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Original Research Article

The Extract of *Acacia sieberiana* (DC.) Leaf Prevents Blood REDOX Status, Oxidative Lipid and DNA Damage Complications in DMH-induced Pre-Malignant Colon Cancer in Male Rat

Adesola V. Adegbite, Olaniyi T. Adedosu, Jelili A. Badmus*

Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

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ABSTRACT

Alkaloids are important plant phytochemical reported to have diverse pharmacological significance most especially for cancer treatment. This study sought to evaluate protective effects of alkaloid-rich extract of *Acacia sieberiana* leaf on blood reduction and oxidation (REDOX) status, oxidative lipid and DNA damage complications in DMH-induced pre-malignant colon cancer in male rat. Pre-malignant colon cancer was induced using 1, 2-dimethylhydrazine (DMH) in male *Wistar* rats through intraperitoneal injection. Alkaloid-rich leaf extract of *Acacia sieberiana* (50, 100 and 150 mg/kg) and 2 mg/kg doxorubicin (standard drug) were simultaneously administered with DMH intraperitoneally to the animals once per week for 22 weeks. The haematological indices, liver and kidney function tests, serum lipid profile and serum REDOX status were assessed. Serum lipid and DNA oxidative damage biomarkers, and induction of the colon aberrant foci crypt were evaluated. The results indicated that administration of the extract significantly ($p<0.05$) prevented the DMH-induced reduction of packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The extract significantly ($p<0.05$) reversed in a dose dependent manner the increased white blood cells (WBC), platelet (PLT), alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), malondialdehyde (MDA) and 8-hydroxyl-2-deoxyguanosine (8-OHdG) occasioned by DMH. Induction of colon cancer aberrant foci by DMH was not observed in the groups exposed to the extract. The study revealed that the alkaloid extract of the plant has potential to prevent the complications in tissues arising from chemical-induced pre-malignant colon cancer incidence.

Keywords: Oxidative stress, 8-OHdG, *A. sieberiana*, Lipid profiles, and MDA.

Introduction

Colon cancer is ranked third in terms of cause of mortality associated with cancer in the world with sustained increase incidence in developing countries.[1] Development of colon cancer is initiated when there are mutations in the epithelial cells of the large intestine.[2] Based on data released by GLOBOCAN, 2018, colon cancer is the fourth most incidents in the world. Globally, the third most diagnosed type of cancer is colon cancer constituting 11% of all diagnosed cancers.[3] Colon cancer cases are more among men than women and 3-4 times more pronounced in more developed than in developing countries. The expected number of men to be diagnosed of colon neoplasia is about 576,000 while women would be 521,000.[4]

Cancers including colon cancer presently relied on surgical resection in combination with chemotherapy, such as cytotoxic drugs and radiation for its management and treatment. However, chemotherapy and radiation are known to possess severe side effects.

Hence, the discovery of the unique products of natural sources with improved efficacy against cancer and the less injurious impact is highly desired.[5]

The use of natural plant bioactive compound such as alkaloids as pharmaceutical agent has received huge attention as a supportive or alternative therapy.[6] In fact, first and third world countries have shifted focus to substances of herbal source for therapeutic purposes due to their safety and low-cost attributes.[7] Investigations conducted on the plant kingdom for substances with anticancer potential, aided the detection of anticancer alkaloids.[8] Alkaloids are exceptionally varied chemical group that possesses a ring structure with the nitrogen atom. It has been recognized as the most significant biologically dynamic groups obtained from herbs.[9]

Acacia sieberiana is commonly known as paperback thorn and is locally called Daneji in Fulani, Fara kaya in Hausa and Aluki in Yoruba.[10] It is employed locally for the treatment of ailments such as inflammation, tiredness, joint pain etc. This study evaluated the preventive potential of crude alkaloid leaf extract of *Acacia sieberiana*.

Materials and Methods

Collection and identification of plants

Acacia sieberiana leaves were obtained in Ogbomoso, Oyo State, Nigeria during the rainy season (June, 2018). Botanical identification was done at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso. The voucher specimen of the plant leaf with voucher number (LHO 478) was deposited in the Herbarium Unit of the Department.

*Corresponding author. E mail: jabadmus@lautech.edu.ng
Tel: +234803585098

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Processing of plant samples

The leaves were separated from the stalk and air dried at room temperature. The leaves were then crushed into powder form using electric blender. The alkaloid-rich extract was obtained from the leaves of *Acacia sieberiana* using the procedure earlier described by Gonzales and Tolentino.[11] The extraction of the leaves is based on the continuous extraction method using soxhlet apparatus. The pulverized leaves of *A. sieberiana* were macerated overnight in 95% ethanol doped with ammonia in an extraction thimble. The sample was then extracted next day using soxhlet extractor for 4 hr. The ethanol extract was concentrated at 60^0C in Soxhlet distiller. It was further treated with 1.0 N HCl and the alkalinified with ammonia. The resulting extract was washed with chloroform and then concentrated at 60^0C in sohxlet distiller to obtain the alkaloid extract of *A. sieberiana* leaves.

Reagents

1, 2-Dimethylhydrazine, 8-hydroxy-2′-deoxyguanosine and MDA kit was purchased from Sigma-Aldrich Corp. Saint Louis (MO), USA. The 8-hydroxydeoxoguanosine (8-OHdG) ELISA kit (catalog number "E-EL-0028) was purchased from Elabscience Biotechnology Co., Ltd. Houston, Texas, USA, while other chemicals were purchased from Sigma-Aldby (ch Corp. Saint Louis (MO), USA.

Animals

Forty-two healthy male Wistar rats averagely weighing 120 g used for this study were housed in well ventilated cages. They had free access to standard feeds and water *ad libitum.* The rats were handled with respect to the standard operating procedures for the Care and Use of Laboratory Animals (US-NRC 2003). The study was approved by the Ethical Committee on the Use of Laboratory Animals of the Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria with approval number LTH2019-05.

Experimental design

The animals were randomly selected into seven groups (A-G) with six animals per group. Colon cancer induction in the rats was carried out via intraperitoneal injection of Dimethylhydrazine (DMH) using a modified version of a method as previously described.[12] Group A used as negative control was intraperitoneally injected with normal saline alone, Group B designated as the positive control was treated with 20 mg/kg b.w of 1, 2 -dimethyl hydrazine (DMH) weekly and normal feed alone. Group C, D and E were each intraperitoneally injected simultaneously with both 20 mg/kg of DMH and 50, 100 and 150 mg/kg b.w doses of the alkaloids-rich extract of *A. sieberiana* leaf respectively once per week. Group F was treated weekly with 20 mg/kg b.w of DMH and 2 mg/kg doxorubicin (standard anticancer drug) while group G was treated with 150 mg/kg b.w of the extract only per week. The dose of the extract was chosen based on the results of the median lethal dose of the extract (LD_{50}) of the preliminary study not included in this report. The study was conducted for 22 weeks, after which the animals were sacrificed. Ketamine hydrochloride (30 mg/kg b.w) was used to anaesthetize the animals. The blood of the animal was obtained from heart puncture using syringes and transfer into the plain bottle and EDTA vial. The blood containing a plain bottle was centrifuged at 5000 rpm for 10 min and the supernatant was carefully collected as serum into the fresh neat vial.[13] The blood in the EDTA bottle was used for haematological evaluation.

Haematological assessments

The haematology test was conducted using Abacus Junior Vet haematology autoanalyzer. White blood cells (WBC), haemoglobin (Hb), red blood cells (RBC) and platelets (PLT).

Serum antioxidant enzymes

The evaluation of Superoxide dismutase (SOD) was carried out using the method earlier documented by Magnani *et al.*[14] The method utilized the competition between the pyrogallol autoxidation by superoxide anion and dismutation of this radical by the SOD in the serum sample. The inhibition of autoxidation of pyrogallol by the sample in the presence of EDTA (pH 8.2) was read at 420 nm wavelength using Uv-visible spectrophotometry. The estimation of Catalase (CAT) was carried out using the method of Zhou and Kang[15]. The CAT evaluation is a colorimetric method that utilizes an alternative phenol (3,5-dichloro-2-hydroxybenzenesulfonic acid) that oxidatively binds to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP) to generate a red quinoneimine dye (N-(-4-antipyryl)-3-chloro-5-sulfonatep-benzoquinone-monoimine) that absorbs at 520 nm. Glutathione peroxidase (GPx) was estimated as described by Thomas *et al.*[16]The assay monitors oxidation of NADPH to $NADP^+$ during the conversion of oxidized glutathione to reduced form. The decrease in absorbance is immediately measured at 340 nm using Uv-visible spectrophometer.

Lipid profiles estimation

The effect of the alkaloid-rich extract of the plant leaves on lipid profile of rats was determined by analyzing serum level of some biochemical parameters such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), Total Cholesterol (TC) and triglycerides (TG) using RANDOX reagent by following the manufacturer's guide (RANDOX Laboratories LTD, U.K).

Liver function indices estimation

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALKP) were measured according to the method earlier described by Reitman and Frankel.[17] ALT was evaluated by monitoring the concentration of pyruvate hydrazine formed with 2, 4-dinitrophenylhydrazine at 600 nm using spectrophotometer. AST transfers the amino group of aspartate to α-ketoglutarate and the enzyme activity corresponds to the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenylhydrazine at 600 nm wavelength. ALKP catalyzes the hydrolysis of the p-nitrophenyl phosphate to p-nitrophenol at alkaline pH. The product of the reaction (p-nitrophenol) was monitored using spectrophotometer at 405 nm corresponds to the enzyme activity. Total Bilirubin (TBIL) was estimated using Agape reagent by the method of Annino.[18] The sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid which reacts with total bilirubin to form azobilirubin. The total bilirubin level corresponds to formed azobilirubin monitored using spectrophotometer at 546 nm.

Kidney function indices estimation

Creatinine (CRSC) was estimated using RANDOX reagent in accordance with the manufacturer's guide. The serum potassium (K^+) level was determined using the Henry's method as described by Abdulmumin *et al.*[19] The serum potassium level evaluation involves the use of sodium tetraphenylboron to produce a colloidal suspension. The intensity of the turbidity corresponds to serum potassium concentration at 500 nm wavelength. Sodium (Na^+) was estimated by modifying the colorimetric method of Maruna and Trinders.[20]Magnesium uranyl acetate precipitate sodium and proteins to form uranyl magnesium sodium acetate salt. The excess uranyl salt reacts with potassium ferrocynide to produce brown colour of which intensity is inversely proportional to the sodium concentration. The intensity is measured using spectrophotometer at 530 nm wavelength.

Evaluation of Oxidative Lipid and DNA damage

Lipid peroxidation was monitored by quantifying level of malondialdehyde (MDA) generated during lipid peroxidation using the previous method as described by Alam and Fareed.[21] Serum sample was added to Tris-KCl (0.15 mM) and mixture was quenched with addition of 30% trichloroacetic acid (TCA) and 0.75% thiobarbituric acid and mixture was incubated at 80^0C for 45 min. The pink colour chromogen produced was centrifuged at 3000 rpm for 10 min and the supernatant was read at 532 nm wavelength. The level of serum MDA was calculated according to equation 1 below;

$$MDA(unit/mgprotein) = \frac{Absorbance \times total\ volume\ of\ reacting\ mixture}{\text{E532} \times \text{volume of sample} \times \text{mgprotein}} \quad \text{..... equation (1)}$$

Where E532=$1.56X10^5$ $M^{-1}cm^{-1}$ (molar extinction coefficient for MDA)

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The level of 8-oxodeoxyguanosine (8-OHdG) was estimated as a biomarker of oxidative DNA damage using a modified version of an ELISA method previously described by Płachetka *et al.*[22] in accordance with the manufacturer's guide. The microtiter plate provided in the kit was pre-coated with an antigen specific to 8-OHdG and compete with a fixed amount of 8-OHdG in the sample or standard. The colour change was measured spectrophotometrically at a wavelength of 450 nm using microplate reader. The concentration of 8-OHdG in the sample was determined by extrapolating OD of the sample to the standard curve.

Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA) followed by Tukey's post-hoc test. GraphPad Prism version 6.05 for Windows (GraphPad Software, La Jolla California, USA. www.graphpad.com). Data obtained was expressed as mean ± SD of six animals in each group and the levels of significance was set at $p<0.05$.

Results and Discussion

1,2-dimethyl hydrazine (DMH) is the most regularly used substance for initiation and progression of colon cancer in animal models. It involves a multistage process that resembles human colorectal cancer and encompasses a sequence of pathological changes including aberrant cryptic foci formation.[23] Natural plant products have achieved noticeable records in the field of cancer management and treatment. More than 60% of drugs employed clinically for the treatment of cancer are substances developed from plants, marine organisms and microbes. There are still substantial numbers of plant species that are yet to be evaluated for their potential as anticancer agents.[24]

The study assessed the potential protective effects of alkaloid-rich extract of *A. sieberiana* leaves on some blood biochemical indices of male *Wistar* rats with pre-malignant colon cancer induced with DMH. A preliminary study was piloted to identify the safe dose of the alkaloid rich extract of *A. sieberiana*. The result of preliminary lethal dose study, which is not reported here form the basis for the selection of doses of the extract used for the study.

One of the systems that is highly reactive to toxic substances and acts as a key indicator of the physiological and pathological status both in humans and animals is the hematopoietic system.[25] The hematopoietic system response may involve a reduction in the level of blood hemoglobin, decrease in red blood cell (RBC) volume as well as white blood cells (WBC). The observed significant ($p<0.05$) increase of PCV, Hb and RBC by the extract when compared with the group administered DMH alone suggests the potential of the plant extract to protect the body against anaemia (Table 1). Anaemia is a known prevalent condition in cancer incidence most especially in patients undergoing chemotherapy.[26] The potential of the plant extract to revive the level of RBC and PCV back to normal can however be observed to be dose dependent. The observed effects of DMH on RBC, PCV and Hb are in agreement with previously reported studies on the effect of DMH on erythrocyte during colon cancer induction.[27]

Significant increases of WBC and PLT were observed in the group treated with DMH alone. The observed increased WBC in this study has been shown to be associated with systemic inflammation.[28] Also, increased platelet activation plays significant role in the progression of malignancies and cancer-associated thrombosis.[29] The reduction of DMH-induced increase in WBC and Platelet could be related to the alkaloids. Alkaloids have been reported to have anti-inflammatory activity.[30, 31] Similar observations were reported in the study conducted to observe the effect of *A. nilotica* on WBC and PLT in rats.[32]

Carcinogen induces cancer through several modes of actions, including increase oxidative stress. Oxidative damage to lipids, proteins and nucleic acids enhance DNA damage, genome variability, uncontrolled cell proliferation and ultimately lead to pathological complications such as cancer.[33, 34] Enzymatic (SOD, GPx and CAT) and non-enzymatic (glutathione) are known protector of cells against oxidative destruction.[35] The observed reduced serum antioxidant enzyme activities in Group B may be as a result of overwhelming reactive oxygen species generated due to DMH-induced inflammation (Figure 1). The reduction in the serum antioxidant enzyme activities observed in Group B was alleviated by the administration of the extract of *A. sieberiana* leaves in a manner that depict dose dependence. The observed effects of the plant extract are in agreement with earlier documented reports on the effect of species of *Acacia* on serum antioxidant enzymes.[36]

There is conflicting epidemiological correlation between dyslipidemia and incidence of colorectal cancer.[37] The metal-analysis has shown that serum total cholesterol and triglyceride correlate positively while HDL-C negatively correlate to cancer incidence.[37] In this study, the DMH did not induce dyslipidemia, as is shown by non-significant effects on all the serum indices of lipid profile in Group B animals.

Table 1: Effect of alkaloid-rich leaf extract of *Acacia sieberiana* on rats Haematology parameters

Groups	PCV (%)	Hb (g/dl)	WBC (×10^3/µl)	RBC (×10^3/µl)	PLT (×10^3/µl)
A	42.22±2.21^{a}	14.48±0.93^{a}	5.34±0.7^{a}	10.88±0.66^{a}	366.83±36.96^{a}
B	38.59±1.34^{b}	13.93±0.44^{a}	10.97±0.63^{b}	5.77±0.28^{b}	745±63.22^{b}
C	42.93±1.47^{a}	14.39±0.62^{a}	5.35±0.37^{a}	8.11±0.47^{a}	282.5±23.32^{c}
D	43.27±1.6^{a}	14.86±0.58^{a}	5.59±0.5^{a}	9.37±0.82^{a}	325.83± 23.32^{a}
E	45.49±2.55^{a}	15.97±0.85^{a}	5.75±0.3^{a}	9.51±0.46^{a}	347.83±29.17^{a}
F	29.37±1.13^{c}	9.79±0.37^{b}	3.5±0.45^{c}	4.12±0.38^{b}	278.83±25.52^{c}
G	46.7±4.32^{a}	15.61±1.48^{a}	5.92±0.56^{a}	10.23±0.7^{a}	341.5±18.46^{a}

Data are representative of Mean ± SD of six animals per group. Different superscript alphabets on the same row is significantly different at P<0.05. Groups A (Untreated control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract). Packed cell volume (PCV), Hemoglobin (Hb), White blood cells (WBC), Red Blood Cell (RBC), Platelet (PLT), 1, 2-dimethyl hydrazine (DMH), doxorubicin (DOX).

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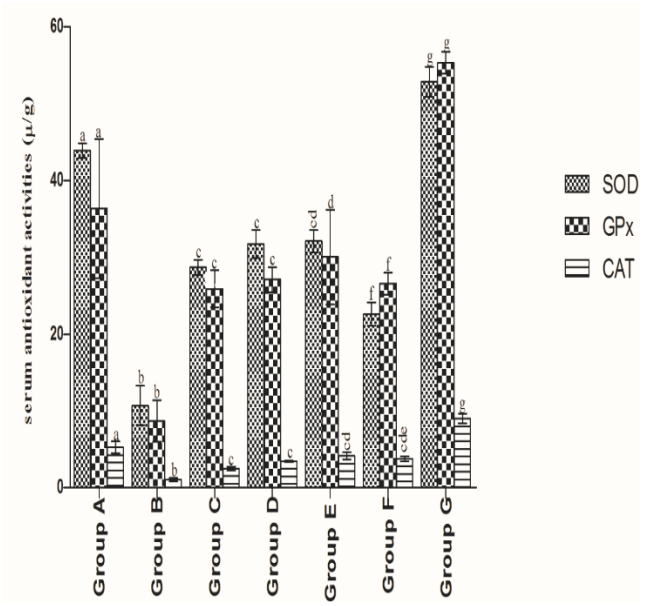

Figure 1: Effects of *Acacia sieberiana* alkaloid-rich extract on serum antioxidant enzyme status in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabet at each designated column representing each enzyme are significantly different at P<0.05. Superoxide dismutase (SOD), Glutathione peroxide (GPx), Catalase (CAT). Groups A (Untreated control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

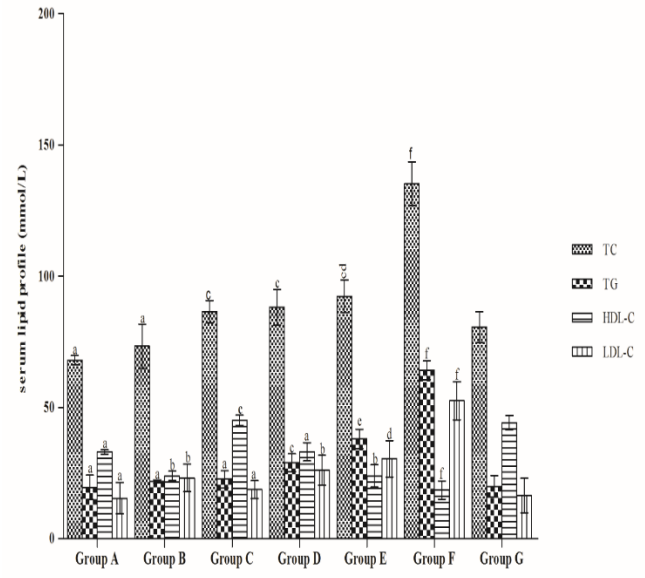

Figure 2: Effects of *Acacia sieberiana* alkaloid-rich extract on serum lipid profile in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabet on each designated column representing each lipid indices are significantly different at P<0.05. Total Cholesterol (TC), Triacylglycerol (TG), High density lipoprotein cholesterol (HDL-C), Low density Lipoprotein cholesterol (LDL-C). Groups A (Untreated control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

This implies that induction of pre-malignant lesion may not be through the deregulation of the lipid profile. The extract also had no effect on the serum lipid profile while significant disruption was observed in the group treated with both DMH and doxorubicin (Figure 2). This result showed the dyslipidemia effect of doxorubicin, which is in accordance with the previous study.[38] The doxorubicin effect on lipid profile is suggestive of its cardiotoxicity.[39, 40]

The major organ in living system responsible for the metabolism of medicines and other substances is liver.[41] Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP) and total bilirubin (TBIL) are the biomarkers used for assessing the effects of hepatotoxic attributes of xenobiotic on the integrity of liver cells.[42, 43] This study shows that DMH induced significantly ($p<0.05$) increase in the level of serum ALT, AST, ALKP and TBIL in Group B and F when compared with other treated groups (Figure 3 and 4). The hepatotoxic effect of DMH observed in this study is in line with earlier documented observation.[44] The extract protected the liver from the DMH induced hepatotoxicity by lowering in a dose dependent manner all the liver function indices. This finding further indicates hepato-protective nature of the alkaloid-rich extract and are in concordance with previous studies.[45-47]

Kidney contributes greatly to waste discharge and osmoregulation.[48] Inability to eliminate toxicants from the body system can result in renal damage.[49] The results as presented in Figure 5 and 6 show that DMH and the extract did not impact any significant effect on the kidney function indices. However, significant (P<0.05) elevation of these parameters was observed in the group treated with doxorubicin when compared with the positive and negative controls. The report on the nephrotoxicity nature of doxorubicin is in agreement with the previously documented study.[50] Non-nephrotoxic effect of the alkaloids-rich extract is in line with the earlier documented observation.[51]

Biomembrane peroxidation stimulated by increased oxidative stress is connected with cancer development and toxicology.[52] Oxidative stress is a consequence of the overwhelming effect of free radicals generated against antioxidant enzymes, leading to the initiation of lipid peroxidation (MDA).[53] The increased level of MDA plays a significant role in the initiation and progression of various disorders such as cancer.[54] In this study, the serum MDA level was observed to be significantly ($p<0.05$) increased in pre-malignant bearing animals (Group B) when compared with Group A (Fig. 7). The observed DMH-induced significant increase in serum MDA level is in line with previously documented study.[55] *A. sieberiana* leaves reversed the elevated serum MDA level occasioned by DMH in a dose dependent manner (Fig. 7). This implies that the extract has potential to protect the lipid component of cell membrane from the oxidative modification.[56]

The interaction between reactive oxygen species (ROS) and bases of DNA strand like guanine generates 8-hydroxyguanosine (8-OHGua) mostly employed as a biomarker of oxidative DNA damage and carcinogenesis.[57] The result of this study as presented in Fig. 8 reveals a dose dependent reversal of DMH-induced elevation of serum 8-OHdG by the extract. The result implies that the extract has the ability to protect DNA from oxidative modification of DNA damage and it could be seen as an indication of possible anticancer potential of the plant.

The aberrant foci crypt has been identified as the intermediate biomarkers indicating the development of colon cancer at pre-malignant stage. The relationship between aberrant foci crypt (ACF) and colon cancer is supported by the histological and molecular features shared by ACF with colonic polyps and adenomas.[58] The development of aberrant crypts was prevented by the alkaloids-rich leaf extract of *A. sieberiana* as observed in Fig. 9. Results of other studies using varieties of plant constituents such as capsaicin and piperlongumine are consistent with the present study.[59, 60]It has been reported that inhibition of aberrant foci crypt may be partly due to decreased cell proliferation in the colonic mucosa.[61]

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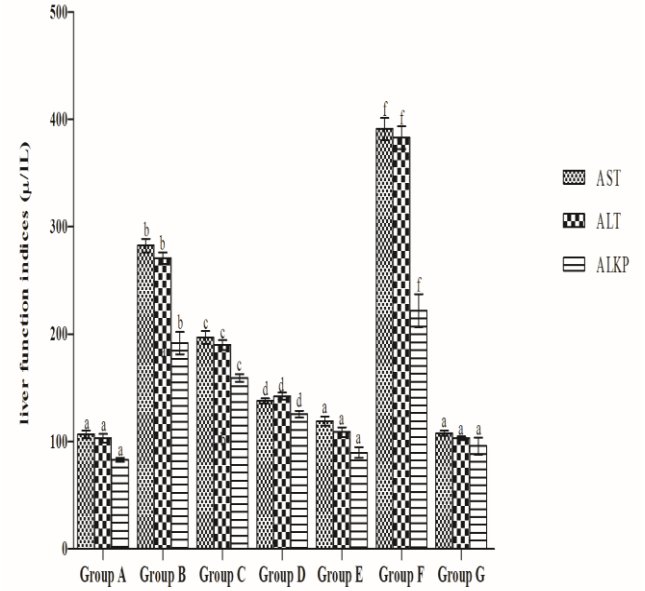

Figure 3: Effects of *Acacia sieberiana* alkaloid-rich extract on Liver function indices in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabet on each designated column representing each enzyme are significantly different at P<0.05. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALKP). Groups A (Untreated control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

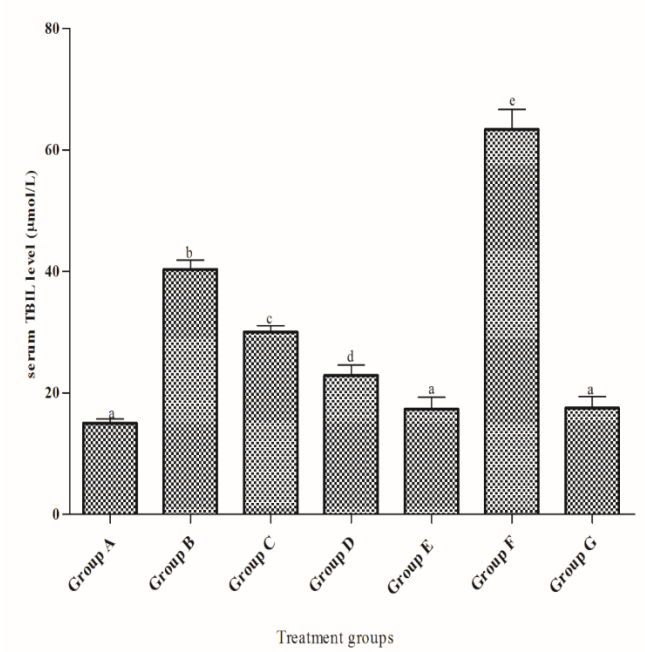

Figure 4: Effects of *Acacia sieberiana* alkaloid-rich extract on Total Bilirubin in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabets on each column are significantly different at P<0.05. Groups A (Negative control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

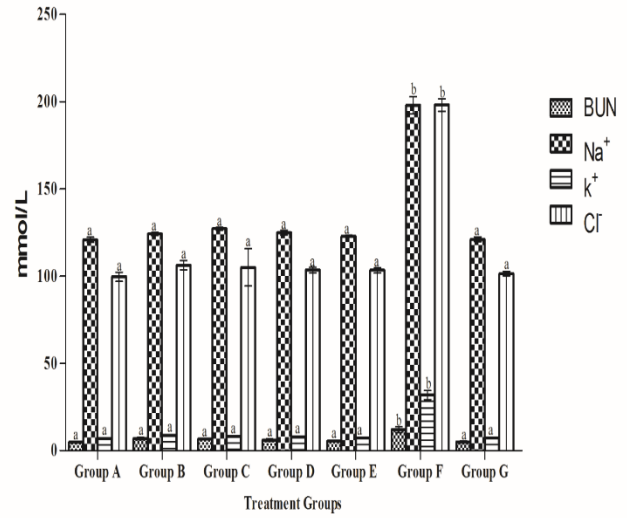

Figure 5: Effects of *Acacia sieberiana* alkaloid-rich extract on kidney funtion indices in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabet on each designated column representing each parameter are significantly different at P<0.05. Sodium ion (Na^+), Potassium ion (K^+), Chloride ion (Cl^-). Groups A (Negative control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

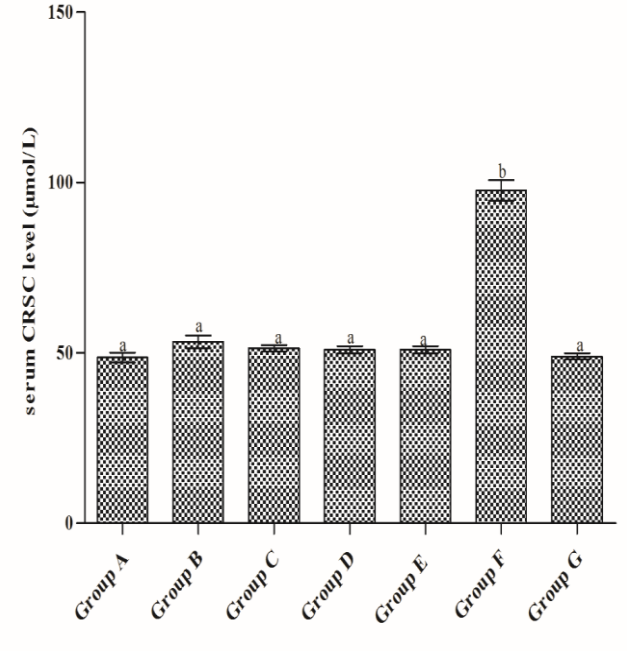

Figure 6: Effects of *Acacia sieberiana* alkaloid-rich extract on serum creatinine in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabet on each column are significantly different at P<0.05. Groups A (Negative control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

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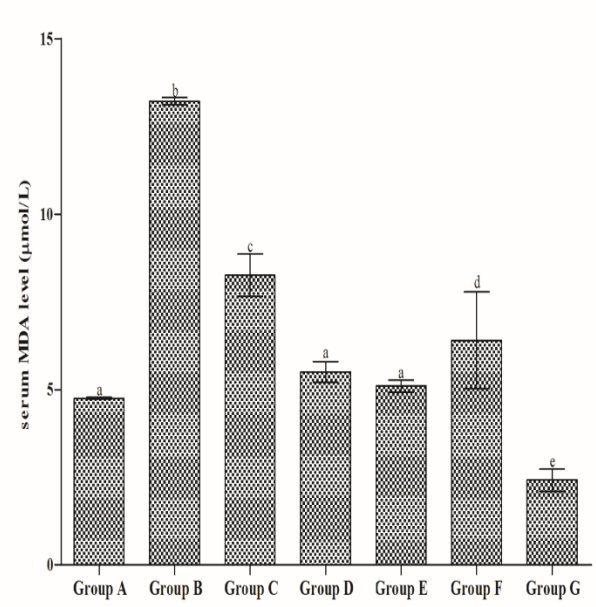

Figure 7: Effects of *Acacia sieberiana* alkaloid-rich extract on serum MDA level in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabet on each column are significantly different at P<0.05. Groups A (Untreated control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

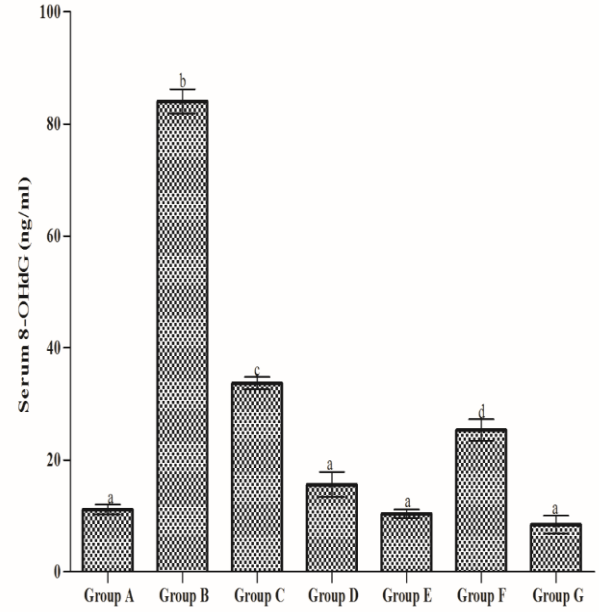

Figure 8: Effects of *Acacia sieberiana* alkaloid-rich extract on serum 8-hydroxyl-2-deoxyguanosine (8-OHdG) level in pre-malignant colon bearing Wistar Rats.
Each column represents mean±SD of six animals per group. Different alphabet on each column are significantly different at P<0.05. Groups A (Untreated control); B (20 mg/kg DMH); C (50 mg/kg extract); D (DMH + 100 mg/kg extract); E (DMH + 150 mg/kg extract); F (DMH + 2 mg/kg DOX); G (150 mg/kg extract).

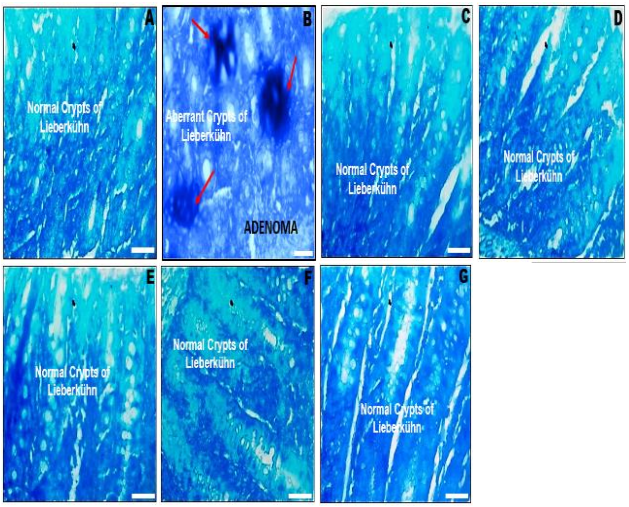

Figure 9 (A-G): Photomicrographs of colon Aberrant crypts general micromorphological presentations in Male adult Wistar rats after methylene blue stain (*Scale bar 50 um*).
(A) Negative control group with normal architecture (**B)** Positive control (DMH treated) group characterized with aberrant crypts (red arrow), **(C), (D), (E), (F) and G** with normal cytoarchitecture for crypts Lieberkühn.

Conclusion

This study revealed the protective potential of the alkaloid-rich leaf extract of *Acacia sieberiana* against DMH-induced serum REDOX dysregulation, oxidative lipid and DNA damage complications in pre-malignant colon cancer in male rats.

Conflict of Interest

The authors declared no conflict of interest.

Authors' Declaration

The authors declare that the report in this article is original and will be responsible for any liability arising from its content.

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