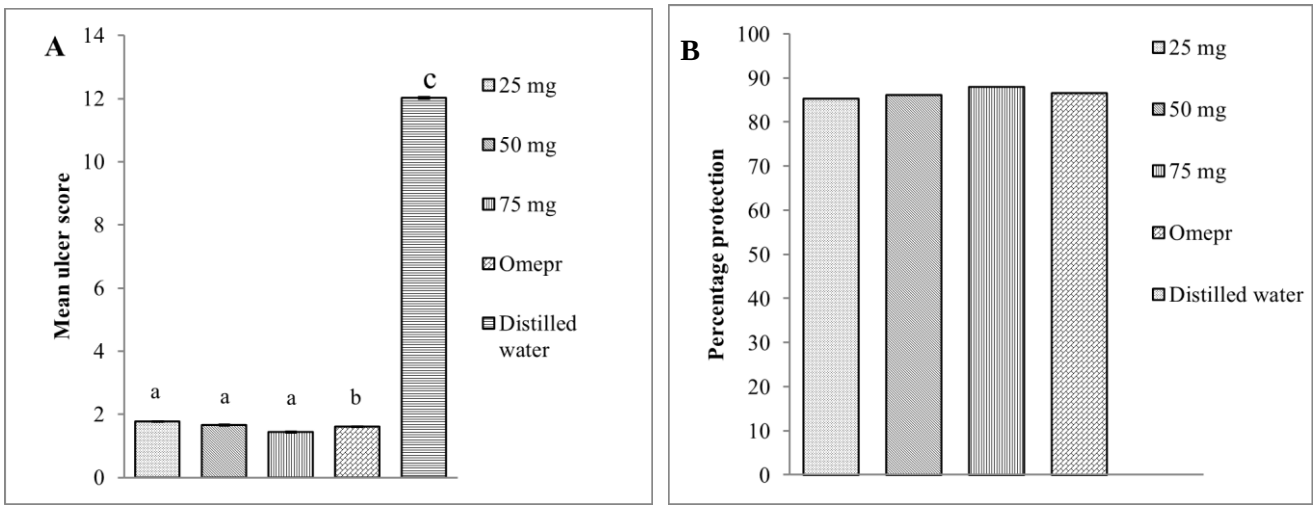

Figure 2: Effect of kolanone on ethanol-induced ulcer in albino rats. **(A)** Mean ulcer score. **(B)** Percentage protection of albino rats.

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Table 1: Antioxidant Effects of Kolanone

Group	Treatment	CAT (mmoles of H_2O_2 consumed min/mg protein)	GPx (mg of GSH consumed/min/mg protein)	TBARS (nmoles of MDA formed/mg protein)	SOD (units[a]/min/mg protein)
1	Kolanone (25 mg/kg)	1.22±0.18*	118.2±0.04	6.01±0.4	4.12±0.03
2	Kolanone (50 mg/kg)	1.29±0.21*	125.3±0.30*	5.72±0.3*	5.22±0.04*
3	Kolanone (75 mg/kg)	1.95±0.04	141.3±0.09*	5.31±0.43*	5.83±0.03*
4	Omeprazole (20 mg/kg)	1.35±0.22*	129.0±0.08*	4.53±0.40*	4.01±0.04*
5	Distilled water (10 ml/kg)	0.61±0.03	112.3±0.05	10.01±0.81	1.98±0.08

[a] *One unit corresponds to the amount of enzyme that gives 50 % inhibition of NBT reduction*
** As compared to negative control (distilled water), P < 0.05*

Kolanone suppressed ulcerogenic tendencies of ethanol in this experiment at 50 mg/kg and 75 mg/kg, an effect suggestive of possible antioxidant potentials (Table 1). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) have the capability to neutralize unstable molecules called free radicals.[26] They disrupt the chain reactions in which free radicals turn other molecules into free radicals like themselves, a process of chain breaking or stabilization.[26] Kolanone also influenced free radical formation.[10] Lower concentrations of peroxidation index (TBARS) was seen and this is associated with an increase in antioxidant enzyme activities. This may be due to replenishment of sulfhydryl levels in the mucosa as increase in levels of -SH groups account for ulcer inhibition.[27]

Earlier reports have shown that most plant extracts and pure compounds isolated from plant origin possess significant antioxidant activities *in vitro*.[26-28] The gastro-protective potentials of kolanone can be linked to its antioxidant activity. Kolanone is a polyisoprenyl benzophenone which is a flavonoids with high antioxidant potentials.

Omeprazole is a proton pump inhibitor and offered protective effect on gastric mucosa while 75 mg/kg of Kolanone produced a more protective effect on the rat gastric mucosa (Figure 2). The results of the *in vivo* studies indicate that the effect of the compound at 50 and 75 mg/kg was similar to that of omeprazole, suggestive of a possible inter-relationship in their mechanism of action. Proton pump inhibitors are capable of producing almost complete suppression of acid secretion. The mechanism of action of omeprazole is such that it binds very specifically to a single subunit of the H^+, K^+-ATPase at the secretory surface of parietal cells and inactivate it.[29] It reduces acid secretion regardless of the source of secretory stimulation. By increasing intragastric pH through inhibition of acid secretion, proton pump inhibitors inhibit activation of pepsin. They are effective in treating peptic ulcer diseases and gastroesophageal reflux with both short and long-term use.[26] With the advancement of information technology and bioinformatics, there is an increasing trend to build resources and databases that report herbal formulations, active components of the herb, and related information.[30] This study showed that Kolanone given orally provided a dose-dependent gastro protection against the effects of ethanol which is a necrotizing agent.

Conclusion

Kolanone exerts its antiulcer activity by inhibiting acid secretions and through cytoprotective effects. Kolanone protected the mucosa through preventing the formation of lesions by various necrotic agents through the inhibition of the K^+-ATPase (Proton pump) activity. It also exhibited antioxidative properties by scavenging the free radicals and reactive oxygen species possibly produced around the mucosal environment. This effect may find a beneficial application in the therapy of gastric ulcer since kolanone possess anti-oxidative and cytoprotective potentials. Kolanone is a likely candidate in the therapy of gastric ulcers and in wound healing.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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