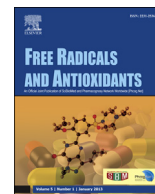


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Original article

N-nitrosodiethylamine induced redox imbalance in rat liver: Protective role of polyphenolic extract of *Blighia sapida* arilli

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ABSTRACT

Aim: This study investigates the reactive oxygen species detoxification potentials of polyphenolic extract of *Blighia sapida* arilli in the liver of N-nitrosodiethylamine-treated rats.**Methods:** Male rats, weighing 158 ± 2.9 g were completely randomized into 7 groups (A–G) of five rats each. Rats in C, D, E and F were administered orally once daily at 24 h interval for 7 days with 1000, 250, 500 and 1000 mg/kg body weight of polyphenolic extract of *B. sapida* arilli respectively. Group G was given 100 mg/kg body weight of vitamin C. On the sixth day, group B, D, E, F and G were administered with 100 mg/kg body weight N-nitrosodiethylamine (NDEA). Group A, which served as the control was treated like the test groups except, that the animals received distilled water only.**Results:** Reactive oxygen species detoxifying enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenase) activities were significantly ($P < 0.05$) induced by *B. sapida* arilli. These inductions significantly ($P < 0.05$) attenuated the decrease in reactive oxygen species detoxifying enzymes mediated by NDEA treatment and compared favourably with vitamin C. NDEA-mediated elevation in the concentrations of oxidative stress biomarkers; malondialdehyde, conjugated dienes, lipid hydroperoxides, protein carbonyl and percentage DNA fragmentation were significantly ($P < 0.05$) lowered by polyphenolic extract of *B. sapida* arilli.**Conclusion:** Overall, the results obtained from this study revealed that the polyphenolic extract from *B. sapida* arilli enhanced the detoxification of reactive oxygen species in NDEA-treated rats. The polyphenols also prevented the peroxidation of lipid, oxidation of proteins as well as fragmentation of DNA component in the liver of rats and hence gave the evidence of possible prophylactic potentials of *B. sapida* arilli.

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1. Introduction

N-nitrosodiethylamine (NDEA), N-nitroso alkyl compound, is a potent hepatotoxin and hepatocarcinogen found in wide variety of foods such as cheese, soybean, smoked, salted and dried fish, cured meat and alcoholic beverages.¹ Microsomal metabolism of NDEA by cytochrome P₄₅₀ monooxidase system leads to the generation of free radicals, lipid-derived and hydroxyl radical² and reactive oxygen species.³ These reactive species causes oxidative damage leading to cytotoxicity, carcinogenicity and mutagenicity.^{4,5}

Reactive oxygen species (ROS), which include the superoxide anion radical ($O^{\cdot-}$), hydroxyl ($\cdot OH$) and peroxy (RO_2) as well as

non-radicals such as hydrogen peroxide (H_2O_2) and peroxy nitrite ($ONOO^-$),⁶ is ubiquitous in living organisms due to the occurrence of oxidative metabolism through mitochondrial respiration.⁷ Overproduction of ROS and reactive nitrogen species (RNS) including nitric oxide is associated with physical exercise, ischemia/reperfusion, chronic inflammation, atherosclerosis, type-2 diabetes, neurodegenerative disorders and cancer.^{8–10}

The deleterious free radical events such as lipid peroxidation, protein oxidation and fragmentation of DNA are rarely the cause of cell death in realistic *in vivo* condition¹¹ since the antioxidant defence arsenal in the liver cells detoxifies ROS and repairs damage resulting from ROS.¹² The antioxidants and cytoprotective activity of some dietary medicinal plants have been reported and their protective capability were alluded to the polyphenolic and flavonoid constituents of the plants.^{13–16}

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Polyphenols and flavonoids are widely distributed in plants most especially cereals, fruits and vegetables.¹⁷ Wise use of the fruits, medicinal plants, and vegetables requires investigations into the phytochemicals and antioxidants as well as the possible medicinal properties and prospective products, such as nutraceuticals and phytomedicines. One of the fruit that is widely consumed in Nigeria is *Blighia sapida* arilli.

B. sapida (König) is a tropical to subtropical plant in the soapberry family (Sapindaceae) and indigenous to equatorial Africa. It is cultivated in the West Indies, Central and South America, and Florida for its edible yellow fruit arils. It is commonly known as Ackee, in Nigeria, it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo). It is an evergreen tree of about 33–40 ft (10–12 m) with a dense crown spreading branches. It's rather handsome, usually with a short trunk of 6 ft (1.8 m) in circumference. The bark is grey and nearly smooth.¹⁸ The flower is small, greenish-white and about 5 mm long.¹⁹ Owonubi²⁰ reported that the fleshy pulp is usually eaten raw, made into soup or fried in oil.

Phytochemical investigation of the extracts of *B. sapida* shows the presence of some groups of phytochemicals such as saponins, reducing sugar, phytosterols, and polyamide.²¹ Isolation of six principal groups of compounds: triterpenes, steroids, and their glycosides (collectively called saponins), sesquiterpenes, quinines, alkaloids, and polyphenols have also been reported.^{22–25} Recently, Parkinson²⁶ ascribed the high antioxidant and high total phenolic content of the ackee pods to six known polyphenols.

Thus, this study reports the capability of the polyphenolic extract of *B. sapida* arilli to promote the detoxification of ROS in the liver of N-nitrosodiethylamine-treated rats.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animals

Two month old, healthy male albino rats (*Rattus norvegicus*) of Wistar strain, weighing 158 ± 2.90 g were obtained from Animal House of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. They were kept in clean plastic cages contained in well-ventilated house conditions with free access to feeds (Capfeed Ltd., Osogbo, Nigeria) and tap water.

2.1.2. Plant materials

Fresh and ripe *B. sapida* fruits were collected at Ido-Osun, Osogbo, Nigeria in November 2011. The plant sample was authenticated and deposited in the Herbarium unit of Forestry Research Institute of Nigeria, Ibadan, Oyo state with Voucher No: FHI. 109506.

2.1.3. Chemicals and assay kits

Diphenylamine 5,5'-Dithio-bis(2-nitrobenzoic acid), guanidine hydrochloride, N-ethyl-maleimide (NEM), and salicylic acid, were procured from Research Organics, Cleveland, Ohio, USA. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-red) and glucose 6-phosphate dehydrogenase (Glc 6-PD) were products of Randox Laboratories Ltd., Co. Antrim, United Kingdom. All other reagents used were supplied by Sigma–Aldrich Inc., St. Louis, USA.

2.2. Methods

2.2.1. Preparation of *B. sapida* arilli's polyphenolic extract

The arilli of *B. sapida* fruits were nipped from the black shiny seeds and freeze-dried using lyophilizer (LTE Scientific Ltd,

Greenfield, Oldham OL37ET). The lyophilized arillis (500 g) were homogenized, exhaustively and successively extracted using hexane, ethyl acetate and methanol. The filtered extracts were concentrated under reduced pressure using rotatory evaporator and kept frozen.

2.2.2. Animal treatment

Thirty-five (35) male rats were completely randomized into seven groups (A–G) of 5 animals each. Rats in groups C, D, E and F were administered orally once daily at 24 h interval for 7 days with 1000, 250, 500 and 1000 mg/kg body weight of polyphenolic extract of *B. sapida* arilli respectively. Group G was given 100 mg/kg body weight of vitamin C. On the sixth day, group B, D, E, F and G were administered with 100 mg/kg body weight of NDEA. Group A, which served as the control was treated like the test groups except, that the animals received distilled water only.

2.2.3. Preparation of serum and tissue homogenates

The serum and tissue homogenates were prepared after the rats were sacrificed using the procedure described by Yakubu et al.²⁷

2.2.4. Biochemical assay

The activities of alkaline phosphatase, alanine and aspartate aminotransferases were determined as described by Wright et al.²⁸ and Bergmeyer et al.^{29,30} respectively. SOD, Catalase, GSH-Px, GSH-red and Glc 6-PD activities were assayed according to the procedures described by Beers & Sizer³¹ Mavis & Stellwagen,³² Misra & Fridovich,³³ Rotruck et al.³⁴ and Kornberg and Horecker³⁵ respectively. The levels of glutathione reduced (GSH) and peroxidised glutathione (GSSG) were determined using the procedures described by Ellman³⁶ and Hissin and Hiff³⁷ respectively. The concentration of protein carbonyl in the liver homogenates was determined according to the procedure described by Levine et al.³⁸ The concentrations of conjugated dienes, lipid hydroperoxides and malondialdehyde were assessed according to the procedures described by Bus et al.³⁹ The quantity of fragmented DNA was quantified according to the procedure described by Burton.⁴⁰

2.2.5. Statistical analysis

Results were expressed as the mean of five determinations \pm SD. Analysis of variance (ANOVA) followed by Tukey–Kramer test for differences between means was used to detect any significant differences ($P < 0.05$) between the treatment groups in this study using StatPlus, 2011 (AnalystSoft Inc., Alexandria, VA, USA).

3. Results

Administration of NDEA alone significantly ($P < 0.05$) reduced the activities of ALP, ALT and AST in the liver with corresponding increase in the activities of these enzymes (ALP, ALT and AST) in the serum (Table 1). This trend was reversed when the polyphenolic extract of *B. sapida* arilli at various doses were administered to NDEA-treated rats, as the activities of the liver and serum enzymes compared favourably ($P > 0.05$) with that of the control and vitamin C pretreated groups (Table 1).

The activities of ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) were significantly ($P < 0.05$) reduced following the administration of 100 mg/kg body weight of NDEA (Table 2). In addition to the increase in the activities of these enzymes following the administration of the polyphenolic extract of *B. sapida* arilli alone, the extract completely attenuated NDEA-mediated decreases in these enzymes (Table 2).

While the level of the non-enzymatic antioxidant glutathione reduced (GSH) was significantly reduced, the concentration of peroxidised glutathione (GSSG) in the liver increased significantly

Table 1Specific activities of hepatic marker enzymes following the administration of polyphenolic extract of *Blighia sapida* arilli to N-nitrosodiethylamine-treated rats.

Treatment/tissues	Alkaline phosphatase		Alanine aminotransferase		Aspartate aminotransferase	
	Liver	Serum	Liver	Serum	Liver	Serum
Distilled water (control)	5.32 ± 0.17 ^a	0.058 ± 0.001 ^a	65.23 ± 1.48 ^a	5.23 ± 0.13 ^a	97.34 ± 1.89 ^a	10.32 ± 0.23 ^a
NDEA-treated	1.25 ± 0.31 ^b	0.166 ± 0.002 ^b	22.05 ± 3.81 ^b	18.23 ± 0.69 ^b	50.52 ± 3.21 ^b	22.23 ± 0.31 ^b
1000 mg/kg body weight of extract	5.39 ± 0.02 ^a	0.054 ± 0.002 ^a	64.24 ± 1.30 ^a	4.73 ± 0.28 ^a	94.42 ± 1.32 ^a	9.37 ± 0.21 ^a
NDEA + 250 mg/kg body weight of extract	3.97 ± 0.13 ^c	0.100 ± 0.001 ^c	31.42 ± 2.06 ^c	13.42 ± 0.23 ^c	64.21 ± 2.32 ^c	18.24 ± 0.22 ^c
NDEA + 500 mg/kg body weight of extract	4.50 ± 0.31 ^a	0.075 ± 0.002 ^c	44.57 ± 1.12 ^d	9.75 ± 0.28 ^c	75.74 ± 2.62 ^c	15.57 ± 0.01 ^c
NDEA + 1000 mg/kg body weight of extract	5.01 ± 0.25 ^a	0.061 ± 0.001 ^a	59.35 ± 1.42 ^a	6.05 ± 0.12 ^a	83.59 ± 2.62 ^b	11.05 ± 0.15 ^a
NDEA + 100 mg/kg body weight of vitamin C	5.60 ± 0.03 ^a	0.056 ± 0.001 ^a	62.70 ± 3.02 ^a	5.72 ± 0.21 ^a	87.07 ± 4.01 ^a	10.72 ± 0.02 ^a

Data are mean of five determinations ± SD. Specific enzyme activities are expressed as nmol min⁻¹mg protein⁻¹. Values carrying superscripts different for the liver and serum of each enzyme are significantly different ($P < 0.05$).

Table 2Specific activities of antioxidant enzymes following the administration of polyphenolic extract of *Blighia sapida* arilli to N-nitrosodiethylamine-treated rats.

Treatments	Superoxide dismutase (nmol min ⁻¹ mg protein ⁻¹)	Catalase (nmol min ⁻¹ mg protein ⁻¹)	Glutathione peroxidase (nmol min ⁻¹ mg protein ⁻¹)	Glutathione reductase (nmol min ⁻¹ mg protein ⁻¹)	Glucose 6-phosphate dehydrogenase (nmol min ⁻¹ mg protein ⁻¹)
Distilled water (control)	66.47 ± 5.24 ^a	30.93 ± 3.76 ^a	346.17 ± 2.14 ^a	55.70 ± 1.52 ^a	24.40 ± 1.30 ^a
NDEA-treated	27.83 ± 2.52 ^b	7.43 ± 1.29 ^b	98.48 ± 2.67 ^b	15.44 ± 0.05 ^b	6.33 ± 0.43 ^b
1000 mg/kg body weight of extract	77.50 ± 2.18 ^c	53.48 ± 1.36 ^c	743.11 ± 2.35 ^c	90.19 ± 1.38 ^c	20.50 ± 1.22 ^c
NDEA + 250 mg/kg body weight of extract	37.06 ± 1.20 ^d	21.08 ± 1.26 ^d	237.92 ± 1.02 ^d	34.35 ± 0.80 ^d	19.24 ± 1.61 ^d
NDEA + 500 mg/kg body weight of extract	49.26 ± 0.64 ^d	23.24 ± 0.42 ^a	285.00 ± 2.26 ^c	39.89 ± 1.32 ^c	20.85 ± 1.24 ^d
NDEA + 1000 mg/kg body weight of extract	62.75 ± 1.40 ^a	32.18 ± 1.26 ^a	320.28 ± 1.62 ^a	47.82 ± 1.08 ^f	21.64 ± 1.23 ^a
NDEA + 100 mg/kg body weight of vitamin C	64.95 ± 1.06 ^a	26.92 ± 1.82 ^a	336.15 ± 2.44 ^a	40.45 ± 1.26 ^f	24.03 ± 1.05 ^a

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

($P < 0.05$) and GSH:GSSG ratio decreased significantly following the administration of NDEA (Table 3). The polyphenolic rich extract of *B. sapida* arilli significantly ($P < 0.05$) reversed the NDEA-mediated alterations in the levels of these non-enzymatic antioxidants (Table 3).

NDEA administration resulted to significant ($P < 0.05$) increase in the levels of lipid peroxidised products (conjugated dienes, lipid hydroperoxides and malondialdehyde) in the liver of rats (Table 4). The NDEA-mediated increase in the lipid peroxidation products were significantly ($P < 0.05$) reduced in the liver of rats by the polyphenolic extract of *B. sapida* arilli.

In a similar vein, carbonylation of protein resulting from the oxidation of liver protein significantly ($P < 0.05$) increased in the liver of NDEA-treated rats. Treatment of rats with only polyphenolic rich extract of *B. sapida* arilli produced no change in the level of protein carbonyl, but significantly ($P < 0.05$) reduced the protein carbonyl level in the liver of NDEA-treated rats (Table 5).

The extent of DNA damage in the liver as assessed by the measurement of DNA fragmentation in NDEA-treated rats increased significantly ($P < 0.05$). *B. sapida* significantly ($P < 0.05$) reversed the NDEA-mediated increase in DNA fragmentation and it compared significantly ($P < 0.05$) with the control and vitamin C treated groups (Table 5).

4. Discussion

The interactions of dietary antioxidants, with one or more biomolecules at cellular or molecular level or both, lead to the induction of certain enzymes, as well as inhibition or inactivation of some enzymes.¹³ In this study, the capability of the polyphenolic extract of *B. sapida* arilli to promote reactive oxygen species detoxification in the liver of NDEA-treated rats was investigated.

ALP is a marker enzyme for plasma membrane such that any alteration in the level of this enzyme shows compromise of the integrity of the plasma membrane.⁴¹ The significant reduction in the specific activity of ALP in the liver of rats with a corresponding increase in the serum is an indication of loss of integrity in the liver plasma membrane. The decrease in the specific activity of ALP in the liver of rats might have resulted from the peroxidation of the polyunsaturated fatty acids of the plasma membrane by ROS ($O_2^{\cdot-}$, $\cdot OH$, RO_2 , H_2O_2 and $ONOO^-$) generated during NDEA metabolism.³ The capability of polyphenolic extract of *B. sapida* arilli to prevent NDEA-mediated alteration in ALP could be attributed to the free radical and ROS scavenging capability of *B. sapida* arilli made possible by flavonoids, polyphenol present in *B. sapida* arilli.

The reduction in the specific activities of ALT (cytosolic) and AST (cytosolic and mitochondrial) in the liver of NDEA-treated rats is

Table 3Levels of non-enzymic antioxidants following the administration of polyphenolic extract of *Blighia sapida* arilli to N-nitrosodiethylamine-treated rats.

Treatments	Glutathione (reduced) (nmol mg protein ⁻¹)	Glutathione (oxidized) (nmol mg protein ⁻¹)	GSH:GSSG ratio
Distilled water (control)	60.90 ± 0.54 ^a	3.67 ± 0.16 ^a	16.59 ± 0.23 ^a
NDEA-treated	26.25 ± 0.62 ^b	18.93 ± 0.13 ^b	1.39 ± 0.21 ^b
1000 mg/kg body weight of extract	90.66 ± 0.42 ^c	3.84 ± 0.16 ^a	26.44 ± 0.01 ^c
NDEA + 250 mg/kg body weight of extract	51.74 ± 0.82 ^d	12.87 ± 0.18 ^c	4.17 ± 0.04 ^d
NDEA + 500 mg/kg body weight of extract	56.36 ± 0.91 ^a	8.59 ± 0.15 ^d	6.84 ± 0.01 ^e
NDEA + 1000 mg/kg body weight of extract	57.12 ± 0.03 ^e	4.48 ± 0.08 ^a	15.38 ± 0.08 ^a
NDEA + 100 mg/kg body weight of vitamin C	57.32 ± 0.62 ^a	5.02 ± 0.04 ^e	11.42 ± 0.03 ^f

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

Table 4
Levels of lipid peroxidation products following the administration of polyphenolic extract of *Blighia sapida* arilli to N-nitrosodiethylamine-treated rats.

Treatments	Conjugated dienes (nmol mg protein ⁻¹)	Lipid hydroperoxide (nmol mg protein ⁻¹)	Malondialdehyde (nmol mg protein ⁻¹)
Distilled water (control)	51.58 ± 0.86 ^a	49.81 ± 0.45 ^a	5.69 ± 0.43 ^a
NDEA-treated	118.59 ± 3.51 ^b	123.92 ± 0.48 ^b	15.70 ± 0.23 ^b
1000 mg/kg body weight of extract	51.86 ± 1.26 ^a	50.23 ± 0.60 ^a	5.62 ± 0.18 ^a
NDEA + 250 mg/kg body weight of extract	90.98 ± 1.34 ^c	85.83 ± 0.15 ^c	9.90 ± 0.21 ^c
NDEA + 500 mg/kg body weight of extract	74.24 ± 1.06 ^d	77.42 ± 0.12 ^c	7.49 ± 0.28 ^d
NDEA + 1000 mg/kg body weight of extract	62.42 ± 0.61 ^e	58.27 ± 0.70 ^a	6.22 ± 0.46 ^e
NDEA + 100 mg/kg body weight of vitamin C	58.58 ± 0.84 ^e	59.01 ± 0.26 ^d	5.89 ± 0.32 ^a

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

not surprising, as the pattern of alterations on ALP revealed that the integrity of the plasma membrane had been compromised. Damage to plasma membrane will consequentially lead to leakage of cytosolic content of the cell to the external milieu. The capability of the extract to reverse this trend in a manner similar to vitamin C suggests antioxidant potential of the extract. This amelioration may be added to the capability of the extract to scavenge ROS (O_2^- , $\cdot OH$, RO_2 , H_2O_2 and $ONOO^-$) generated during NDEA metabolism.³

Oxidative damage to cellular macromolecules (lipids, proteins, DNA etc.) arising from redox imbalances is normally counteracted by ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD).¹³ The reduction in the specific activities of these ROS detoxifying enzymes could have resulted from the excessive mobilization of the antioxidant enzymes towards the detoxification of ROS (O_2^- , $\cdot OH$, RO_2 , H_2O_2 and $ONOO^-$) during NDEA carcinogenesis.³ This could lead to uncontrolled oxidative attack on the cellular macromolecules resulting to oxidative damage and cell death. Similar reduction in the activities of these enzymes (SOD, CAT, GSH-Px and GSH-Red) during NDEA hepatocarcinogenesis were reported to be due to the excessive ROS generation.^{42–44} Thus, the significant attenuation of the NDEA-mediated reductions in the specific activities of ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) by the polyphenolic rich extract of *B. sapida* arilli might have resulted from the capability of the extract to scavenge the ROS generated during NDEA metabolism. It might also have resulted from the capability of *B. sapida* to induce ROS detoxifying enzymes. Reports have shown attenuation of NDEA-mediated decrease in the antioxidant enzymes by medicinal plants and plant components.^{4,45}

The significant ($P < 0.05$) reduction in the level of GSH, a non-enzymatic antioxidant playing complementary role in the prevention of oxidative damage resulting from ROS generated during NDEA metabolism might have resulted from the depletion of GSH-Px and GSH-Red, as they have direct relationship with GSH.⁴⁶ Conversely, the NDEA-mediated increase in the level of GSSG might have resulted from oxidation of GSH or mobilization of GSH towards production of GSH-Px. The reduction in GSH:GSSG ratio

Table 5
Levels of protein carbonyl and fragmented DNA following the administration of polyphenolic extract of *Blighia sapida* arilli to N-nitrosodiethylamine-treated rats.

Treatments	Protein carbonyl (nmol mg protein ⁻¹)	Fragmented DNA (%)
Distilled water (control)	4.81 ± 0.11 ^a	5.07 ± 0.58 ^a
NDEA-treated	8.05 ± 0.18 ^b	84.81 ± 1.25 ^b
1000 mg/kg body weight of extract	4.18 ± 0.03 ^a	5.00 ± 0.11 ^a
NDEA + 250 mg/kg body weight of extract	6.06 ± 0.02 ^b	57.16 ± 2.13 ^c
NDEA + 500 mg/kg body weight of extract	5.17 ± 0.03 ^c	41.66 ± 0.21 ^d
NDEA + 1000 mg/kg body weight of extract	4.54 ± 0.05 ^a	22.35 ± 0.16 ^e
NDEA + 100 mg/kg body weight of vitamin C	4.76 ± 0.04 ^a	15.33 ± 0.17 ^e

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

following administration of NDEA is an indication that the liver cell is prone to oxidative attack. Thus, the preservation of the levels of GSH, high GSH:GSSG and low GSSG in the liver of NDEA-treated rats by the polyphenolic rich extract of *B. sapida* arilli shows the possible antioxidant potentials.

Lipid peroxidation induces disturbance of fine structures, alteration of integrity, fluidity, and permeability, and functional loss of biomembranes, modifies low-density lipoprotein (LDL) to proatherogenic and proinflammatory forms, and generates potentially toxic products.⁴⁷ Elevation in the status of lipid peroxidation in the liver during NDEA treatment has been reported.^{48,49} Thus, the significant increase in the levels of lipid peroxidised products (conjugated dienes, lipid hydroperoxides and malondialdehydes) shows indiscriminate oxidative assaults on the cellular lipids. This increase (most especially conjugated dienes) could result to mutation and carcinogenesis.^{50,51} The capability of *B. sapida* extract to reverse the NDEA-mediated increase in conjugated dienes, lipid hydroperoxide and malondialdehyde might have resulted from the ROS scavenging activity of the extract. It might have also resulted from the capability of the extract to promote the detoxification (through the induction of antioxidant enzymes) of ROS, which could cause the peroxidation of polyunsaturated fatty acids of plasma membrane. Pradeep et al⁴⁵ also reported similar reduction in level of lipid peroxidised products following the administration of Silymarin to NDEA-treated rats.

Protein carbonyl content, indicator of irreversible oxidative damage leading to protein oxidation,⁵² may have lasting detrimental effects on cells and tissues.⁵³ Thus, the significant increase in protein carbonyl, a marker of protein oxidation in NDEA-treated rat could have resulted from the oxidation of protein by the free radicals and ROS generated during NDEA metabolism. The attenuation of NDEA-mediated increase in the level of protein carbonyl by the polyphenolic extract of *B. sapida* arilli further shows the possible ROS scavenging and its capability to promote the detoxification of ROS via the induction of antioxidant enzymes.

Oxidative stress and accumulation of calcium ion have been reported to mediate DNA fragmentation.⁵⁴ This damage, which usually results from OH^- , can lead to either the arrest or induction of transcription, induction of signal transduction pathways, replication errors and genomic instability, all of which are associated with carcinogenesis.⁵⁵ Thus, the significant increase in the level of fragmented DNA in the liver of NDEA-treated rat shows the genotoxicity arising from NDEA treatment. It also denotes possible initiation of carcinogenesis. The reduction in the level of fragmented DNA in the liver of NDEA-treated rat by the polyphenolic extract of *B. sapida* shows the antioxidants and antigenotoxic role of the extract.

5. Conclusion

The results from the study show that the polyphenolic extract of *B. sapida* arilli enhanced the detoxification of N-nitrosodiethylamine possibly by increasing the activities of the reactive oxygen

species detoxifying enzymes, thus preventing the oxidation and fragmentation of cellular macromolecules such as DNA, lipids and proteins. Hence, the consumption of *B. sapida* arilli is encouraged because of its prophylactic potentials.

Conflicts of interest

All authors have none to declare.

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