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Research Article

Chemopreventive Influence of Kolaviron on 1,2-Dimethylhydrazine Induced Plasma Carbonyl Content and Non-Enzymatic Antioxidant Status in Rat Colon Carcinogenesis

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Abstract: Colon cancer is the second leading cause of cancer death worldwide with diet playing a prominent role in disease initiation and progression. We have investigated the chemopreventive efficacy of kolaviron on plasma protein oxidation and non-enzymatic antioxidant status on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis. Male albino Wistar rats were randomly divided into six groups. Group 1 served as control, animals have access to water and the rodent feeds for 8 weeks plus 1mM EDTA-saline injection subcutaneous (s.c) once a week for 4 weeks. Group 2 rats served as kolaviron (KV) control received 100 mg/kg bodyweight of kolaviron per oral (p.o.) every day. Group 3 served as gallic acid (GA) control, received pellet diet and 50 mg/kg bodyweight of gallic acid p.o. every day. Group 4 served as carcinogen control, received pellet diet and 30 mg/kg bodyweight of 1,2-dimethylhydrazine (DMH) subcutaneous injection once a week for 4 weeks to induce colon carcinogenesis. Group 5 rats received DMH injection and kolaviron 100 mg/kg bodyweight. Group 6 rats received DMH injection and gallic acid 50 mg/kg bodyweight. The results of this experiment shows significant decrease levels of Vitamin C and Total Antioxidant Capacity in plasma of carcinogen exposed groups as compared with control group. Again the levels of carbonyl content and Lipid Hydroperoxide were significantly higher as compared with the control group. Supplementation with kolaviron or gallic acid increases the levels of Vitamin C and Total Antioxidant Capacity but decreases the levels of carbonyl content and Lipid Hydroperoxide significantly as compared with the DMH group. These findings suggest that both kolaviron and gallic acid can significantly reduce the formation of plasma free radicals which are crucial in colon carcinogenesis and effectively modulate the levels of vitamin C and Total Antioxidant Capacity in rats.
Keywords: Colon cancer¸ carcinogenic, 1,2-dimethylhydrazine (DMH), Antioxidant activity, kolaviron.

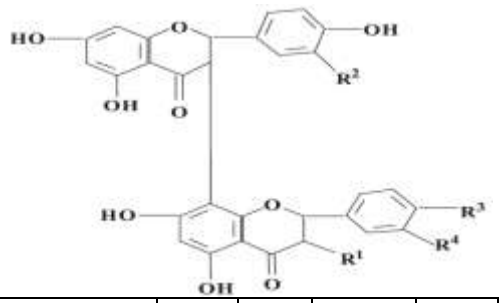
INTRODUCTION

Cancer of the colon is one of the most common cancers in developed countries and its chemoprevention is of great interest throughout the world [1]. During the early stages, neoplastic development by environmental genotoxic and non-genotoxic carcinogens act predominantly by triggering the free radical mediated damage [2]. The carcinogen, 1,2-dimethylhydrazine (DMH) is an alkylating agent, the metabolic events of which are believed to occur in the liver with the formation of active intermediates such as azoxymethane and methylazoxymethanol, that are subsequently transported to the colon via bile or the blood [3]. The decomposition of methylazoxymethanol results in the formation of methyldiazonium ions, which generate reactive carbonium ions capable of methylating DNA, RNA, or protein of colonic epithelial cells [4]]. Moreover, previous reports have pointed out the tendency of DMH to produce free radicals in blood, plasma and large bowel of experimental animal models[5].

An increase in the reactive oxygen metabolites (superoxide radical and hydrogen peroxide, precursors of a number of oxygen-derived radicals including hydroxyl radicals) in tumor cells are noted in the early stages, and the overproduction and/or the inability to destroy them may result in severe damage to cell molecules and structures. During carcinogenesis, reactive oxygen radicals may damage specific genes that control the growth and differentiation of cells [6]. Recently, a general scheme has been proposed to describe the role of oxy-radicals during the initiation and promotion stages of carcinogenesis, which leads to decreased PUFA content in the tumor subcellular membranes. This in turn would diminish the lipid peroxidizability of these membranes, reducing the production of lipid hydroperoxides in later stages of carcinogenesis and may confer an advantage to overwhelm oxidative stress [7].

Cells are equipped with an impressive repertoire of antioxidant enzymes as well as non-enzymic small antioxidant molecules [8]. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are considered to be the primary antioxidant enzymes, since they are involved in the direct elimination of reactive oxygen species. The non-enzymic small molecular antioxidants including sulfhydryl compounds such as glutathione (GSH), ascorbate and α-tocopherol also play a significant role in reducing the oxidative stress. Since these antioxidants work co-operatively, a change in any one of them may break the equilibrium and cause cell damage leading to malignancy[9].

Bitter kola (Garcinia kola) belongs to the family of plants called Guttiferae and the genus Garcinia. Garcinia kola seeds have been shown to contain a complex mixture of polyphenolic compounds, biflavonoids, prenylated benzophenones and xanthones which account for the majority of its effects [10-11]. Kolaviron (KV) is an extract from the seeds of Garcinia kola, containing a complex mixture of biflavonoids and polyphenols [12]. A number of studies have confirmed the antioxidative and anti-inflammatory effects of kolaviron in chemically-induced toxicity, animal models of diseases and in cell culture [13-15]. Although the chemopreventive effect of kolaviron has been reported in aflatoxin B1-induced genotoxicity and hepatic oxidative damage and 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation animal models [16-17], no study has addressed the effect of Kolaviron on plasma lipid peroxidation and non-enzymatic antioxidant status on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis. In the present study, we investigated the effects of kolaviron in modulating plasma Vitamin C, Total Antioxidant Capacity, lipid hydroperoxide and carbonyl content. The primary objective of this study was to assess the influence of kolaviron on DMH-induced colon carcinogenesis by correlating carbonyl content in plasma and antioxidant status.



	R1	R2	R3	R4
GB 1	OH	H	OH	H
GB 2	OH	H	OH	OH
Kolaflavanone	OH	H	OMe	OH

Fig-1 structure of kolaviron

MATERIALS AND METHODS

Animals

Four-week-old, maleWistar rats were obtained from Pharmacology Animal House, Niger Delta University, quarantined for 1 week and allocated randomly to experimental and control groups. Animals were maintained as per the principles and guidelines of the Ethical Committee of Animal Care of Niger Delta University. The animals were housed five per cage in a specific pathogen-free animal room under controlled conditions of a 12 h light/12 h dark cycle, with temperature of 22±1 °C and relative humidity of 50±10% till the end of 8 weeks.

Extraction of kolaviron

Garcinia kola seeds purchased from a local market in Yenagoa, Nigeria, were certified at the department of Botany, Niger Delta University, Nigeria. Peeled seeds were sliced, pulverized with an electric blender and dried at 40 ^{0}C in a drying oven. Powdered seeds were extracted with light petroleum ether (boiling point 40–60 ^{0}C) in a soxhlet for 24 h. The defatted dried marc was repacked and extracted with acetone. The extract was concentrated and diluted twice its volume with water and extracted with ethylacetate (6 x300 mL). The concentrated ethylacetate yielded kolaviron as a golden yellow solid shown in fig -1. [10].

Chemicals

1,2-Dimethylhydrazine dihydrochloride was purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Experimental design

Male albino Wistar rats were randomly divided into six groups. Group 1 served as control, animals have access to water and the rodent feeds for 8 weeks plus 1mM EDTA-saline injection subcutaneous (s.c) once a week for 4 weeks.Group 2 rats served as kolaviron (KV) control received 100 mg/kg bodyweight of kolaviron per oral (p.o.) every day. Group 3 served as gallic acid (GA) control, received pellet diet and 50 mg/kg bodyweight of gallic acid p.o. every day. Group 4 served as carcinogen control, received pellet diet and 30 mg/kg bodyweight of 1,2-dimethylhydrazine (DMH) subcutaneous injection once a week for 4 weeks to induce colon carcinogenesis. Group 5 rats received DMH injection and kolaviron 100 mg/kg bodyweight. Group 6 rats received DMH injection and gallic acid 50 mg/kg bodyweight.

Preparation of Plasma

After sacrificing the rats, the blood was collected in 5.0 mL heparinized tubes, and the plasma was separated by centrifugation at 800xg for 5 min at 4 ^{0}C. plasma was stored for biochemical assays.

Total Antioxidant Capacity measurements

A colorimetric method using a Randox Assay Kit (Randox Laboratories Ltd, Antrim, UK) was used to measure the TAC. The assay is based on the incubation of plasma samples with 2, 2'-azino-di-[3-ethylbenzthiazoline sulphonate 6 diammonium salt (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation $ABTS^+$ which has a relatively stable blue-green color that is measured at 600 nm. Antioxidants present in the assayed plasma samples may or may not inhibit the oxidation of ABTS to $ABTS^+$ (cause suppression of the color production) to a degree that is proportional to their concentration. The capacity of the assayed sample antioxidants was compared with that of standard Trolox, a water soluble tocopherol analogue, which is widely used as a traditional standard for TAC measurement assays, and the assay results were normalized and reported as Trolox equivalent antioxidant capacity (TEAC) and defined as the nanomolar concentration of the Trolox anitoxidant capacity of a calibration curve.

Protein carbonyl contents were determined according to the methods of Uchida et al [18]. The plasma sample was treated with an equal volume of 0.1% (w/v) 2,4-dinitrophenyl hydrazine in 2N HCl and incubated for 1 h at room temperature and then treated with 20% TCA. After centrifugation, the precipitate was washed three times with ethanol-ethyl acetate and dissolved in 8M guanidine hydrochloride in 133 mM Tris solution containing 13 mM EDTA. The absorbance was recorded at 365 nm. The results were expressed as nmol/ml based on the molar extinction coefficient of 22,000 M^{-1} cm^{-1} for aliphatic hydrazones.

Vitamin C was determined by the method of Omaye et al [19]. Briefly, 0.5 ml of plasma was mixed thoroughly with 0.5 ml of water, 1.0ml of 10% TCA and centrifuged at 3500×*g* for 20min. 1.0ml of the supernatant was subsequently treated with 0.2 ml of 75 µl of DTC reagent (2 g of dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 ml 5M H_2SO_4) and incubated at 37 ◦C for 3 h. Then 1.5ml of 65% sulphuric acid was added mixed well and the solution was allowed to stand at room temperature for another 30 min. The colour developed was read at 520 nm. Vitamin C levels were expressed as mg/ml plasma from a standard.

Estimation of lipid hydroperoxide

FOX 2 method was used to measure the levels of lipid hydroperoxides (LOOH) in plasma samples[20-21]. FOX 2 Reagent was prepared by mixing following reagents: (i) 0.1 ml of 2.5 mM ammonium ferrous sulphate (dissolved in 35.71 mM H_2SO_4), (ii) 0.6 ml of 35.71 mM H_2SO_4, (iii) 0.1 ml of 1 mM xylenol Orange and (iv) 0.1 ml distilled water. To 0.9 ml of FOX 2 reagent 100 µl of plasma sample was added, vortexed and incubated at room temperature for 1 hour. Then it was centrifuged to remove any flocculated materials and absorbance of supernatant was read at 552 nm against a blank. Cumene hydroperoxide was taken as the standard and a linear curve was obtained in the concentration range of 25-200 nmols/ml. The LOOH content was expressed as nmol lipid hydroperoxide/ml.

RESULTS

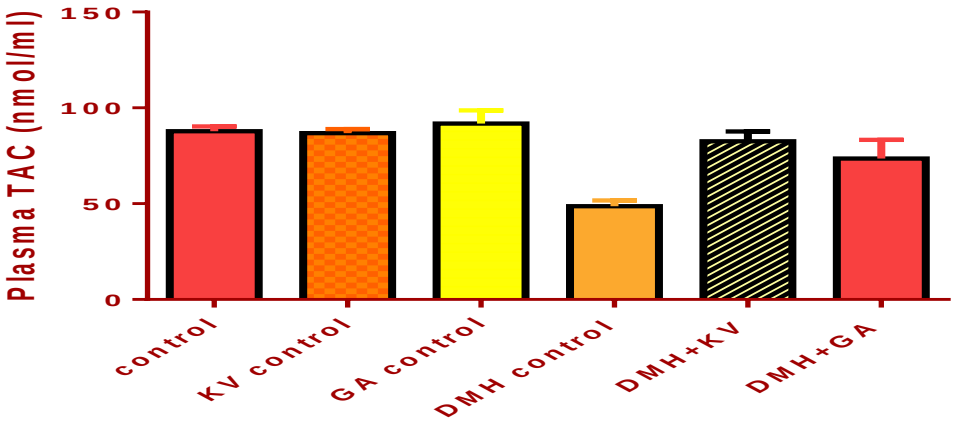


Fig-1: Effect of Kolaviron, Gallic Acid supplementation on DMH induced colon carcinogenesis on Plasma Total antioxidant level of control and experimental groups. The values are the means ± S.D. represented by vertical bars for 5 values. Statistically significant differences between control and other treatment groups are indicated by *(P<0.05) and between DMH treated group (DMH) and Kolaviron or Gallic acid supplemented groups (DMH+KV, DMH+GA) by ** (P<0.05) or *(P<0.05) respectively.**

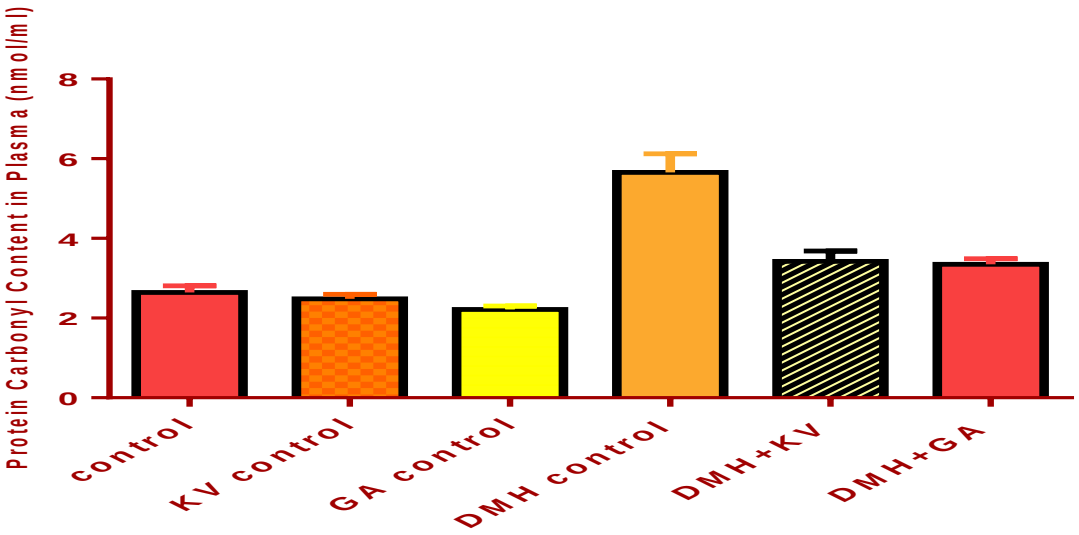


Fig-2: Effect of Kolaviron, Gallic Acid supplementation on DMH induced colon carcinogenesis on Protein carbonyl level in Plasma of control and experimental groups. The values are the means ± S.D. represented by vertical bars for 5 values. Statistically significant differences between control and other treatment groups are indicated by *(P<0.05) and between DMH treated group (DMH) and Kolaviron or Gallic acid supplemented groups (DMH+KV, DMH+GA) by ** (P<0.05) or *(P<0.05) respectively.**

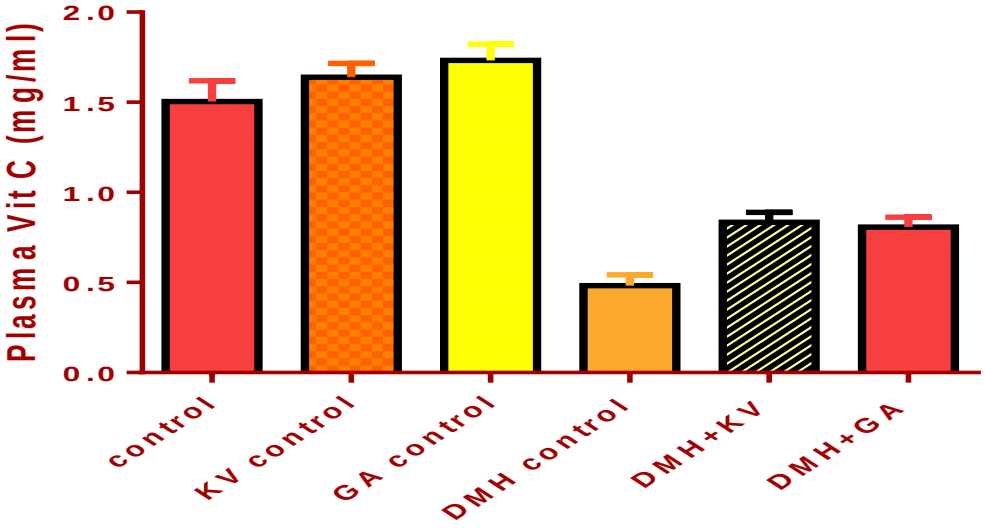


Fig-3:Effect of Kolaviron, Gallic Acid supplementation on DMH induced colon carcinogenesis on Plasma Vitamin C level of control and experimental groups. The values are the means ± S.D. represented by vertical bars for 5 values. Statistically significant differences between control and other treatment groups are indicated by *(P<0.05) and between DMH treated group (DMH) and Kolaviron or Gallic acid supplemented groups (DMH+KV, DMH+GA) by ** (P<0.05) or *(P<0.05) respectively**

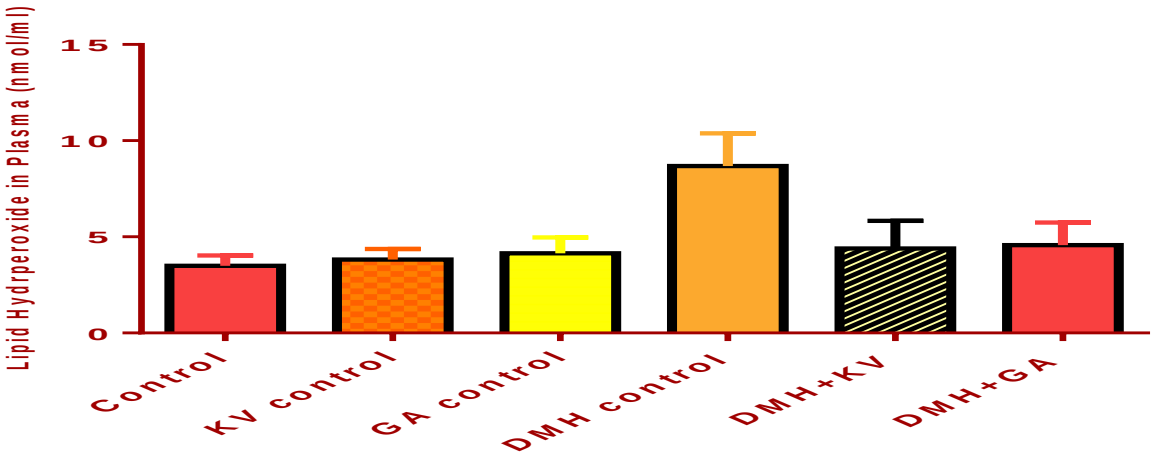


Fig- 4: Effect of Kolaviron, Gallic Acid supplementation on DMH induced colon carcinogenesis on lipid hydroperoxide in plasma of control and experimental groups. The values are the means ± S.D. represented by vertical bars for 5 values. Statistically significant differences between control and other treatment groups are indicated by *(P<0.05) and between DMH treated group (DMH) and Kolaviron or Gallic acid supplemented groups (DMH+KV, DMH+GA) by ** (P<0.05) or *(P<0.05) respectively**

DISCUSSION

Colon Cancer can be inhibited at different stages of its development [22].The results of the present study demonstrated that Kolaviron and gallic acid supplementation during the initiation phase of DMH-induced colon carcinogenesis in plasma of rats significantly modulated the levels of Total Antioxidant Capacity, protein carbonyl content, Vitamin C and lipid hydroperoxide.

DMH is a procarcinogen and after metabolic activation it results in the formation of methyl free radical which is known to induce oxidative stress. DMH also generates hydroxyl radical or hydrogen peroxide in the presence of metal ion that may play a part in the initiation of lipid peroxidation [23].

Earlier reports suggest that tumour cells produce substantial amount of H_2O_2 that are released into circulation [24]. In addition superoxide (O2•−) and hydroxyl radicals (•OH) are also released into circulation resulting in increased susceptibility of the plasma and RBC's to lipid peroxidation in DMH-treated rats. Kolaviron and gallic acid are known to possess antioxidant property and scavenge free radicals like superoxide and hydroxyl radicals 15, 25]. In our study we observed decreased levels of lipid hydroperoxide and protein carbonyl contents in Kolaviron and gallic acid supplementation. The plasma levels of both lipid hydroperoxide (LOOH) and protein carbonyl contents in DMH-treated rats were significantly higher than ($p \leq 0.5$) the Kolaviron and gallic acid supplementation groups. The enhanced lipid peroxidation in the circulation of rats bearing DMH induced colon tumours reflect excessive free radical generation aggravated by a decreased efficiency of the host antioxidant defence mechanisms. These observations are consistent with earlier studies which show that tumour cells generate and release peroxides into the circulation which can subsequently oxidize GSH[26]. Tumour cells also sequester antioxidants from circulation to promote tumour growth. This may be one of the reasons for the declined antioxidant status with enhanced lipid peroxidation in the circulation of the DMH treated rats. Kolaviron and gallic acid supplementation to DMH exposed rats significantly enhanced the antioxidants and decreased the lipid peroxides in circulation, which means that Kolaviron and gallic acid protects cells from DMH induced neoplastic transformation.

Our results have demonstrated decreased levels of the water soluble antioxidant ascorbate (vitamin C) and total antioxidant capacity level in DMH treated plasma of rats as compared to normal plasma which correlate with previous studies [29]. Cancer cells readily take up vitamin C in the oxidized form through glucose transporters (GLUTs) [27], but the precise function of vitamin C in neoplastic tissues is unknown. On Kolaviron and gallic acid supplementation, the levels of vitamins C and total antioxidant capacity levels were higher significantly. Therefore, the decreased concentrations of plasma vitamins C and total antioxidant capacity levels in DMH administered animals may be due to increased utilization of these antioxidants to counter lipid peroxidation. Increased plasma concentrations of Vitamin C ant TAC in Kolaviron and gallic acid supplementation animals may be because Kolaviron and gallic acid scavenges the free radicals involved in the process of carcinogenesis thus enhancing the concentrations of plasma vitamin C and total antioxidant capacity.

Reactive oxygen species (ROS) exert an oxidative damaging effect by reacting with nearly every molecule in the living system including protein leading to protein oxidation [28]. These lesions, if unrepaired, may contribute to mutagenesis progressing into carcinogenesis. Hence, a new approach to the control of colon cancer is critically needed. In the present study, treatment with Kolaviron and gallic acid decreased protein carbonyl content, in DMH-induced colon cancer significantly ($p \leq 0.5$).

Our results strongly suggest that administration of Kolaviron and gallic acid during the initiation of colon carcinogenesis significantly inhibited and decreased circulatory lipid hydroperoxide and protein carbonyl contents, but increases the levels of total antioxidant capacity and vitamin C levels. The study has revealed that Kolaviron and gallic acid has no toxic effects and has protective role against DMH induced colon carcinogenesis.

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