Original Article

Effects of Aqueous Root Bark Extracts of *Anogeissus leiocarpus* (DC) Guill & Perr and *Terminalia avicennioides* Guill & Perr on Redox and Haematological Parameters of Diethylnitrosamine-Administered Rats

Amadu Kayode Salau[*1], *Musa Toyin Yakubu*[2], *Adenike Temidayo Oladiji*[2]

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ABSTRACT

Background: This study investigated the protective effects of aqueous extracts of *Anogeissus leiocarpus* (DC) Guill & Perr (Combretaceae) and *Terminalia avicennioides* Guill & Perr (Combretaceae) root barks, as well as their 1:1 (w/w) mixture on liver redox and haematological parameters of diethylnitrosamine-treated rats.

Methods: Rats were orally administered distilled water, diethylnitrosamine (30 mg/kg body weight once a week on weeks 3 and 4), curcumin (200 mg/kg body weight), extracts and 1:1 mixture (200, 400 and 800 mg/kg body weight) for 4 weeks. Malondialdehyde, markers of oxidative stress and hematological indices were evaluated.

Results: The extracts and their mixture significantly (P<0.05) reversed the diethylnitrosamine-induced alterations in the levels of liver malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glucose 6-phosphate dehydrogenase, glutathione, vitamin C and platelet counts. The other haematological parameters (red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell count, lymphocyte count and neutrophil count) were not affected by diethylnitrosamine and extracts.

Conclusion: The extracts possess antioxidant, hepatoprotective and haemoprotective activities that compared well with curcumin. These activities were better exhibited by the mixture than the individual extracts.

Keywords: Antioxidants, Combretaceae, Curcumin, Diethylnitrosamine, Oxidation-reduction, Oxidative stress.

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INTRODUCTION

Various chemical agents, whether domestic or industrial, to which man is exposed have deleterious effects on man. Such effects could be local, causing damage to a part of the body, or systemic, damaging various parts of the body. Exposure to chemicals could also be from industrial effluents, vehicular exhausts, and pollution from burning materials. One of the many chemicals that man is exposed to is diethylnitrosamine.

Diethylnitrosamine (DEN) or *N*-nitroso diethylamine (NDEA) is an *N*-nitroso alkyl compound, a potent hepatotoxin and hepatocarcinogen in experimental animals, which causes severe hepatic injury [1, 2]. It occurs widely in the environment, is formed endogenously from the interaction of ingested nitrate or nitrite with secondary amines, and is formed in food products [3]. DEN is found in tobacco smoke, water, agricultural chemicals, cosmetics, pharmaceutical products and a wide variety of foods like cheese, soybean, smoked, salted and dried fish, cured meat and alcoholic beverages [2, 4]. It distorts the cellular antioxidant system and causes lipid peroxidation [2, 5]. One of the ways of preventing or reversing such oxidative damage is using antioxidant agents, which are present in a wide variety of plants such as *Anogeissus leiocarpus* and *Terminalia avicennioides* both of the Combretaceae family.

A. leiocarpus is known as chewing stick tree (English) and *Ayin* (Yoruba, southwestern

1. Department of Chemical Sciences, Fountain University, Osogbo, Nigeria.
2. Department of Biochemistry, University of Ilorin, Nigeria.
*Corresponding Author: E-mail: ka.salaudeen@gmail.com

Nigeria). The presence of flavonoids, tannins, phenolics, saponins and other phytochemicals in the aqueous root bark extract of this plant has been reported [6].

T. avicennioides is known as *Baushe* (Hausa, Northern Nigeria), *Idi* (Yoruba) and *Edo* (Igbo, Southeast Nigeria). The roots contain phenolics, tannins, saponins and alkaloids [6].

Previously, studies have been carried out on these plants. Aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides* as well as their mixture have been reported to inhibit the proliferation of Ehrlich ascite carcinoma cells *in vitro* and to possess antioxidant properties [6, 7]. The plants and their mixture have been reported to increase the activities of antioxidant enzymes and other non-enzyme antioxidants in the liver of normal rats as well as inhibit free radicals *in vitro* [7]. Their protective effects on DEN-mediated fluctuations of liver enzymes and functional indices have also been reported [8]. Studies on the effects of these plants and their mixture on hepatic redox status and haematological parameters of DEN-treated rats have not been reported.

Due to the fact that man is exposed to a variety of dangerous chemicals that induce oxidative stress in the system, this study was designed to investigate the protective effects of aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides* as well as their mixture on hepatic redox status and haematological parameters of DEN-treated rats using curcumin as a reference antioxidant.

MATERIALS AND METHODS

Plant Materials

Fresh roots of *A. leiocarpus* and *T. avicennioides* were obtained from a farmland in Offa, Nigeria in December 2010 and authenticated at the IFE Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria, with voucher numbers 13775 and 15428, respectively.

Experimental Animals

Male albino rats (181.98±9.36 g) of Wistar strain were obtained from the Animal House of Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in aluminium cages placed in well-ventilated house with optimum conditions (temperature: 22 ±3^{0}C; photoperiod: 12 hours natural light and 12 hours dark; humidity: 40-45%) and were allowed free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water. The study was conducted at the Biochemistry and Nutrition Unit of Department of Chemical Sciences, Fountain University, Osogbo, Nigeria.

Chemicals and Reagents

Vitamin C, DEN, curcumin and glutathione were products of Sigma-Aldrich, St. Louis, USA. Assay kits for the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRed), and glucose 6-phosphate dehydrogenase (G6PDH) were products of Randox Laboratories, UK. All other reagents were of analytical grade and prepared in all glass-distilled water.

Preparation of Extracts

The aqueous extracts were prepared as described previously [6-8]. Fresh root barks of both plants were separately oven-dried at 40 ^{o}C for 3 weeks and pulverised with an AKIRA blender (model: BL-1531, Indonesia). A known amount (300 g) of each powder was extracted in 5 L of distilled water and placed on orbital shaker maintained at 300 rpm for 24 hours. This was then filtered with Whatman No. 1 filter paper and the filtrate concentrated on water bath to yield 28.5 g of *A. leiocarpus* (9.5%) and 30 g of *T. avicennioides* (10%). A 1:1 (w/w) mixture of both extracts was also prepared for use in the present study.

Animal Grouping and Preparation of Liver Supernatants

Eighty-four male albino rats were randomly assigned into twelve groups (A-L) of seven rats each and treated orally with 0.5 ml of distilled water, extracts or their mixture (1:1 w/w) daily for 28 days as follows:

Group A - received distilled water only (daily) for 4 weeks;

Group B –received distilled water (daily) for 4 weeks and DEN (30 mg/kg body weight in normal saline) on day 15 and day 22 (weeks 3 and 4);

Group C –pre-treated with curcumin a reference antioxidant agent (200 mg/kg body

weight in olive oil) daily for 4 weeks and DEN (as in group B above);

Group D –pre-treated with 200 mg/kg body weight *A. leiocarpus* extract daily for 4 weeks and DEN (as in group B above);

Group E - pre-treated with 400 mg/kg body weight *A. leiocarpus* extract daily for 4 weeks and DEN (as in group B above);

Group F - pre-treated with 800 mg/kg body weight *A. leiocarpus* extract daily for 4 weeks and DEN (as in group B above);

Group G - pre-treated with 200 mg/kg body weight *T. avicennioides* extract daily for 4 weeks and DEN (as in group B above);

Group H - pre-treated with 400 mg/kg body weight *T. avicennioides* extract daily for 4 weeks and DEN (as in group B above);

Group I - pre-treated with 800 mg/kg body weight *T. avicennioides* extract daily for 4 weeks and DEN (as in group B above);

Group J - pre-treated with 200 mg/kg body weight of the mixture daily for 4 weeks and DEN (as in group B above);

Group K - pre-treated with 400 mg/kg body weight of the mixture daily for 4 weeks and DEN (as in group B above); and

Group L - pre-treated with 800 mg/kg body weight of the mixture daily for 4 weeks and DEN (as in group B above).

The rats were sacrificed 24 hours after the last doses by placing them in a glass jar containing cotton wool soaked in diethyl ether before their jugular veins were cut for blood collection. Blood was collected into clean bottles containing anticoagulant for haematological analysis. Liver supernatants were prepared as previously described [8, 9] and used for the biochemical assays.

The study was approved by the Ethical Committee on the Care and Use of Experimental Animals of the College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria. The animals were also handled according to the Guidelines of the National Institute of Health (NIH; Bethesda, Maryland, USA), Guide for the Care and Use of Laboratory Animals [10].

Biochemical Assays and Haematological Analysis

The biochemical assays were carried out using standard methods as described for malondialdehyde (MDA) [11], SOD [12], catalase [13], GPx [14], GRed [15], G6PDH [16], glutathione [17] and vitamin C [18]. Haematological parameters, namely red blood cells (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM) and platelets (PLT) were determined on a haematology analyzer, SysmexHaematology Systems (SysmexAmeria Inc., model no. KX-21N, Kobe, Japan).

Statistical Analysis

The group mean (n = 7) ±S.D for each analysis was calculated and significant differences were determined by Analysis of Variance (ANOVA) and Tukey's Post Hoc test for multiple comparisons at 95% confidence level using SPSS software (SPSS Inc., Chicago, IL, USA).

RESULTS

Liver MDA in DEN-treated rats was significantly (P<0.05) elevated but pre-treatment with *A. leiocarpus* at 800 mg/kg body weight produced MDA levels that compared favourably with distilled water- and curcumin-treated control animals (Table 1). In addition, all the doses of the mixture of the extracts produced MDA levels that compared favourably (P>0.05) with the curcumin- and distilled water- treated animals (Table 1).

DEN significantly (P<0.05) reduced the activities of liver SOD, catalase, GPx, GRed and G6PDH of the animals (Figures 1-5). This trend of reductions were significantly (P<0.05) reversed following pre-treatment of the animals with the extracts and their mixture (Figures 1-5) in a dose-dependent manner. Interestingly, the 800 mg/kg body weight of the individual extracts and their mixture produced SOD and catalase activities that compared well with the curcumin- and distilled water-treated animals (Figures 1 & 2) except for the 200 mg/kg body weight of *A. leiocarpus* and the 200 and 400 mg/kg body weight of *T. avicennioides* (Figure 3). The 200 mg/kg body weight of *A. leiocarpus* produced GPx activities that were still significantly (P<0.05) less than the distilled water- and curcumin-treated animals (Figure 4).

The 400 mg/kg body weight of the mixture also produced G6PDH activity, which compared well with the controls (Figure 5).

Reduced glutathione (GSH) and vitamin C concentrations were significantly (P<0.05) decreased following the administration of DEN. These reductions were significantly (P<0.05) reversed after pre-treatment with the extracts and their mixture, though the values produced were still lower than those of the controls (Table 1). The elevations in the levels of GSH by *A. leiocarpus* and *T. avicennioides* were not comparable with that of curcumin (Table 1). Furthermore, the 800 mg/kg body weight of the mixture of the extracts produced levels of GSH that compared well with that of curcumin (Table 1). The 400 mg/kg body weight of *A. leiocarpus* and the 800 mg/kg body weight of the individual extracts also produced vitamin C concentrations that compared well with the distilled water-treated control animals, while 400 and 800 mg/kg body weight of mixture of the extracts produced vitamin C concentrations that were comparable to the curcumin-treated animals (Table 1). Administration of DEN and the extracts did not significantly (P>0.05) alter the Hb, RBC, PCV, MCV, MCH, MCHC, WBC, LYM and NEU when compared with the control (Table 2). PLT were, however, significantly (P<0.05) elevated following the administration of DEN and this was reversed by the individual extracts and their mixture. The 800 mg/kg body weight of all the extracts as well as the 400 mg/kg body weight of the mixture of the extracts produced platelet counts that compared favorably with the curcumin- and distilled water-treated animals (Table 2).

Table 1. Liver malondialdehyde (MDA), reduced glutathione and vitamin C contents of diethylnitrosamine (DEN)-treated rats pre-treated with aqueous root bark extracts of *A. leiocarpus*, *T. avicennioides* and their mixture.

	A. leiocarpus	*T. avicennioides*	Mixture
Liver malondialdehyde (nmol MDA/mg protein)			
I	3.05±0.11^{a}	3.05±0.11^{a}	3.05±0.11^{a}
II	7.59±0.53^{d}	7.59±0.53^{c}	7.59±0.53^{b}
III	2.97±0.31^{a}	2.97±0.31^{a}	2.97±0.31^{a}
IV	4.64±0.42^{c}	4.30±0.09^{b}	3.40±0.37^{a}
V	4.03±0.19^{b}	4.00±0.25^{b}	3.39±0.24^{a}
VI	3.71±0.56^{b}	3.27±0.12^{a}	2.85±0.18^{a}
Liver reduced glutathione (nmol/mg protein)			
I	30.00±0.51^{bc}	30.00±0.51^{c}	30.00±0.51^{b}
II	23.87±0.45^{a}	23.87±0.45^{a}	23.87±0.45^{a}
III	36.72±0.61^{e}	36.72±0.61^{e}	36.72±0.61^{d}
IV	27.71±1.09^{b}	26.19±0.99^{b}	29.11±0.17^{b}
V	31.21±0.97^{c}	32.33±1.06^{d}	34.45±0.24^{c}
VI	33.29±0.86^{d}	33.01±0.12^{d}	37.51±0.37^{d}
Liver vitamin C (mg/dL)			
I	54.09±0.32^{a}	54.09±0.32^{a}	54.09±0.32^{a}
II	49.32±0.16^{b}	49.32±0.16^{b}	49.32±0.16^{b}
III	58.15±0.76^{c}	58.15±0.76^{c}	58.15±0.76^{c}
IV	52.57±0.13^{d}	51.97±0.57^{d}	55.64±0.10^{a}
V	53.99±1.10^{a}	53.19±0.78^{d}	57.18±0.37^{c}
VI	55.57±0.97^{a}	56.32±0.72^{a}	59.31±1.61^{c}

Values are mean of 7 determinations±Standard Deviation. Values carrying different superscripts down the column for each parameter are significantly different (P<0.05). *A. leiocarpus* = *Anogeissus leiocarpus*, *T. avicennioides* = *Terminalia avicennioides,* I = control, II = Diethyl nitrosamine (30 mg/kg bd wt), III = DEN+Curcumin (200 mg/kg bd wt), IV = DEN+Extract (200 mg/kg bd wt), V = DEN+Extract (400 mg/kg bd wt), VI = DEN+Extract (800 mg/kg bd wt)

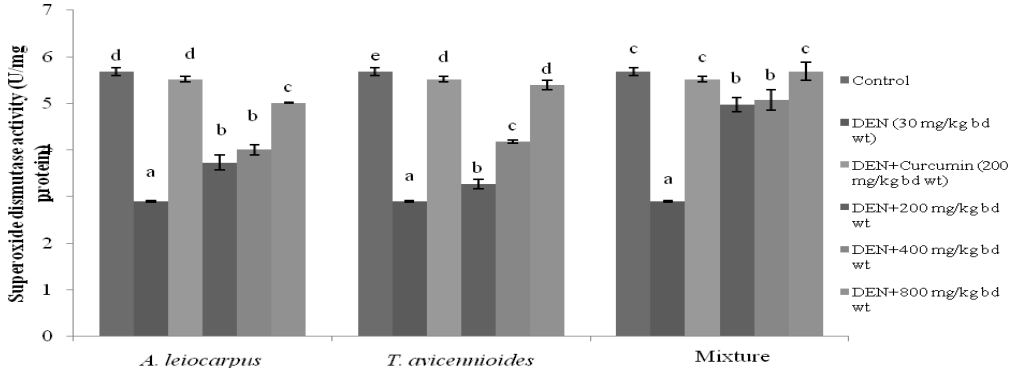

Figure 1. Liver superoxide dismutase activities of diethylnitrosamine (DEN)-treated rats pre-treated with aqueous root bark extracts of *A. leiocarpus, T. avicennioides* and their mixture. Bars carrying different letters for each treatment are significantly different (P<0.05).

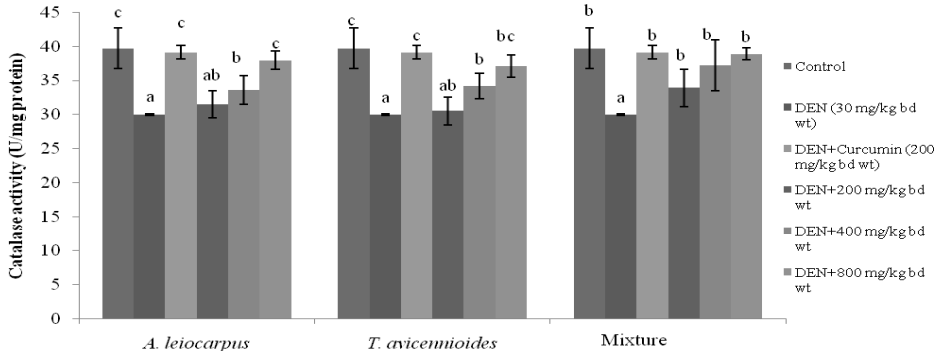

Figure 2. Liver catalase activities of diethylnitrosamine (DEN)-treated rats pre-treated with aqueous root bark extracts of *A. leiocarpus, T. avicennioides* and their mixture. Bars carrying different letters for each treatment are significantly different (P<0.05).

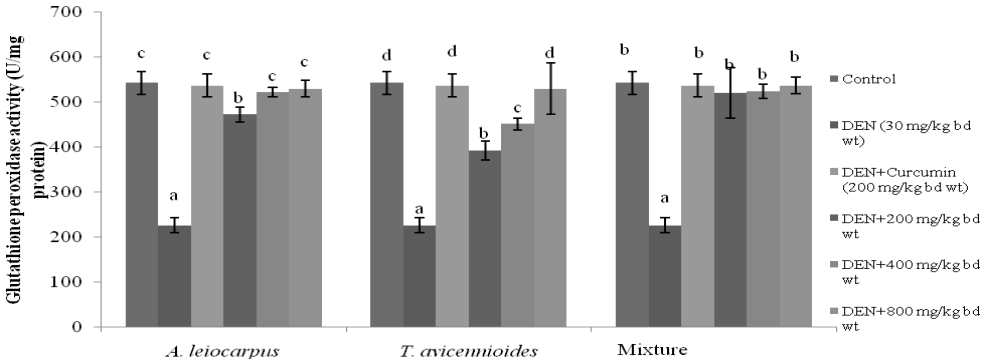

Figure 3. Liver glutathione peroxidase activities of diethylnitrosamine (DEN)-treated rats pre-treated with aqueous root bark extracts of *A. leiocarpus, T. avicennioides* and their mixture. Bars carrying different letters for each treatment are significantly different (P<0.05).

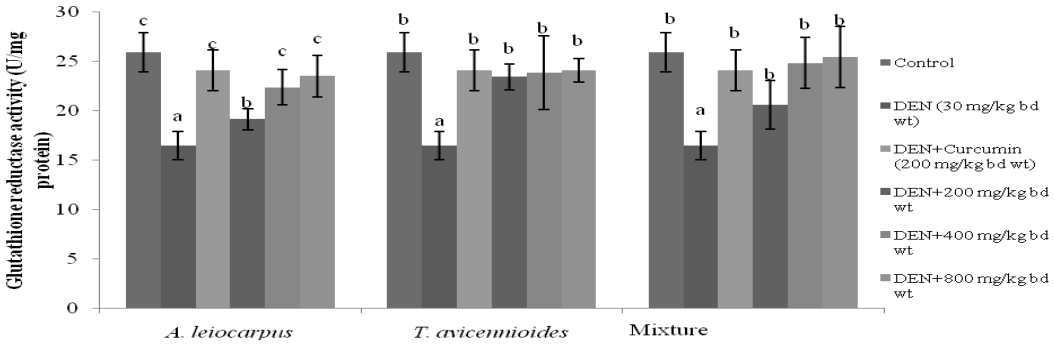

Figure 4. Liver glutathione reductase activities of diethylnitrosamine (DEN)-treated rats pre-treated with aqueous root bark extracts of *A. leiocarpus, T. avicennioides* and their mixture. Bars carrying different letters for each treatment are significantly different (P<0.05).

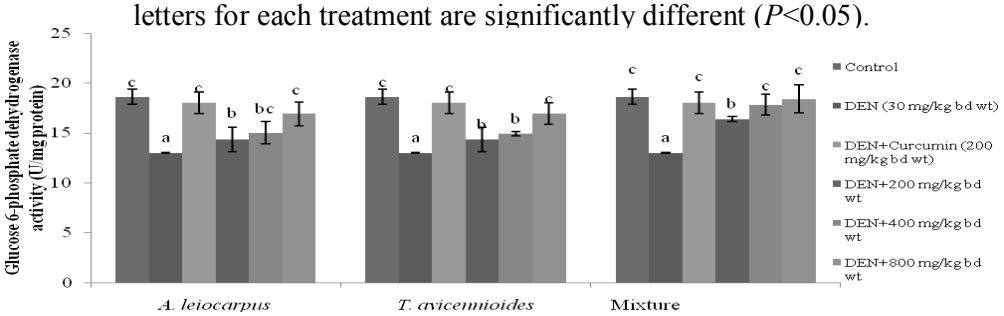

Figure 5. Liver glucose 6-phosphate dehydrogenase activities of diethylnitrosamine (DEN)-treated rats pre-treated with aqueous root bark extracts of *A. leiocarpus, T. avicennioides* and their mixture. Bars carrying different letters for each treatment are significantly different (P<0.05).

Table 2. Haematological parameters of diethylnitrosamine (DEN)-treated rats pre-treated with aqueous root bark extracts of *A. leiocarpus*, *T. avicennioides* and their mixture.

	A. leiocarpus	*T. avicennioides*	Mixture	*A. leiocarpus*	*T. avicennioides*	Mixture
	Haemoglobin (g/dL)			**Red Blood Cells (x10^6/µl)**		
I	12.80±0.02^a	12.80±0.02^a	12.80±0.02^a	7.70±0.56^a	7.70±0.56^a	7.70±0.56^a
II	12.60±0.40^a	12.60±0.40^a	12.60±0.40^a	7.14±0.76^a	7.14±0.76^a	7.14±0.76^a
III	13.07±0.35^a	13.07±0.35^a	13.07±0.35^a	8.03±0.55^a	8.03±0.55^a	8.03±0.55^a
IV	12.56±0.12^a	13.18±0.70^a	13.09±0.20^a	6.98±0.01^a	7.53±0.10^a	8.45±0.50^a
V	12.19±0.77^a	13.31±0.34^a	13.03±0.16^a	7.06±0.14^a	7.77±0.75^a	7.28±1.17^a
VI	12.91±0.64^a	1300±0.21^a	13.10±0.11^a	7.61±0.62^a	7.93±0.87^a	8.06±0.31^a
	Packed Cell Volume (%)			**Mean Cell Volume (fL)**		
I	45.23±0.86^a	45.23±0.86^a	45.23±0.86^a	65.87±0.15^a	65.87±0.15^a	65.87±0.15^a
II	44.97±0.25^a	44.97±0.25^a	44.97±0.25^a	65.33±0.72^a	65.33±0.72^a	65.33±0.72^a
III	44.67±0.59^a	44.67±0.59^a	44.67±0.59^a	65.67±0.67^a	65.67±0.67^a	65.67±0.67^a
IV	43.75±0.32^a	4400±0.12^a	45.20±2.01^a	64.76±0.46^a	65.34±0.21^a	66.03±0.16^a
V	43.19±0.84^a	43.59±0.26^a	45.01±1.18^a	65.11±0.98^a	65.37±1.52^a	64.93±1.10^a
VI	44.18±0.73^a	45.61±0.29^a	45.37±1.50^a	65.17±0.65^a	64.61±0.14^a	65.27±1.05^a
	Mean Cell Haemoglobin (pg)			**Mean Cell Haemoglobin Conc. (g/dL)**		
I	18.20±1.29^a	18.20±1.29^a	18.20±1.29^a	25.70±0.56^a	25.70±0.56^a	25.70±0.56^a
II	18.01±1.30^a	18.01±1.30^a	18.01±1.30^a	25.43±1.01^a	25.43±1.01^a	25.43±1.01^a
III	18.33±0.76^a	18.33±0.76^a	18.33±0.76^a	25.30±1.04^a	25.30±1.04^a	25.30±1.04^a
IV	17.46±0.21^a	18.05±0.58^a	18.47±0.15^a	25.99±0.17^a	26.02±0.34^a	26.31±0.70^a
V	16.85±0.21^a	17.13±0.04^a	17.97±0.55^a	26.10±0.53^a	26.31±0.72^a	26.00±0.62^a
VI	18.51±0.29^a	18.00±0.71^a	18.47±0.35^a	26.30±0.10^a	25.9±0.19^a	25.00±0.170^a
	White Blood Cells (x10^3/µL)			**Lymphocytes (%)**		
I	15.87±0.25^a	15.87±0.25^a	15.87±0.25^a	86.93±0.78^a	86.93±0.78^a	86.93±0.78^a
II	16.63±0.90^a	16.63±0.90^a	16.63±0.90^a	89.70±1.36^a	89.70±1.36^a	89.70±1.36^a
III	15.10±0.90^a	15.10±0.90^a	15.10±0.90^a	86.43±1.21^a	86.43±1.21^a	86.43±1.21^a
IV	15.15±0.39^a	14.69±0.23^a	14.67±0.15^a	87.12±0.09^a	86.50±0.12^a	86.00±0.56^a
V	15.49±0.17^a	15.34±0.42^a	15.63±1.06^a	86.01±0.11^a	86.91±0.78^a	86.77±1.11^a
VI	1500±0.24^a	14.90±1.09^a	14.53±0.54^a	86.91±0.64^a	86.25±1.72^a	86.07±1.08^a
	Platelets (x10^3/µL)			**Neutrophils (%)**		
I	846.00±22.08^c	846.00±22.08^b	846.00±22.08^b	12.97±0.86^a	12.97±0.86^a	12.97±0.86^a
II	975.91±29.06^a	975.91±29.06^a	975.91±29.06^a	12.2±0.41^a	12.2±0.41^a	12.2±0.41^a
III	849.33±23.44^c	849.33±23.44^b	849.33±23.44^b	13.17±0.20^a	13.17±0.20^a	13.17±0.20^a
IV	950.01±27.00^a	940.98±22.11^a	924. 00±26.00^{ab}	13.10±0.71^a	13.81±0.90^a	13.13±0.64^a
V	905.01±14.52^b	898.91±24.78^{ab}	886.67±47.00^b	11.94±0.65^a	12.00±0.73^a	12.09±0.19^a
VI	873.90±19.21^{bc}	870.12±27.65^b	857.67±17.39^b	13.24±0.92^a	13.54±0.31^a	13.61±0.92^a

Values are mean of 7determinations±Standard Deviation. Values carrying different superscripts down the column for each parameter are significantly different (P<0.05). *A. leiocarpus* = *Anogeissus leiocarpus*, *T. avicennioides* = *Terminalia avicennioides,* I = control, II = Diethyl nitrosamine (30 mg/kg bd wt), III = DEN+Curcumin (200 mg/kg bd wt), IV = DEN+Extract (200 mg/kg bd wt), V = DEN+Extract (400 mg/kg bd wt), VI = DEN+Extract (800 mg/kg bd wt).

DISCUSSION

Rats were pre-treated with aqueous root bark extracts of *A. leiocarpus*, *T. avicennioides* and their mixture (1:1 w/w) and were thereafter challenged with DEN, a potent hepatotoxin, which induced alterations in redox parameters of the liver and some haematological indices. The extracts prevented the DEN-induced effects in a manner comparable with curcumin, a reference antioxidant.

MDA is a decomposition product of lipid peroxides, which result from lipid peroxidation [19]. It is an indicator of lipid peroxidation and has been used as an index of tissue toxicity [20]. MDA in the cell can attack intracellular macromolecules, including DNA, leading to mutagenicity and carcinogenicity [4]. The significant increase in liver MDA concentration by DEN might indicate increased peroxidation of the unsaturated fatty acids in the cellular membrane of the liver. DEN produces excessive ROS [5], which attack the unsaturated fatty acids in the cellular membrane thereby causing lipid peroxidation. The ability of the extracts and their mixture to attenuate the lipid peroxidation is an indication that they could have blocked the lipid peroxidation pathway by acting as antioxidants.

This might be attributed to the presence of tannins, flavonoids and phenolics, which are reportedly present in the extracts [6] and might have acted as ROS scavengers and chain breaker in the process of lipid peroxidation. Reduction of cellular MDA would also reduce the risk of cancer. Increase in MDA concentration by DEN reported in this study is similar to previous findings [2, 4].

DEN has been reported to induce oxidative stress and retrogradation of the antioxidant defence mechanism due to overproduction of ROS, lipid peroxidation and damage to many enzymes involved in DNA repair, which result in organ disorders, especially the liver [2, 5]. Increased ROS production by DEN would shift the redox balance of the cell in favour of ROS, thereby depleting the antioxidant system. The redox status of cells is maintained by a set of enzymic and non-enzymic antioxidants. The antioxidant enzymes SOD, CAT, GPx, GRed and G6PD are involved in a coordinated process to prevent oxidative damage by ROS. SOD dismutates superoxide radical to H_2O_2, which is further decomposed to water and oxygen by catalase [19]. GPx catalyzes the reduction of hydroperoxide (ROOH) with concomitant oxidation of glutathione. The oxidized glutathione is then reduced by GRed, oxidizing NADPH in the process. G6PDH converts the oxidized $NADP^+$ to the reduced form (NADPH) by oxidizing glucose 6-phoshate. This coordinated set of reactions eventually lead to reduction of cellular ROS concentration. The excessive production of highly oxidized and oxidizing free radicals in cells has been variously reported to reduce the activities of these enzymes. Furthermore, the induction of these enzymes by chemical compounds has also been reported to be associated with *in vivo* antioxidant activity [4, 7].

The reduction in the activities of SOD by DEN is an indication of excessive ROS production. Since SOD converts superoxide radical to H_2O_2, reduced SOD activity will lead to the participation of superoxide in the Fenton Reaction to produce hydroxyl radicals, which will oxidatively damage DNA, lipids and proteins [19] and may lead to the development of cancer. The ability of the extracts to reverse this trend is an indication of cytoprotective activity against ROS and antioxidant activity of the extracts as reported in previous studies [6, 7].

Catalase catalyzes the decomposition of H_2O_2 to water. The reduction in the activity of this enzyme by DEN would also lead to the participation of hydrogen peroxide in the Fenton Reaction to produce hydroxyl radicals as mentioned earlier in this study. The ability of the extracts to reverse this DEN-induced reduction in catalase activity is an indication of *in vivo* antioxidant activity against oxidative stress.

GPx catalyses the detoxification of organic hydroperoxides and H_2O_2. It also catalyses the reduction of disulphide bonds in proteins by glutathione. Glutathione is consequently oxidised to its oxidised form (GSSG). DEN-induced reduction of GPx activity will lead to the accumulation of H_2O_2 and organic hydroperoxides in the cells. This may consequently lead to oxidative stress. The attenuation of this trend by the extracts further indicates antioxidant activity.

GRed catalyses the conversion of GSSG to GSH using NADPH and, in the process, provides protection to cellular constituents against ROS. The reduction of the activity of this enzyme by DEN will lead to ROS-induced oxidative damage. The ability of the extracts to reverse this trend is also an indication of antioxidant activity.

G6PDH recycles the NADPH consumed by GRed so that cellular GSH pool can be replenished. DEN-induced decrease in G6PDH activity will lead to accumulation of $NADP^+$ and GSSG as well as depletion of GSH, which can lead to oxidative stress. The reversal of this DEN-induced oxidative stress by the extracts is also an indication of *in vivo* antioxidant activity.

The non-enzyme antioxidants glutathione and vitamin C act in a complementary manner to the antioxidant enzymes. Glutathione reduces the disulphide bonds of cytoplasmic proteins to cysteines, thereby forming GSSG, which is reduced by GRed to GSH. Cellular glutathione consists of GSSG and GSH; increase in GSSG/GSH ratio is an indication of oxidative stress. The decrease in the levels of reduced glutathione following DEN administration is suggestive of oxidative stress.

Vitamin C or ascorbic acid donates electrons to oxidants, forming the stable dehydroascorbate, and therefore, acting as an antioxidant. The DEN-induced decrease in the vitamin C content will make the cells prone to attack by ROS. The reversal of this trend by the extracts further corroborates the earlier reported antioxidant activity of the extracts.

DEN-induced decrease in the enzymic and non-enzymic antioxidant systems would lead to

damage of cellular biomolecules, including DNA, by ROS, which could lead to mutagenesis and then carcinogenesis. The attenuation of this DEN-induced damage by the extracts could mean that the extracts may act as cancer preventing agents via antioxidant mechanism. This further corroborates the reduction in MDA, reported in this study. These results agree with previous reports [2, 4, 7] that DEN causes decrease in antioxidant systems, and that extracts possessing *in vivo* antioxidant and anticarcinogenic activities can reverse such processes and induce the increase in concentrations of cellular antioxidants. These activities could be attributed to the antioxidant phytochemicals previously reported to be present in the extracts [6]. The preventive activities exhibited by the extracts were, in most of the cases, produced by the 800 mg/kg body weight of the individual extracts, which compared well with the reference antioxidant curcumin and the control. The mixture, especially, produced the complete reversal of the DEN-induced liver damage.Haematological parameters can be used to monitor interactions of chemicals with the biological system *in vivo* in order to assess their systemic effects. RBCs contain haemoglobin, which carries oxygen to the tissues and returns CO_2 to the lungs during respiration. RBC, Hb and MCH are all indices of red blood cells and their reduction can indicate anaemia just as an increase can indicate increased rate of erythropoiesis, i.e. red blood cell synthesis [21]. White blood cells defend the body against infections or any foreign body. Elevated values may indicate a boost in immunological activity or a pathological condition [22]. The lack of any effect on these parameters by DEN is an indication that the balance between the rates of synthesis and destruction of these blood cells were not affected. It also means that the immune system of the animals was not affected. These findings are in contrast with that of a previous study [23], which reported a reduction in RBC, Hb, PCV, MCV, MCH, MCHC and total white blood cell count by a single intraperitoneal administration of 200 mg/kg body weight of DEN. This difference might be related to differences in doses and routes of administration of DEN.

Platelets activate the blood clotting mechanism [24] and function mainly in the formation of mechanical plugs during normal haemostatic response to vascular injury [25]. The elevated platelet counts could have resulted from liver injury caused by DEN. The reversal of this elevation of platelet count by the extracts further indicates that the extracts possess haemoprotective effects. This could be due to the presence of flavonoids in the extracts, which have been implicated in the prevention of platelet aggregation [26].

This study is limited by the fact that the active ingredients have not yet been identified, which would provide a direction for the isolation and production of potent hepatoprotective agents in the future.

CONCLUSION

Aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides* as well as their mixture possess antioxidant activities exhibited by their hepatoprotective and haemoprotective activities against hepatic redox and haemostatic imbalances caused by DEN in rats. These activities have been compared well with curcumin and better exhibited by the mixture than the individual extracts.

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