



Polyphenolic extract of *Sorghum bicolor* grains enhances reactive oxygen species detoxification in *N*-nitrosodiethylamine-treated rats

Taofeek O. Ajiboye^{a,*}, Yesirat O. Komolafe^a, Oyelola B. Oloyede^a, Simiat M. Ogunbode^b,
Moriām D. Adeoye^c, Ibrahim O. Abdulsalami^c, Quadri O. Nurudeen^d

^a Antioxidants, Free Radicals and Toxicology Research Laboratory, Biochemistry and Nutrition Unit, Department of Chemical Sciences, Fountain University, Osogbo, Nigeria

^b Nutritional Biochemistry Research Laboratory, Biochemistry and Nutrition Unit, Department of Chemical Sciences, Fountain University, Osogbo, Nigeria

^c Industrial and Environmental Chemistry Unit, Department of Chemical Sciences, Fountain University, Osogbo, Nigeria

^d Phytomedicine, Toxicology and Reproductive Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria

Received 7 October 2012; received in revised form 24 February 2013; accepted 27 February 2013

Abstract

Reactive oxygen species detoxification potentials of *Sorghum bicolor* polyphenolic extract was investigated in the liver of *N*-nitrosodiethylamine-treated rats. Male rats, weighing (135 ± 5.5) 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 d with 500, 125, 250 and 500 mg/kg body weight of polyphenolic extract of *S. bicolor*, respectively. Group G was given 100 mg/kg body weight of vitamin C. On the sixth day, groups 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. 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 *S. bicolor*. These inductions significantly ($P < 0.05$) attenuated the NDEA-mediated decrease in reactive oxygen species detoxifying enzymes 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 *S. bicolor* polyphenolic extract. Overall, the results obtained from this study revealed that the polyphenolic extract of *S. bicolor* grains 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 *S. bicolor* grains.

© 2013 Beijing Academy of Food Sciences. Production and hosting by Elsevier B.V. All rights reserved.

Keywords: Antioxidant; *Sorghum bicolor*; Reactive oxygen species; *N*-nitrosodiethylamine; Detoxification; Polyphenols

1. Introduction

N-nitrosodiethylamine (NDEA) is a potent hepatocarcinogenic nitrosamine present in tobacco smoke, ground water with high level of nitrates, cheddar cheese, cured and fried meals, soya beans, alcoholic beverages, occupational settings,

cosmetics, agricultural chemicals and pharmaceutical agents [1,2]. In the liver, cytochrome P₄₅₀ (CYP2E1) activates *N*-nitrosodiethylamine [3] to form electrophilic and reactive oxygen species [4], which causes oxidative damage leading to cytotoxicity, carcinogenicity and mutagenicity [5,6].

Oxygen-derived radicals known as reactive oxygen species (ROS) include the highly reactive superoxide ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$) and peroxy ($RO_2\cdot$) as well as non-radicals such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) [7]. The productions of these reactive species ($O_2^{\cdot-}$, $\cdot OH$, $RO_2\cdot$, H_2O_2 and $ONOO^-$) are usually in response to endogenous and exogenous stimulus [8]. Regardless of the origin, increased ROS production or oxidative stress results to either activation of specific signal transduction pathways or damage to cellular components resulting to adaptive and maladaptive molecular responses, respectively [9]. Cell death arising from carbonylation of protein, peroxidation of lipids and fragmentation of

* Corresponding author. Tel.: +234 8035844608.

E-mail address: ajiboyeyong@yahoo.com (T.O. Ajiboye).

Peer review under responsibility of Beijing Academy of Food Sciences.



Production and hosting by Elsevier

DNA are consequential effects of ROS-induced oxidative stress [10]. Furthermore, excessive ROS production has been reported to stimulate oncogenesis *via* alterations in redox regulated signaling pathways suggesting that the redox state plays a critical role in signal transduction, cellular proliferation, differentiation and apoptosis [11,12].

Antioxidant defense arsenal in liver cells is responsible for the detoxification of ROS and repair damage resulting from ROS [13]. Thus, catastrophic free radical events such as lipid peroxidation, protein oxidation and fragmentation of DNA are rarely the cause of cell death in realistic *in vivo* condition [14]. However, when the antioxidant defense arsenals are overwhelmed, ROS cause direct damage to proteins, lipids, and nucleic acids, leading to cell death [15]. Consumptions of dietary antioxidants complement the cellular defense system to prevent oxidative damage to cellular macromolecules. Recently, we have reported the antioxidants and cytoprotective activities of some dietary medicinal plants and alluded the protective role to the polyphenolic and flavonoid constituents of the plants [16–19]. Sorghum bicolor grains represent one of the common cereals that is widely consumed in Nigeria because of the good amount of antioxidant, carbohydrate and protein contents.

Sorghum (*S. bicolor* (L.) Moench) is an important staple food in developing countries of the semi-arid tropics. It is the world's fifth most important cereal, with higher protein content than corn [20]. It is particularly important as human food resource and folk medicine in Asia and Africa. Studies have shown that sorghum has antioxidant activity, anti-carcinogenic effects, anti-mutagenic effects, cholesterol-lowering effects and can reduce the risk of cardiovascular disease [21,22]. Most of these activities have been shown to be due to the presence of numerous flavonoids, phenolics and anthocyanins in sorghum. Recently, Ajiboye et al. [23] reported that *S. bicolor* grains extract protected NDEA-induced oxidative stress in rat microsomes *in vitro* [23].

Phytochemical constituents of sorghum include phenolic compounds, polyflavonols and thiols, anthocyanins and tannins. Several flavonoids have been identified and characterized in sorghum over the years. Recently, 3-deoxyanthocyanidin, flavone, and flavanone levels were reported in red/black sorghum genotypes [22]. Despite these myriad studies on *S. bicolor*, there is little or no literature that describes the effects of the polyphenolic rich extract of *S. bicolor* grains on ROS detoxifying enzymes *in vivo*. This study thus investigates the capability of polyphenolic rich-extract of *S. bicolor* grains to promote ROS detoxification in the liver of NDEA-treated rats.

2. Materials and methods

2.1. Materials

2.1.1. Plant materials

Red variety of *S. bicolor* grains were obtained from Igbona market, Osogbo, Nigeria and was authenticated by Prof. F.A. Oladele of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen was deposited in the herbarium.

2.1.2. Experimental animals

Two-month-old, healthy male albino rats (*Rattus norvegicus*) of Wistar strain, weighing (135 ± 5.5) 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. The animals were used according to the Guidelines of National Research Council Guide for the Care and Use of Laboratory Animals [24] and in accordance with the principles of Good Laboratory Procedure (GLP) [25].

2.1.3. Chemicals and assay kits

Diphenylamine 5,5'-Dithio-bis(2-nitrobenzoic acid), guanidine hydrochloride, and *N*-ethyl-maleimide (NEM) 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 polyphenolic rich-extract of Sorghum bicolor grains

Finely ground *S. bicolor* grains were defatted exhaustively in hexane for 24 h with constant shaking. The residue was re-extracted exhaustively with methanol for 48 h. The filtered extract was concentrated under reduced pressure using rotary evaporator (R-200, BUCHI, Flawil, Switzerland) and kept frozen till further use.

2.2.2. Animal treatment

Thirty-five 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 d with 500, 125, 250 and 500 mg/kg bodyweight of polyphenolic extract of *S. bicolor* grains, respectively. Group G was given 100 mg/kg bodyweight of vitamin C. On the sixth day, groups B, D, E, F and G were administered with 100 mg/kg bodyweight 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 rats were sacrificed 24 h after their last daily doses using the anesthetic method described by Yakubu et al. [26]. Under diethyl ether anesthesia, rats were made to bleed through their cut jugular veins (slightly displaced to prevent blood from being contaminated with interstitial fluid) into centrifuge tubes. The blood samples were allowed to clot for 15 min and centrifuged at $33.5 \times g$ for 15 min to obtain the sera. The sera were frozen and used within 12 h of preparation for the biochemical assay. Liver excised from the animals were blotted in tissue paper, cut

Table 1

Specific activities of hepatic marker enzymes following the administration of polyphenolic extract of *Sorghum bicolor* to *N*-nitrosodiethylamine-treated rats.

Treatment/tissues	Alkaline phosphatase		Alanine aminotransferase		Aspartate aminotransferase	
	Liver	Serum	Liver	Serum	Liver	Serum
Distilled water (control)	5.77 ± 0.52 ^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	1.55 ± 0.31 ^b	0.168 ± 0.002 ^b	22.05 ± 3.81 ^b	18.23 ± 0.69 ^b	50.52 ± 3.21 ^b	22.26 ± 0.11 ^b
500 mg/kg body weight of extract	5.75 ± 0.04 ^a	0.059 ± 0.001 ^a	65.00 ± 2.06 ^a	5.86 ± 0.42 ^a	98.04 ± 2.76 ^a	10.01 ± 0.17 ^a
NDEA + 125 mg/kg body weight of extract	2.47 ± 0.48 ^c	0.123 ± 0.001 ^c	35.49 ± 3.12 ^c	15.73 ± 0.49 ^c	63.81 ± 3.96 ^c	17.15 ± 0.12 ^c
NDEA + 250 mg/kg body weight of extract	3.98 ± 0.23 ^a	0.092 ± 0.003 ^c	49.87 ± 3.14 ^d	11.25 ± 0.16 ^c	75.72 ± 1.81 ^c	15.92 ± 0.34 ^c
NDEA + 500 mg/kg body weight of extract	5.23 ± 0.61 ^a	0.061 ± 0.001 ^a	61.15 ± 2.83 ^a	5.89 ± 0.25 ^a	89.51 ± 6.84 ^b	11.03 ± 0.51 ^a
NDEA + 100 mg/kg body weight of vitamin C	5.60 ± 0.17 ^a	0.056 ± 0.002 ^a	62.70 ± 5.18 ^a	5.72 ± 0.11 ^a	87.07 ± 2.12 ^a	10.72 ± 0.32 ^a

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

thinly with sterile scalpel blade and then homogenized in ice-cold 0.25 mol/L sucrose solution (1:5, w/v). The homogenates were centrifuged at 800 × *g* at 4 °C for 10 min to obtain the supernatant that was kept frozen at -20 °C before being used for the various biochemical assays.

2.2.4. Biochemical assay

The activities of alkaline phosphatase (ALP), alanine and aspartate aminotransferases (ALT and AST) were determined as described by Wright et al. [27] and Bergmeyer et al. [28,29], respectively. SOD, Catalase, GSH-Px, GSH-red and Glc 6-PD activities were assayed according to the procedures described by Misra and Fridovich [30], Beers and Sizors [31], Rotruck et al. [32], Mavis and Stellwagen [33] and Kornberg and Horecker [34], respectively. The concentration of protein carbonyl in the liver homogenates was determined according to the procedure described by Levine et al. [35]. The concentrations of conjugated dienes, lipid hydroperoxides and malondialdehyde were assessed according to the procedure described by Bus et al. [36]. The quantity of fragmented DNA was quantified according to the procedure described by Burton [37].

2.2.5. Statistical analysis

Results were expressed as the mean of five determinations ± 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

3.1. Hepatocellular enzymes

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 *S. bicolor* grains 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).

3.2. Reactive oxygen detoxifying enzymes

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 ROS detoxifying enzymes following the administration of the polyphenolic extract of *S. bicolor* grains alone, the extract completely attenuated NDEA-mediated decrease in these enzymes (Table 2).

3.3. Non-enzymatic antioxidants

The level of the non-enzymatic antioxidant glutathione reduced (GSH) was significantly reduced following the administration of NDEA. While the concentration of peroxidised glutathione (GSSG) in the liver increased significantly ($P < 0.05$), GSH:GSSG ratio decreased significantly following

Table 2

Specific activities of antioxidant enzymes following the administration of polyphenolic extract of *Sorghum bicolor* to *N*-nitrosodiethylamine-treated rats.

Treatments	Superoxide dismutase	Catalase	Glutathione peroxidase	Glutathione reductase	Glucose 6-phosphate dehydrogenase
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 ± 1.05 ^b	6.33 ± 0.43 ^b
500 mg/kg body weight of extract	98.77 ± 3.77 ^c	52.91 ± 3.52 ^c	512.17 ± 4.90 ^c	108.02 ± 1.38 ^c	32.23 ± 1.82 ^c
NDEA + 125 mg/kg body weight of extract	36.34 ± 2.15 ^d	18.52 ± 1.53 ^d	164.92 ± 1.51 ^d	20.86 ± 1.99 ^d	12.55 ± 1.37 ^d
NDEA + 250 mg/kg body weight of extract	45.72 ± 1.82 ^d	29.08 ± 0.20 ^a	292.98 ± 5.68 ^e	39.84 ± 1.06 ^e	18.60 ± 1.44 ^d
NDEA + 500 mg/kg body weight of extract	60.66 ± 2.70 ^a	30.59 ± 2.18 ^a	323.90 ± 12.86 ^a	45.08 ± 1.24 ^f	20.89 ± 1.62 ^a
NDEA + 100 mg/kg body weight of vitamin C	64.95 ± 3.58 ^a	26.92 ± 1.46 ^a	336.15 ± 4.24 ^a	40.45 ± 1.62 ^f	24.03 ± 1.00 ^a

Table 3

Levels of non-enzymic antioxidants following the administration of polyphenolic extract of *Sorghum bicolor* to *N*-nitrosodiethylamine-treated rats.

Treatments	Glutathione (reduced)	Glutathione (oxidized)	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
500 mg/kg body weight of extract	101.52 ± 0.35 ^c	3.84 ± 0.42 ^a	26.44 ± 0.19 ^c
NDEA + 125 mg/kg body weight of extract	53.71 ± 0.36 ^d	12.87 ± 0.21 ^c	4.17 ± 0.11 ^d
NDEA + 250 mg/kg body weight of extract	58.72 ± 0.31 ^a	8.59 ± 0.45 ^d	6.84 ± 0.31 ^e
NDEA + 500 mg/kg body weight of extract	68.92 ± 0.40 ^e	4.48 ± 0.26 ^a	15.38 ± 0.48 ^a
NDEA + 100 mg/kg body weight of vitamin C	57.32 ± 0.91 ^a	5.02 ± 0.41 ^e	11.42 ± 0.23 ^f

Table 4

Levels of lipid peroxidised products following the administration of polyphenolic extract of *Sorghum bicolor* to *N*-nitrosodiethylamine-treated rats.

Treatments	Conjugated dienes	Lipid hydroperoxide	Malondialdehyde
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
500 mg/kg body weight of extract	50.92 ± 2.43 ^a	49.14 ± 0.35 ^a	5.28 ± 0.24 ^a
NDEA + 125 mg/kg body weight of extract	85.48 ± 1.62 ^c	80.93 ± 0.31 ^c	11.83 ± 0.10 ^c
NDEA + 250 mg/kg body weight of extract	69.59 ± 0.58 ^d	71.56 ± 0.22 ^c	9.37 ± 0.14 ^d
NDEA + 500 mg/kg body weight of extract	56.32 ± 0.32 ^e	54.77 ± 0.35 ^a	6.16 ± 0.23 ^e
NDEA + 100 mg/kg body weight of vitamin C	58.58 ± 0.40 ^e	59.01 ± 0.53 ^d	5.89 ± 0.13 ^a

the administration of NDEA (Table 3). The polyphenolic rich extract of *S. bicolor* grains significantly ($P < 0.05$) reversed the NDEA-mediated alterations in the levels of these non-enzymatic antioxidants (Table 3).

3.4. Lipid peroxidation

NDEA administration resulted to significant ($P < 0.05$) increase in the levels of lipid peroxidation 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 *S. bicolor* grains.

3.5. Protein oxidation

Carbonylation of protein resulting from the oxidation of liver protein significantly ($P < 0.05$) increased in the liver of NDEA-treated rats. Although, treatment of rats with only polyphenolic rich extract of *S. bicolor* grains produced no change in the level of protein carbonyl, it significantly ($P < 0.05$) reduced the protein carbonyl level in the liver of NDEA-treated rats (Table 5).

3.6. DNA fragmentation

The extent of DNA damage in the liver as assessed by the measurement of DNA fragmentation in NDEA-treated rats increased significantly ($P < 0.05$). *S. bicolor* 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

Dietary antioxidants elicit protective activities by interacting with biomolecules at cellular and molecular levels to induce cytoprotective enzymes or inhibits/inactivates those involve in carcinogen activation. In this study, the capability of the polyphenolic extract of *S. bicolor* grains to enhance reactive oxygen species detoxification in the liver of NDEA-treated rats was investigated.

4.1. Hepatocellular enzymes

Alkaline phosphatase is a marker enzyme for plasma membrane such that any alteration in the level of this enzyme shows

Table 5

Levels of protein carbonyl and fragmented DNA following the administration of polyphenolic extract of *Sorghum bicolor* to *N*-nitrosodiethylamine-treated rats.

Treatments	Protein carbonyl	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
500 mg/kg body weight of extract	3.66 ± 0.13 ^c	5.05 ± 0.31 ^a
NDEA + 125 mg/kg body weight of extract	7.52 ± 0.12 ^b	60.09 ± 3.21 ^c
NDEA + 250 mg/kg body weight of extract	6.10 ± 0.33 ^c	47.48 ± 0.42 ^d
NDEA + 500 mg/kg body weight of extract	5.13 ± 0.25 ^a	15.19 ± 0.13 ^e
NDEA + 100 mg/kg body weight of vitamin C	4.76 ± 0.24 ^a	15.33 ± 0.37 ^e

compromise of the integrity of the plasma membrane [38]. The significant reduction in specific activity of ALP in the liver of rats with a corresponding increase in serum is an indication of loss of integrity in the liver plasma membrane. The decrease in specific activity of ALP in the liver of rats might have resulted from peroxidation of polyunsaturated fatty acids of the plasma membrane by ROS ($O_2^{\bullet-}$, $\bullet OH$, $RO_2\bullet$, H_2O_2 and $ONOO^-$) generated during NDEA metabolism [4]. The capability of polyphenolic extract of *S. bicolor* grains to prevent NDEA-mediated alteration in ALP could be attributed to free radical and ROS scavenging capability of *S. bicolor* made possible by flavonoids, phenolics, anthocyanins and thiols present in *S. bicolor* grain [21].

The reduction in specific activities of ALT (cytosolic) and AST (cytosolic and mitochondrial) in the liver of DEN treated rats is not surprising, as the pattern of alterations on ALP revealed that integrity of the plasma membrane had been compromised. Damage to plasma membrane will consequentially lead to leakage of cellular cytosolic content 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 capability of the extract to scavenge ROS ($O_2^{\bullet-}$, $\bullet OH$, $RO_2\bullet$, H_2O_2 and $ONOO^-$) generated during NDEA metabolism [4].

4.2. Reactive oxygen detoxifying enzymes

Oxidative damage to cellular macromolecules (lipid, protein, DNA, etc.) arising from redox imbalances is normally counteracted by ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) [16]. The reduction in the specific activities of these ROS detoxifying enzymes could have resulted from the excessive mobilization of antioxidant enzymes towards the detoxification of ROS ($O_2^{\bullet-}$, $\bullet OH$, $RO_2\bullet$, H_2O_2 and $ONOO^-$) during NDEA carcinogenesis [4]. These reductions 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) were reported to be due to the excessive generation of ROS during NDEA hepatocarcinogenesis [1,39,40]. Thus, the significant attenuation of NDEA-mediated reduction in specific activities of ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) by the polyphenolic rich extract of *S. bicolor* grains might have resulted from capability of the extract to scavenge ROS generated during NDEA metabolism. It might also have resulted from the capability of *S. bicolor* to induce ROS detoxifying enzymes. Reports have shown attenuation of NDEA-mediated decrease in the antioxidant enzymes by medicinal plants and plant components [5,41].

4.3. Non-enzymatic Antioxidant

The significant ($P < 0.05$) reduction in the level of GSH, a non-enzymatic antioxidant playing complementary role in 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 [42]. Conversely, NDEA-mediated increase

in the level of GSSG might have resulted from the oxidation of GSH or mobilization of GSH towards the production of GSH-Px. The reduction in GSH:GSSG ratio following the administration of NDEA indicates 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 *S. bicolor* grains shows the possible antioxidant potentials.

4.4. Lipid peroxidation

Elevation in the status of lipid peroxidation in liver during NDEA treatment has been reported [43,44]. Thus, the significant increase in the levels of lipid peroxidation products (conjugated dienes, lipid hydroperoxides and malondialdehydes) shows indiscriminate oxidative assaults on the cellular lipids. These increase (most especially conjugated dienes) could result to mutation [45]. The capability of *S. bicolor* 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 al. [6] also reported similar reduction in level of lipid peroxidised products following the administration of Silymarin to NDEA-treated rats.

4.5. Protein oxidation

Protein carbonyl content, an indicator of irreversible oxidative damage leading to protein oxidation [46], may have lasting detrimental effects on cells and tissues [47]. 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 *S. bicolor* grains further shows possible ROS scavenging and its capability to promote the detoxification of ROS via the induction of antioxidant enzymes.

4.6. DNA fragmentation

Oxidative stress and accumulation of calcium ion have been reported to mediate DNA fragmentation [48]. This damage, which usually results from $OH^{\bullet}$, can lead to either arrest or induction of transcription, induction of signal transduction pathways, replication errors and genomic instability, all of which are associated with carcinogenesis [49]. 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 *S. bicolor* grains shows the antioxidants and antigenotoxic role of the extract.

5. Conclusion

The results from this study show that the polyphenolic extract of *S. bicolor* grains enhanced the detoxification of *N*-nitrosodiethylamine possibly by enhancing the activities of reactive oxygen species detoxifying enzymes, thus preventing the oxidation and fragmentation of cellular macromolecules such as DNA, lipids and proteins. Hence, the consumption of *S. bicolor* grains as staple food is encouraged because of its prophylactic potentials.

Acknowledgement

Part of this paper was presented at the 8th Congress of Toxicology in Developing Countries (8CTDC) under the auspices of International Union of Toxicology (IUTOX) September 10–13, 2012: at Centara Grand at Central Ladprao, Bangkok, Thailand.

References

- [1] V. Sivaramakrishnan, P.N.M. Shilpa, V.R. Praveen Kumar, et al., Attenuation of *N*-nitrosodiethylamine-induced hepatocellular carcinogenesis by a novel flavonol-Morin, *Chemico-biological Interactions* 171 (2008) 79–88.
- [2] C. Gupta, A. Vikram, D.N. Tripathi, et al., Antioxidant and antimutagenic effect of quercetin against DEN induced hepatotoxicity in rat, *Phytotherapy Research* 24 (2010) 119–128.
- [3] F.P. Guengerich, D.H. Kim, M. Iwasaki, Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects, *Chemical Research in Toxicology* 4 (1991) 168–179.
- [4] O. Teufelhofer, W. Parzefall, E. Kainzbauer, et al., Superoxide generation from Kupffer cells contributes to hepatocarcinogenesis: studies on NADPH oxidase knockout mice, *Carcinogenesis* 26 (2005) 319–329.
- [5] P.J. Wills, V. Suresh, M. Arun, et al., Antiangiogenic effect of *Lygodium flexuosum* against *N*-nitrosodiethylamine-induced hepatotoxicity in rats, *Chemico-biological Interactions* 164 (2006) 25–38.
- [6] K. Pradeep, C.V.R. Mohan, K. Gobianand, et al., Silymarin modulates the oxidant–antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats, *European Journal of Pharmacology* 560 (2007) 110–116.
- [7] M. Bains, E.D. Hall, Antioxidant therapies in traumatic brain and spinal cord injury, *Biochimica et Biophysica Acta (BBA): Molecular Basis of Disease* 1822 (2012) 675–684.
- [8] M. Ushio-Fukai, Y. Nakamura, Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy, *Cancer Letters* 266 (2008) 37–52.
- [9] L. Covarrubias, D. Hernández-García, D. Schnabel, et al., Function of reactive oxygen species during animal development: passive or active? *Developmental Biology* 320 (2008) 1–11.
- [10] E.R. Stadtman, R.L. Levine, Protein oxidation, *Annals of the New York Academy of Sciences* 899 (2006) 191–208.
- [11] M. Valko, C.J. Rhodes, J. Moncol, et al., Free radicals, metals and antioxidants in oxidative stress-induced cancer, *Chemico-biological Interactions* 160 (2006) 1–40.
- [12] J. Chandra, A. Samali, S. Orrenius, Triggering and modulation of apoptosis by oxidative stress, *Free Radical Biology and Medicine* 29 (2000) 323–333.
- [13] H. Jaeschke, A. Ramachandran, Reactive oxygen species in the normal and acutely injured liver, *Journal of Hepatology* 55 (2011) 227–228.
- [14] H. Jaeschke, Reactive oxygen and mechanisms of inflammatory liver injury: present concepts, *Journal of Gastroenterology and Hepatology* 26 (Suppl. 1) (2011) 173–179.
- [15] T.O. Ajiboye, Redox status of the liver and kidney of 2,2-dichlorovinyl dimethyl phosphate (DDVP) treated rats, *Chemico-biological Interactions* 185 (2010) 202–207.
- [16] T.O. Ajiboye, A.K. Salau, M.T. Yakubu, et al., Acetaminophen perturbed redox homeostasis in Wistar rat liver: protective role of aqueous *Pterocarpus osun* leaf extract, *Drug and Chemical Toxicology* 33 (2010) 77–87.
- [17] T.O. Ajiboye, M.T. Yakubu, A.K. Salau, et al., Antioxidant and drug detoxification potential of aqueous extract of *Annona senegalensis* leaves in carbon tetrachloride-induced hepatocellular damage, *Pharmaceutical Biology* 48 (2010) 1361–1370.
- [18] T.O. Ajiboye, M.T. Yakubu, A.T. Oladiji, Electrophilic, free radical and reactive oxygen species scavenging and detoxification potentials of *Lophira alata* stem bark extract, *Free Radicals and Antioxidants* 1 (2011) 40–47.
- [19] T.O. Ajiboye, *In vivo* antioxidant potentials of *Piliostigma thonningii* (Schum) leaves: studies on hepatic marker enzyme, antioxidant system, drug detoxifying enzyme and lipid peroxidation, *Human & Experimental Toxicology* 30 (2011) 55–62.
- [20] L.F. Dowling, C. Arndt, B.R. Hamaker, Economic viability of high digestibility sorghum as feed for market broilers, *Agronomy Journal* 94 (2002) 1050.
- [21] C.S. Kwak, S.C. Park, S.J. Lim, et al., Antioxidative and antimutagenic effects of Korean buckwheat, sorghum, millet and Job's tears, *Journal of The Korean Society of Food Science and Nutrition* 33 (6) (2004).
- [22] L. Dykes, L.M. Seitz, W.L. Rooney, et al., Flavonoid composition of red sorghum genotypes, *Food Chemistry* 116 (2009) 313–317.
- [23] T.O. Ajiboye, Y.O. Komolafe, H.O.B. Oloyede, et al., Diethylnitrosamine-induced redox imbalance in rat microsomes: protective role polyphenolic rich extract from *Sorghum bicolor* grains, *Journal of Basic and Clinical Physiology and Pharmacology* 24 (1) (2013) 41–49.
- [24] National Research Council, Guide for the Care and Use of Laboratory Animals, 8th ed., National Academies Science Press, 2011, pp. 161–196.
- [25] World Health Organization (WHO), Basic OECD Principles of GLP, World Health Organization, Geneva, Switzerland, 1998, Available at: www.who.int/tdrold/publications/publications/pdf/pr15/info.pdf (access date: 24.08.09).
- [26] M.T. Yakubu, M.A. Akanji, A.T. Oladiji, Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf, Ex Hiern) stem in male albino rats, *Asian Journal of Andrology* 7 (2005) 399–404.
- [27] P.J. Wright, P.D. Leathwood, D.T. Plummer, Enzymes in rat urine. Alkaline phosphatase, *Enzymologia* 42 (1972) 317–327.
- [28] H.U. Bergmeyer, M. Horder, R. Rej, Approved recommendation on IFCC methods for measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase, *Journal of Clinical Chemistry and Clinical Biochemistry* 24 (1986) 481–489.
- [29] H.U. Bergmeyer, M. Horder, R. Rej, Approved recommendation on IFCC methods for measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase, *Journal of Clinical Chemistry and Clinical Biochemistry* 24 (1986) 497–508.
- [30] H.P. Misra, I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, *Journal of Biological Chemistry* 247 (1972) 3170–3175.
- [31] R.F. Beers, I.W. Sizer, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, *Journal of Biological Chemistry* 195 (1952) 133–140.
- [32] J.T. Rotruck, A.L. Pope, H.F. Ganther, et al., Selenium: biochemical role as a component of glutathione peroxidase, *Science* 179 (1973) 588–590.
- [33] R.D. Mavis, E. Stellwagen, Purification and subunit structure of glutathione reductase from bakers' yeast, *Journal of Biological Chemistry* 243 (1968) 809–814.
- [34] A. Kornberg, B.L. Horecker, in: S.D. Colowick, N.O. Kaplan (Eds.), *Methods Enzymol*, Academic Press, New York, 1955, p. 823.
- [35] R.L. Levine, D. Garland, C.N. Oliver, et al., Oxygen Radicals in Biological Systems. Part B. Oxygen Radicals and Antioxidants, Elsevier, 1990.
- [36] J.S. Bus, L.G. Costa, E. Hodgson, et al. (Eds.), *Current Protocols in Toxicology*, John Wiley & Sons Inc., Hoboken, NJ, USA, 2001.
- [37] K. Burton, A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, *Biochemical Journal* 62 (1956) 315–323.
- [38] M.A. Akanji, O.A. Olagoke, O.B. Oloyede, Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system, *Toxicology* 81 (1993) 173–179.

- [39] G. Ramakrishnan, H.R.B. Raghavendran, R. Vinodhkumar, et al., Suppression of *N*-nitrosodiethylamine induced hepatocarcinogenesis by silymarin in rats, *Chemico-biological Interactions* 161 (2006) 104–114.
- [40] A.S. Yadav, D. Bhatnagar, Modulatory effect of spice extracts on iron-induced lipid peroxidation in rat liver, *BioFactors* (Oxford, England) 29 (2007) 147–157.
- [41] K. Pradeep, C.V.R. Mohan, K. Gobianand, et al., Silymarin modulates the oxidant–antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats, *European Journal of Pharmacology* 560 (2007) 110–116.
- [42] E. Kozar, S. Evans, J. Barr, et al., Glutathione, glutathione-dependent enzymes and antioxidant status in erythrocytes from children treated with high-dose paracetamol, *British Journal of Clinical Pharmacology* 55 (2003) 234–240.
- [43] D. Nakae, Y. Kobayashi, H. Akai, et al., Involvement of 8-hydroxyguanine formation in the initiation of rat liver carcinogenesis by low dose levels of *N*-nitrosodiethylamine, *Cancer Research* 57 (1997) 1281–1287.
- [44] Y. Sánchez-Pérez, C. Carrasco-Legleu, C. García-Cuellar, et al., Oxidative stress in carcinogenesis. Correlation between lipid peroxidation and induction of preneoplastic lesions in rat hepatocarcinogenesis, *Cancer Letters* 217 (2005) 25–32.
- [45] S. Das, Vitamin E in the genesis and prevention of cancer: a review, *Acta Oncologica* (Stockholm, Sweden) 33 (1994) 615–619.
- [46] M. Chevion, E. Berenshtein, E.R. Stadtman, Human studies related to protein oxidation: protein carbonyl content as a marker of damage, *Free Radical Research* 33 (Suppl.) (2000) S99–S108.
- [47] I. Dalle-donne, R. Rossi, D. Giustarini, et al., Protein carbonyl groups as biomarkers of oxidative stress, *Clinica Chimica Acta* 329 (2003) 23–38.
- [48] A. Amin, A.A. Hamza, Oxidative stress mediates drug-induced hepatotoxicity in rats: a possible role of DNA fragmentation, *Toxicology* 208 (2005) 367–375.
- [49] M.S. Cooke, M.D. Evans, M. Dizdaroglu, et al., Oxidative DNA damage: mechanisms, mutation, and disease, *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 17 (2003) 1195–1214.