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APPRAISAL OF THE ANTIOXIDATIVE POTENTIAL OF *ALOE BARBADENSIS* M. ON ALCOHOL-INDUCED OXIDATIVE STRESS

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ABSTRACT

This investigation estimated the anti-oxidative potential of *Aloe barbadensis* gel extracts in rats against alcohol-induced oxidative stress. Thirty male albino rats (5 each per group) were included in the experiments. Group A (positive control) and B (negative control) were administered 4 mg.kg^{-1} body weight distilled water and 50 % alcohol respectively for 21 days. Groups C and D were administered 50 % alcohol for the first 14 days followed by co-administration of 125 mg and 250 mg.kg^{-1} body weight extract with alcohol respectively for the last 7 days. Groups E and F were administered distilled water for the first 14 days followed by co-administration of 125 and 250 mg.kg^{-1} body weight *Aloe barbadensis* gel extracts with distilled water respectively for the last 7 days. The administration of alcohol resulted in a significant ($P < 0.05$) decrease in the specific activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH) levels, while cholesterol (CHO), triglycerides (TAG), nitric oxide (NO) and malondialdehyde (MDA) concentrations were significantly increased when compared to the controls. Co-mobilization with *Aloe barbadensis* gel extracts for 7 days significantly reversed the deleterious effects of alcohol in the treated groups when compared to the alcohol group. This study indicated that *Aloe barbadensis* probably possesses anti-oxidative effects against alcohol–induced oxidative stress in rats.

Key words: alcohol; *Aloe barbadensis*; antioxidant; free radicals; lipid; oxidative stress

INTRODUCTION

Alcohol, which may be recognized as the most widely used psychoactive substances after caffeine is primarily metabolized in the liver to mainly acetaldehyde and generate toxic effects through the production of free radicals [16, 25, 31]. The acetaldehyde is further metabolized to acetate by acetaldehyde dehydrogenase (ALDH) and xanthine oxidase with the generation of some free radicals, namely superoxide radicals [27, 40, 46].

Free radicals are atoms with unpaired electrons in excited states which lead to further generation of more reactive oxygen species (ROS) and reactive nitrogen species (RNS) by chain reaction resulting in oxidative stress which has been implicated in the pathophysiology of many chronic diseases [39, 40, 46]. Although the production of free radicals is a normal physiological process, imbalances between its production and antioxidants system could lead to oxidative stress with the perturbation of various biological membrane functions and structural changes [23, 52]. Thus, there is a need for external antioxidant supplementation that could curb oxidative stress through the use of medicinal plants due to their therapeutic potentials.

Medicinal plants have gained tremendous interest of various researchers as an alternative, cheap and easily accessible regimen for the treatment of oxidative stress-related diseases [40, 42]. Despite this increased interest in the pharmacotherapeutic potentials offered by medicinal plants, only a few have received proper scientific scrutiny. Thus, *Aloe barbadensis* can be rightly considered as a plant of interest [1, 7, 11, 26].

Aloe vera (*Aloe barbadensis* Miller) is a perennial, succulent plant belonging to the Liliaceae family, which has been used as a source of food and medicine around the world for thousands of years [11, 40]. *A. barbadensis* is often referred to as a "healing" plant with the gel commonly used as an antioxidant gel in some industries [11, 26, 40]. The plant consists of two parts, the outer green rind including the vascular bundles and the parenchyma containing the gel, which consists of different types of polysaccharides that are responsible for its therapeutic properties [1, 11, 26]. Various studies have revealed that *A. barbadensis* gel possesses many pharmaceutical activities, including: antimicrobial, anticancer, anti-diabetic, antiulcer, hepato-protective, free radical scavenging and immune-modulatory activities [1, 3, 7, 38]. To the best of our knowledge, the physiological functions, as well as the mechanism through which lyophilized *A. barbadensis* gel extract exerts its anti-oxidative effect on alcohol-induced oxidative stress in male albino rats, are yet unclear and there has not been any established validated therapeutic antioxidant drug against alcoholic liver diseases, except therapeutic management regime [11, 40]. This study was, therefore designed to estimate the anti-oxidative potential of *A. barbadensis* on alcohol-induced oxidative stress in male albino rats by comparing it with only positive/normal control group using specific activities of; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), as well as the concentrations of cholesterol (CHO), triglycerides (TAG), nitric oxide (NO), malondialdehyde (MDA) and reduced glutathione (GSH) in various tissues of animal models.

MATERIALS AND METHODS

Plant collection and authentication

Fresh aloe vera (*Aloe barbadensis* M.) plants were obtained from Pure and Applied botany garden and authenticated by a Botanist (Professor D. A. Agboola) in the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria. The plant was identified and authenticated with herbarium number FUNAABH0028.

Extraction of aloe vera gel

The aloe vera plants were washed thoroughly with clean water, and then the inner parts (gel) of the leaves were removed into a clean container and blended using an electric blender. The blended gel was stored in a frozen state and lyophilized at Covenant University, Ota, Ogun State, Nigeria.

Experimental animals

The approval of the departmental animal ethical committee (FUNAAB-BCH) was obtained prior to the experiments. The rats were handled with care according to the guide for the care and use of laboratory animal's manuals [37]. Fifty-four male albino rats weighing between 180 and 250 g were purchased from the Department of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria for this study. The animals were acclimatized for two weeks before the commencement of the experiments. They were housed in plastic cages with good ventilation and were supplied with standard pellets and clean water ad libitum.

A. barbadensis lethal dose (LD50) test

Aloe vera gel extracts LD50 was determined according to the method described by Chinedu et al. [9]. Three rats each were used for the 3 stages of the experiment. Lyophilized aloe vera gel (1500 mg.kg^{-1} body weight) was administered in the first stage and when no sign of toxicity

or death was seen, the dose (3000 mg.kg^{-1} body weight) was given to another 3 rats and then was increased to 5000 mg.kg^{-1} body weight when no death or sign of toxicity was observed in the second stage. Again when no sign of toxicity or death was seen at the third stage (final stage) of testing, it was concluded that the LD50 of the extract was more than 5000 mg.kg^{-1} body weight and hence had a high degree of safety [9].

Experimental design (Table 1)

Forty-five male albino rats weighing 180—250 g were first divided into two groups of 15 animals and 30 animals. The 15 animals were orally given 4 ml.kg^{-1} body weight of distilled water for the first two weeks (14 days) of the experiment, while the other 30 animals were orally administered 4 ml.kg^{-1} body weight of 50 % ethanol for the first two weeks (14 days) of the experiment. After the first two weeks, the 15 animals were further divided into three groups (A, E and F) of 5 animals each; group A served as the positive control and were further administered 4 ml.kg^{-1} body weight of distilled water for another one week (7 days); while group E and F were co-administered 125 mg and 250 mg.kg^{-1} body weight aloe vera gel extract with distilled water respectively for another one week (7 days) of the experiments. Twenty-four animals were selected from the 30 alcohol-treated group and 10 of the animals were further administered the same quantity and concentration of alcohol for another one week which served as the negative control group (B). The remaining 14 alcohol-treated animals were also divided into 2 groups (C and D) of 7 animals each and co-treated with 125 mg and 250 mg.kg^{-1} body weight aloe vera gel extract respectively after 30 minutes of alcohol administration for another one week (7 days) of the experiment (see groups description in Table below). On the 22nd day, 30 animals (5 each per group) were sacrificed after an overnight fasting under a light anesthesia. Blood samples were then collected into clean plain tubes, allowed to stand and clot for 15 minutes and centrifuged at 3000 rpm for 10 minutes to obtain the serum using the refrigerated centrifuge (BK-THRI16, Biobase Industry Shandong). The rats were then dissected and some tissues (kidney, liver and stomach, brain, testes and heart) were excised for biochemical examination, using 10 % homogenate.

Table 1. The groups of rats and their treatment (A—F)

Groups /Treatment	Groups
Distilled water for 21 days	A
50 % Alcohol for 21 days	B
50 % Alcohol (14 days) followed by alcohol + aloe vera (7 days)	C (125 mg.kg^{-1}) and D (250 mg.kg^{-1})
Distilled water (14 days) followed by alcohol + aloe vera (7 days)	E (125 mg.kg^{-1}) and F (250 mg.kg^{-1})

Biochemical assays

The malondialdehyde (MDA) concentration was determined according to the method of Fraga et al. [17]. The nitric oxide (NO) level was assayed by using Griess reagent as described by Sild and Horak [47]. The reduced glutathione (GSH) concentration was determined according to the method described by Moron et al [36]. The specific activities of glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) were determined according to the methods described by Rotruck et al. [43], Calberg and Mannervick [5], Marklund and Marklund [34] and Hadwan and Abed [22] respectively. The isolation of HDL (high-density lipoproteins) and VLDL (very low-density lipoproteins) + LDL (low-density lipoproteins) from the serum was performed according the methods described by Gidez et al. [19]. Triacylglycerol (TAG) and cholesterol concentrations were determined in the serum and the lipoproteins (HDL and VLDL + LDL) isolated, using Randox standard Laboratory kits.

Statistical analysis

The results are presented as the mean ± standard error of the mean. The level of homogeneity among the groups were assessed using a one-way analysis of variance (ANOVA). Where homogeneity occurred, the Duncan test was used to differentiate between the groups. All analyses were done using SPSS (Statistical Package for Social Science version 20.0) with $P < 0.05$ considered statistically significant ($n = 5$).

RESULTS

***Aloe barbadensis* acute toxicity test (LD50)**

No sign of toxicity or death was observed at the three stages of the acute toxicity test of *A. barbadensis* (the rats were administered 1500 mg, 3000 mg and 5000 mg.kg^{-1}

body weight gel extracts respectively). Since no sign of toxicity or mortality was perceived at the maximum dose administered given, the LD50 of *A. barbadensis* is considered greater than 5000 mg.kg^{-1} body weight and hence has a high degree of safety [9].

Effects of *A. barbadensis* on the lipid peroxide levels

The extent of oxidative damage by alcohol consumption was assessed by determining the malondialdehyde (MDA) concentration as an index of lipid peroxidation level in all tissues examined. Significant (P < 0.05) increases were observed in the serum, brain, liver, heart, kidney, testes and stomach when compared to the controls (Fig. 1). Co-treatment with *A. barbadensis* resulted in marked (P < 0.05) reductions in the MDA levels respectively as compared to the alcohol-treated groups, with 125 mg.kg^{-1} body weight of extract causing 41.7 % (serum), 45.6 % (brain) and 43 % (testis) reduction in the respective tissues. The administrations of the aloe vera extract only showed little or no difference when compared with control groups.

Effects of *A. barbadensis* on nitric oxide (NO) levels

Fig. 2 shows the effects of the *A. barbadensis* on NO levels in the alcohol-intoxicated rats. There was a significant (P < 0.05) increase (4.5 %, 3.2 %, 3.6 %, 4.1 %, 5 %, 4.5 % and 4.5 %) in the serum, brain, liver, heart, kidney, testes and stomach respectively of the alcohol treated group (group B) when compared to the control (group A). However, *A. barbadensis* co-treatment significantly lowered the NO levels back to comparable level of the control (group A), while administration of aloe vera extract only showed little or no difference when compared with control (group A).

Effects of *A. barbadensis* on reduced glutathione (GSH) levels

The endogenous non-enzymatic antioxidant status of the cell was assessed using the reduced glutathione (GSH) concentration as an index following alcohol administration in rats as depicted in figure 3. Significant (P < 0.05) percentage decreases were observed in the concentrations of GSH in serum (35.9 %), brain (54.5 %), liver (27.9 %), heart (34 %), kidney (33.7 %), testes (64 %) and stomach (35 %) of the alcohol treated animals (group B) when compared to the control (group A). However, 125 mg.kg^{-1} body weight of *A. barbadensis* co-treatment caused 14.5 %, 55.7 %, 32.6 %, 67 % and 84.6 % increase in the serum, liver, heart, testis and stomach, respectively, while 250 mg.kg^{-1} body weight caused 86.8 % and 59 % increase in the brain and kidney respectively when compared to the alcohol treated group (group B).

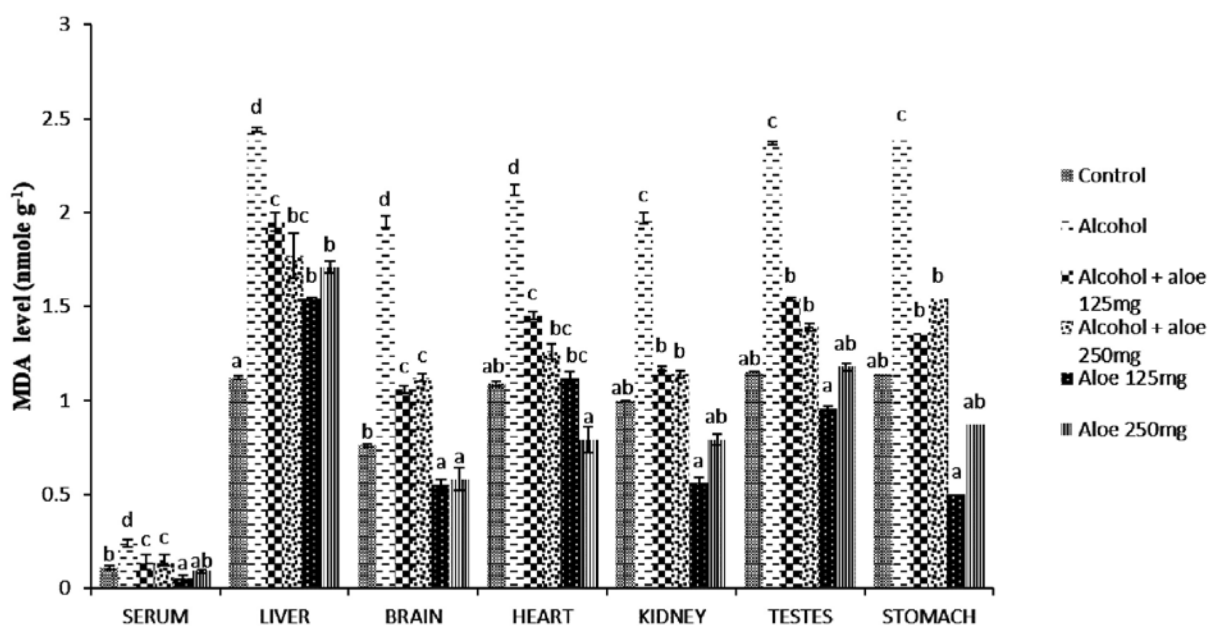

Fig. 1. Effects of *A. barbadensis* on MDA levels in alcohol intoxicated male albino rats
These data were expressed, as the mean ± SEM. The columns with different small alphabets at the top are significantly different at P < 0.05. Alcohol + aloe 125 mg—Alcohol + 125 mg.kg^{-1} body weight *A. barbadensis*; Alcohol + aloe 250 mg—Alcohol + 250 mg.kg^{-1} body weight *A. barbadensis* (n = 5)

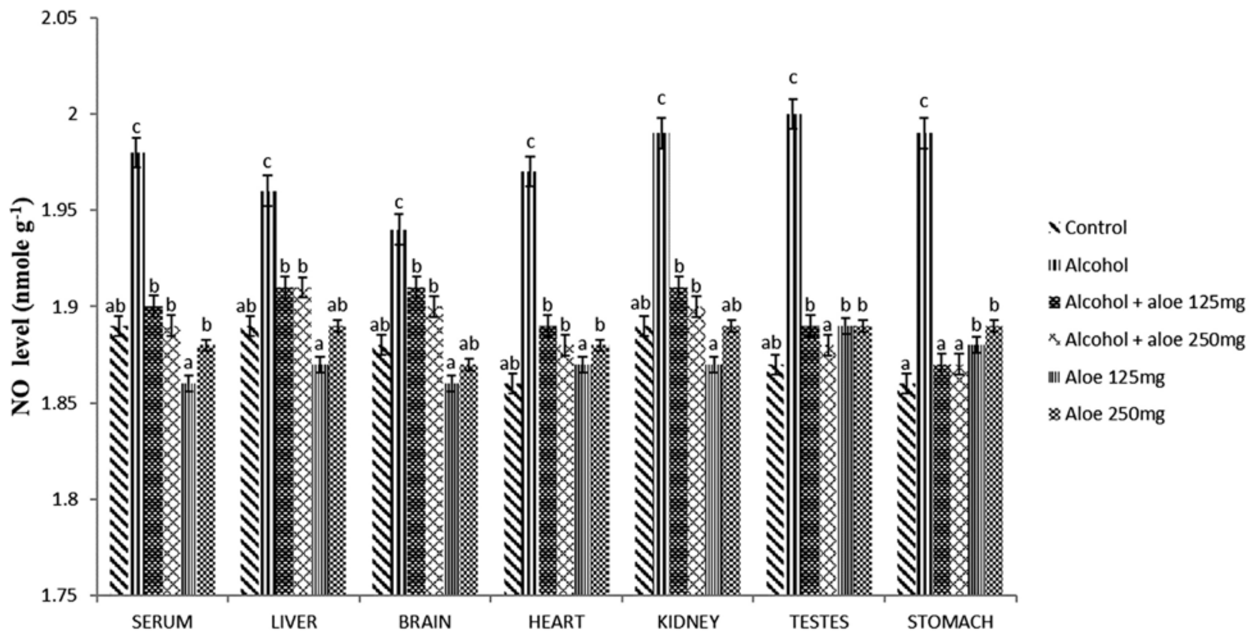

Fig. 2. Effects of *A. barbadensis* on NO levels in alcohol intoxicated male albino rats
These data were expressed, as the mean ± SEM. The columns with different small alphabets at the top are significantly different at $P < 0.05$. Alcohol + aloe 125 mg—Alcohol + 125 mg.kg^{-1} body weight *A. barbadensis*; Alcohol + aloe 250 mg—Alcohol + 250 mg.kg^{-1} body weight *A. barbadensis* (n = 5)

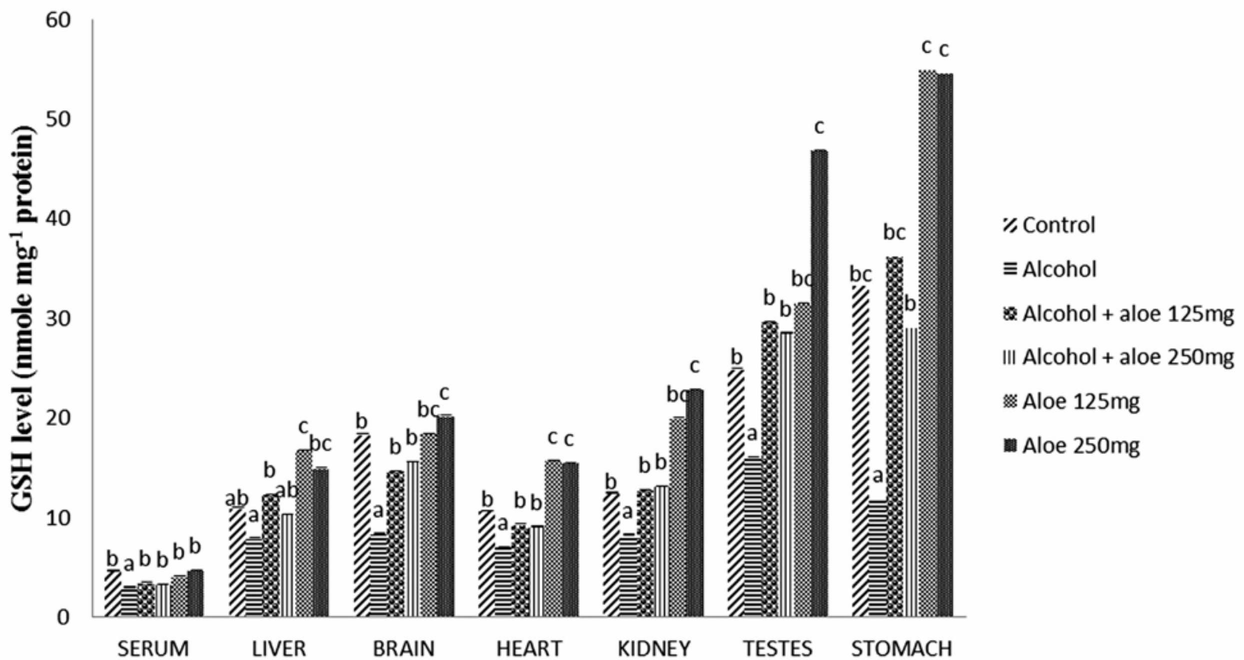

Fig. 3. Effects of *A. barbadensis* on GSH levels in alcohol intoxicated male albino rats
These data were expressed, as the mean ± SEM. The columns with different small alphabets at the top are significantly different at $P < 0.05$. Alcohol + aloe 125 mg—Alcohol + 125 mg.kg^{-1} body weight *A. barbadensis*; Alcohol + aloe 250 mg—Alcohol + 250 mg.kg^{-1} body weight *A. barbadensis* (n = 5)

Effects of *A. barbadensis* gel extract on serum, hepatic and renal antioxidant enzymes

The cellular enzymatic antioxidant status of the animals was estimated using the specific activities of SOD, CAT, GPx and GR in the serum, liver and kidney (Table 2). Note that GR was not done in the serum in Table 2. Alcohol ingestion resulted in a significant (P < 0.05) percentage decrease in serum SOD (40.7 %), CAT (31 %) and GPx (14.6 %) specific activities as compared to the control (group A) respectively. An estimated 34 %, 29 %, 34.8 % and 56.6 % decrease in the hepatic SOD, CAT, GPx and GR specific activities respectively as well as 15.9 %, 33 %, 79.9 % and 74.7 % decrease in renal SOD, CAT, GPx and GR respectively were observed. The observed decrease in the specific activities of these enzymes was significantly elevated in *A. barbadensis* co-administered groups in the investigated tissues. The extract (125 $mg.kg^{-1}$ body weight) elevated serum SOD, CAT and GPx specific activities by 71 %, 82.3 % and 10.8 %; while hepatic SOD, CAT and GPx were increased by 65 %, 95 % and 67 %; and renal SOD, CAT and GPx by 71.4 %, 64 % and 79.3 %, respectively, when compared to the alcohol-treated group (group B). The effect of the extract at 250 $mg.kg^{-1}$ body weight was not significantly different from that of the 125 $mg.kg^{-1}$ body weight dose.

Effects of *A. barbadensis* gel extracts on the brain and cardiac antioxidant enzymes.

Table 3 shows the effects of *A. barbadensis* on the brain and heart SOD, CAT, GPx and GR specific activities following alcohol and/or *A. barbadensis* treatments. The alcohol-treated group showed 20 %, 51.6 % and 38 % significant (P < 0.05) reduction in the brain SOD, CAT and GPx specific activities respectively. Cardiac specific activities were reduced by 30 %, 42 % and 59 % respectively when compared to the control. However, co-treatment with 125 $mg.kg^{-1}$ body weight of *A. barbadensis* caused an estimated 46 %, 99 % and 22.9 % increase in the brain SOD and CAT specific activities respectively; while the cardiac enzymes (SOD, CAT and GPx) specific activities elevated by 61 %, 42 % and 74.8 % respectively when compared to the alcohol-treated group. The effect of the 250 $mg.kg^{-1}$ body weight dose was not significantly different from the 125 $mg.kg^{-1}$ body weight dose, with exception only in the heart GR specific activities.

Effects of *A. barbadensis* gel extracts on testis and stomach antioxidant enzymes

Following alcohol administration, a significant (P < 0.05) decrease (39.9 %—SOD, 26.8 % CAT, 35 %—GPx and 37 %—GR) was observed in the testis while an estimated decrease

Table 2. Effects of *A. barbadensis* gel extract on antioxidant enzymes specific activities in serum, liver and kidney of alcohol-induced oxidative stress rats

Parameter		Control	Alcohol	Alcohol + aloe 125 mg	Alcohol + aloe 250 mg	Aloe 125 mg	Aloe 250 mg
Serum ($U.mg^{-1}$ protein)	SOD	0.59±0.03^{b}	0.35±0.02^{a}	0.60±0.03^{b}	0.58±0.02^{b}	0.69±0.02^{c}	0.74±0.02^{c}
	CAT	4.23±0.09^{b}	2.88±0.12^{a}	5.25±0.15^{b}	5.56±0.29^{b}	6.73±0.30^{c}	5.96±0.25^{c}
	GPx	3.89±0.20^{b}	3.32±0.12^{a}	3.68±0.21^{b}	3.42±0.17^{b}	3.57±0.08^{b}	4.06±0.25^{c}
Liver ($U.mg^{-1}$ protein)	SOD	11.35±0.37^{b}	7.49±0.39^{a}	12.37±0.74^{b}	11.21±0.32^{b}	16.45±0.43c	15.43±0.26^{c}
	CAT	83.78±2.40^{b}	59.37±2.88^{a}	116.0±6.64^{b}	112.0±3.39^{b}	93.37±2.69^{b}	126.0±2.99^{c}
	GPx	6.32±0.24^{b}	4.12±0.17^{a}	6.89±0.32^{b}	5.88±0.39^{b}	7.69±0.37^{c}	8.05±0.09^{c}
	GR	0.83±0.05^{b}	0.36±0.02^{a}	1.21±0.07^{b}	1.08±0.06^{b}	1.25±0.04^{b}	1.50±0.05^{c}
Kidney ($U.mg^{-1}$ protein)	SOD	11.75±0.45^{ab}	9.88±0.38^{a}	14.41±0.26^{b}	15.53±0.12^{b}	17.60±0.13^{b}	20.46±0.97^{c}
	CAT	173.0±1.59^{b}	115.7±5.69^{a}	190.3±2.52^{b}	184.8±8.75^{b}	213.5±7.59^{c}	217.4±7.51^{c}
	GPx	21.14±1.09^{b}	11.75±0.39^{a}	21.07± 0.56^{b}	21.87±0.59^{b}	30.41±2.07^{c}	29.23± 1.66^{c}
	GR	2.97±0.17^{b}	0.75± 0.03^{a}	2.81±0.13^{b}	3.31± 0.12^{b}	4.81±0.20^{c}	5.04±0.20^{c}

These data were expressed as the mean s ± SEM (n = 5). Values with different alphabets across the row for each parameter are significantly different at P < 0.05. Alcohol + aloe 125 mg—Alcohol + 125 $mg.kg^{-1}$ body weight aloe vera; Alcohol + aloe 250 mg—Alcohol + 250 $mg.kg^{-1}$ body weight aloe vera; Aloe 125 mg—125 $mg.kg^{-1}$ body weight aloe vera; Aloe 250 mg—250 $mg.kg^{-1}$ body weight aloe vera

Table 3. Effects of *A. barbadensis* extract on antioxidant enzymes specific activities in the brain and heart of alcohol-intoxicated male albino rats

Parameter		Control	Alcohol	Alcohol + aloe 125 mg	Alcohol + aloe 250 mg	Aloe 125 mg	Aloe 250 mg
Brain [U.mg^{-1} protein]	SOD	5.66±0.21^{ab}	4.51±0.14^{a}	6.61±0.38^{b}	7.15±0.41^{b}	8.17±0.31^{c}	9.07±0.56 c
	CAT	89.74±4.4^{b}	43.40±0.97^{a}	87.22±3.41^{b}	91.9±4.34^{b}	102.2±2.53^{b}	106.89±3.96c
	GR	1.86± 0.08^{ab}	0.73± 0.02^{a}	3.07± 0.06^{b}	2.75± 0.16^{b}	4.32±0.15^{c}	5.08±0.08c
	GPx	4.24±0.21c	2.62±0.16^{a}	3.22±0.20^{b}	3.02±0.13^{b}	4.35±0.21^{c}	4.65±0.22 c
Heart [	SOD	8.38± 0.28^{b}	5.83±0.29^{a}	9.43± 0.27^{b}	9.51±0.26^{b}	12.66±0.5^{c}	11.73±0.71 c
	CAT	115.0±7.20^{b}	66.18±2.25^{a}	117.8± 2.96^{b}	123.5±7.31^{b}	121.2±5.68^{c}	136.3±1.29 c
	GR	7.43±0.14^{b}	4.66±0.15^{a}	8.15± 0.27^{b}	6.90± 0.23^{b}	10.42± 0.34^{c}	10.06± 0.27 c
	GPx	0.58±0.04^{b}	0.16±0.01^{a}	0.91±0.02^{c}	0.83±0.03^{b}	0.89±0.04^{c}	0.92±0.02c

These data were expressed as the means ± SEM (n = 5). Values with different number of alphabets across the row for each parameter are significantly different at p < 0.05. Alcohol + aloe 125 mg—Alcohol + 125 mg.kg^{-1} body weight *A. barbadensis*; Alcohol + aloe 250mg—Alcohol + 250 mg.kg^{-1} body weight *A. barbadensis*; Aloe 125 mg—125 mg.kg^{-1} body weight; Aloe 250 mg—250 mg.kg^{-1} body weight aloe vera

Table 4. Effects of *A. barbadensis* extracts on antioxidant enzymes specific activities in the testis and stomach of alcohol-intoxicated male albino rats

Parameter		Control	Alcohol	Alcohol + aloe 125 mg	Alcohol + aloe 250 mg	Aloe 125 mg	Aloe 250 mg
Testis [U.mg^{-1} protein]	SOD	36.21±2.01^{b}	21.75±0.97^{a}	47.78±1.23^{b}	44.20±1.21^{b}	58.64±2.19^{c}	65.10±2.71^{c}
	CAT	352.9±9.78a^{b}	258.1±16.0^{a}	432.9±9.64^{b}	433.7±6.37^{b}	437.2±5.37^{b}	452.5±11.01^{c}
	GPx	34.96±1.24^{b}	26.59±1.46^{a}	42.04±0.24^{bc}	33.77±1.81^{b}	50.60±1.24^{c}	52.51±0.77^{c}
	GR	21.57±1.31^{b}	13.58±0.42^{a}	29.35±0.94^{bc}	28.01±0.79^{bc}	39.67±1.88^{c}	37.01±1.66^{c}
Stomach [U.mg^{-1} protein]	SOD	36.21±2.01^{ab}	21.75±0.97^{a}	47.78±1.23^{b}	44.20±1.21^{b}	58.64±2.19^{c}	65.10±2.71^{c}
	CAT	364.2±9.38^{b}	235.1±5.92^{a}	446.1±11.63^{bc}	360.2±13.37^{b}	474.1±10.03^{c}	524.8±5.11^{c}
	GPx	38.40±1.21^{ab}	29.17±1.73^{a}	55.55±1.46^{b}	53.57±1.09^{b}	76.17±0.95^{c}	74.95±1.55^{c}
	GR	14.41±0.36^{b}	6.16±0.14^{a}	15.07±0.33^{b}	15.72±0.36^{b}	15.54±0.69^{bc}	18.04±0.49^{c}

These data were expressed as the means ± SEM (n = 5). Values with different alphabets across the row for each parameter are significantly different at P < 0.05. Alcohol + aloe 125 mg—Alcohol + 125 mg.kg^{-1} body weight *A. barbadensis*; Alcohol + aloe 250 mg—Alcohol + 250 mg.kg^{-1} body weight *A. barbadensis*; Aloe 125 mg—125 mg.kg^{-1} body weight; Aloe 250 mg—250 mg.kg^{-1} body weight aloe vera

of 37.9 %, 35 %, 31.6 % and 57 % manifested in the stomach SOD, CAT, GPx and GR of the rats respectively when compared to the control. Co-treatment with *A. barbadensis* (both doses) however caused a significant (P < 0.05) increment in the testis and stomach SOD, CAT, GPx, and GR specific activities respectively as compared to the alcohol group (Table 4).

Effects of *A. barbadensis* on serum and lipoproteins TAG concentration

Figure 4 depicts the effects of *A. barbadensis* extracts on the serum triacylglycerol (TAG), HDL-TAG and VLDL+LDL-TAG in alcohol-intoxicated rats. Alcohol ingestion caused a significant increase in the serum TAG (hypertriglyceridemia) while there were no significant differ-

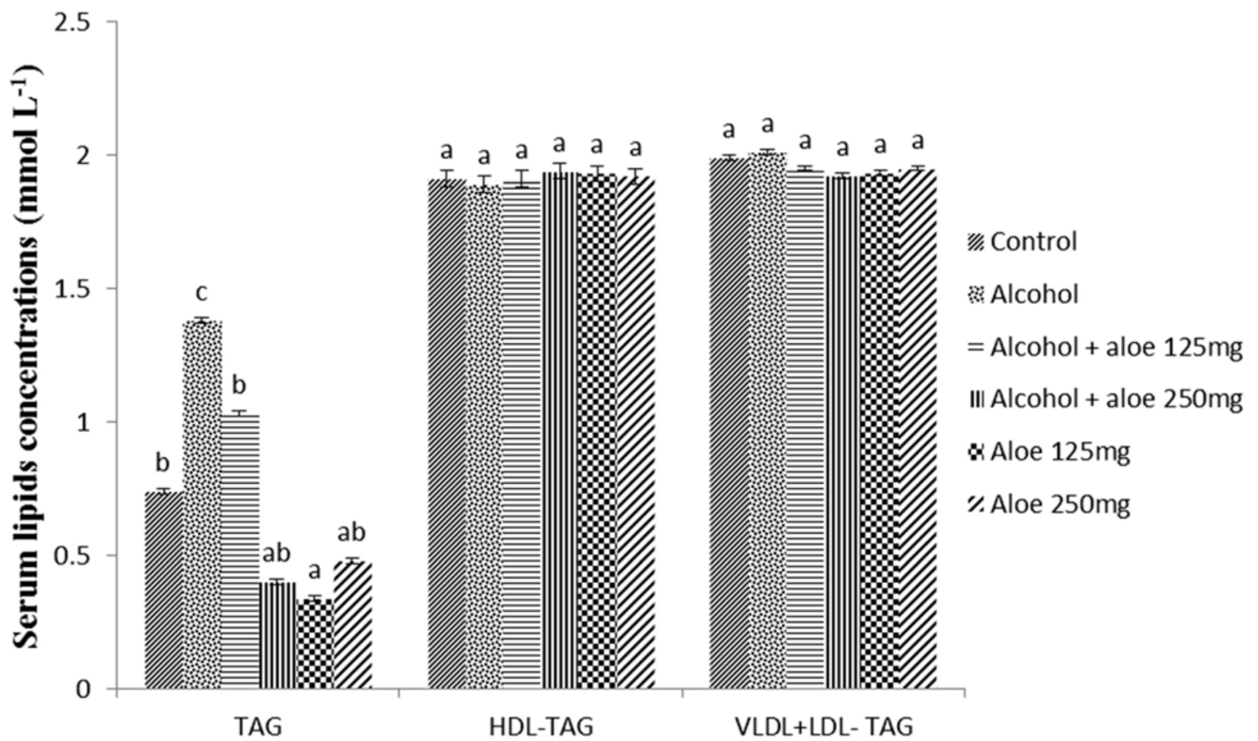

Fig. 4. Effects of A. barbadensis on serum TAG, HDL-TAG and VLDL+ LDL-TAG levels in alcohol-intoxicated male albino rats
These data were expressed as the means ± SEM. Column with different small alphabets at the top are significantly different at $p < 0.05$. Alcohol + aloe 125 mg—Alcohol + 125 mg.kg^{-1} body weight *A. barbadensis*; Alcohol + aloe 250 mg—Alcohol + 250 mg.kg^{-1} body weight *A. barbadensis* (n = 5)

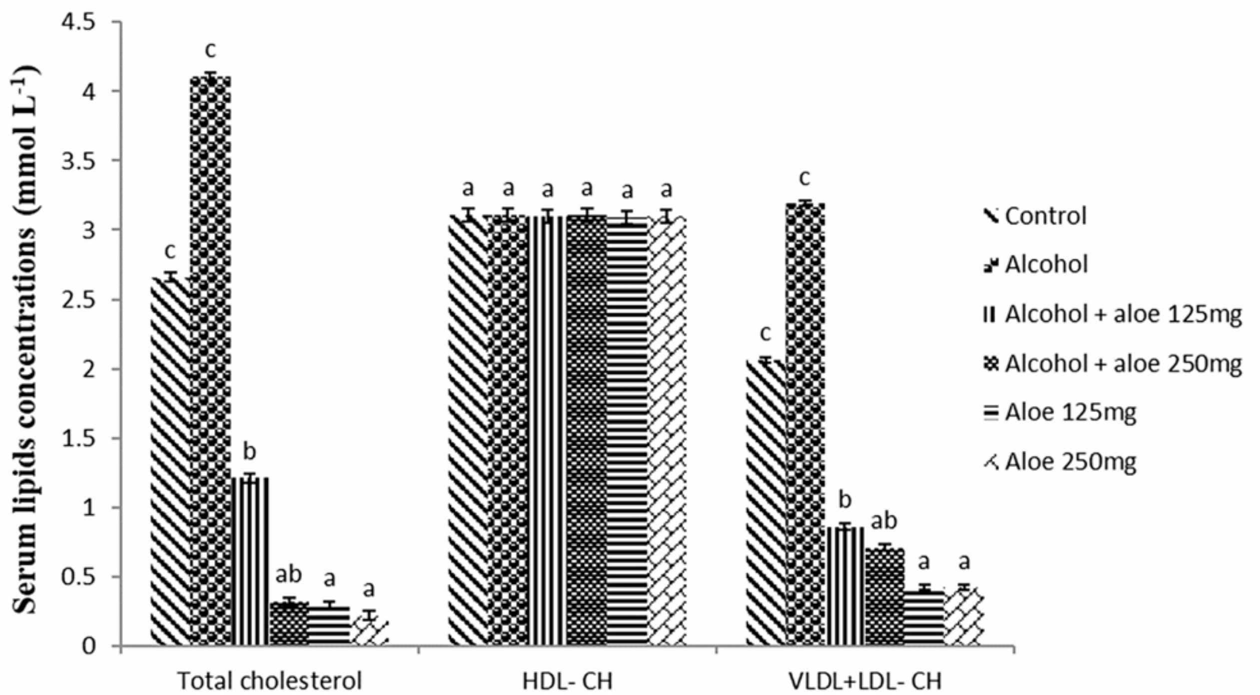

Fig. 5. Effects of *A. barbadensis* on serum cholesterol, HDL-cholesterol and VLDL + LDL-cholesterol levels in alcohol-intoxicated male albino rats
These data were expressed, as the means ± SEM. The columns with different small alphabets at the top are significantly different at $P < 0.05$. Alcohol + aloe 125 mg—Alcohol + 125 mg.kg^{-1} body weight *A. barbadensis*; Alcohol + aloe 250 mg—Alcohol + 250 mg.kg^{-1} body weight *A. barbadensis* (n = 5)

ence in the HDL-TAG and VLDL + LDL-TAG level when compared to the control. The *A. barbadensis* co-mobilized groups (C and D) showed a significant ($p < 0.05$) decrease in the serum TAG levels respectively when compared to the alcohol-treated group. However, the 250 $mg.kg^{-1}$ body weight dose was more effective.

Effects of *A. barbadensis* on serum and lipoprotein cholesterol concentrations

The effects of *A. barbadensis* extracts on the serum cholesterol, HDL-cholesterol and VLDL + LDL-cholesterol in alcohol-intoxicated rats are shown in Figure 5. Alcohol ingestion caused a significant increase in the serum and VLDL + LDL-cholesterol (hypercholesterolemia) while there was no significant difference in the HDL-cholesterol level when compared to the control. The *A. barbadensis* co-mobilized groups showed a significant ($P < 0.05$) decrease in the serum cholesterol and VLDL + LDL-cholesterol levels when compared to the alcohol-treated group respectively, with the 250 $mg.kg^{-1}$ dose proving to be more effective.

DISCUSSION

Cytochrome P450 2E1 (CYP 2E1)—an inducible xenobiotic metabolizing enzyme which is responsible for the metabolism of alcohol in many tissues produces acetaldehyde and at the same time reduces dioxygen to a variety of reactive oxygen species (ROS) such as superoxide anions, hydroxyl and hydroxyethyl radicals [15, 30, 33]. These radicals and lipid peroxides could lead to oxidative stress and inflammation especially when their production overwhelms the antioxidant systems' ability to eliminate them from the system [8, 30, 46]. Malondialdehyde (MDA) and nitric oxide (NO) levels are some of the indices used to measure the degree of peroxidative damage caused by ROS and reactive nitrogen species (RNS) which is capable of altering the membrane structure and function [15, 42]. Reduced glutathione (GSH), an antioxidant and a powerful nucleophile is critical for cellular protection in detoxification of ROS, conjugation and excretion of toxic molecules and control of inflammatory cytokine cascade [4, 42]. The present study sought to evaluate the toxic effects impacted on various tissues of the body by alcohol and possible ameliorative prowess of *A. barbadensis* Miller on such damages.

Our study further confirmed that administration of alcohol resulted in significant ($P < 0.05$) increments in the serum, hepatic and renal MDA and NO levels as well as a marked decrease in GSH concentrations when compared to the control and similar results have been reported by many authors [11, 15, 30, 41, 42]. These increased NO levels observed in this study might be due to inflammation which is seen in alcoholics as a result of inducible nitric oxide synthase (iNOS) that mediates the synthesis of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, and TNF-α and in turn stimulate the synthesis of NO [35, 41]. Similarly, high levels of MDA as seen in the brain especially and other tissues might be due to the presence of easily peroxidizable fatty acids in their membranes [6]. The GSH depletion noticed in various examined organs of alcohol-intoxicated rats might be due to increased utilization of GSH for conjugation processes or the inability of the liver to synthesize it from its precursors [13, 46, 51]. Co-treatment with aloe vera gel extract (125 mg and 250 $mg.kg^{-1}$ body weight) significantly enhanced the antioxidant system by lowering elevated levels of MDA and NO and causing a concomitant increment in the level of GSH. This attenuation of oxidative stress markers might be due to the ability of the gel extracts to alleviate lipid peroxidation and scavenge free radicals produced following alcohol metabolism which could be attributed to the acetyl groups and reductive nature of the monosaccharide in the gel extracts as reported by Chun–hui et al. [10] and Cui et al. [11].

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anions into hydrogen peroxide (H_2O_2) and oxygen (O_2); the H_2O_2 is then converted by catalase (CAT) or by glutathione peroxidase (GPx) to water (H_2O) and oxygen. The GPx uses GSH in producing an oxidized glutathione (GSSG) which is then converted back to GSH by glutathione reductase (GR) using NADPH—a cyclic antioxidant processes occurring in the cell [42, 46]. Significant reductions in the specific activities of SOD, CAT, GPx and GR in all examined tissues following alcohol administration indicates oxidative stress which might be as a result of free radicals generation [46]. These free radicals are capable of inducing lipid peroxidation by either the inactivation of enzymes or depletion of the non-enzymic antioxidants, such as GSH and NADPH [42]. The improved antioxidant status of the *A. barbadensis* co-treated groups (125 mg and 250 $mg.kg^{-1}$ body weight) might be due to the active polysaccharides in the gel as reported by Cui et al. [11]. This polysaccharide inhibits the activation of NF-kB following

stimulation by alcohol via up-regulation of the IkB-alpha leading to lowered stimulation of hepatic inflammation and oxidative stress [11, 24, 48]. Therefore, *A. barbadensis* gel extracts might be considered a potential regimen against alcohol-induced oxidative stress and inflammation.

Lipids and lipoproteins abnormalities have been shown to play a major role in the pathogenesis and progression of various disease conditions [20, 21]. High cholesterol is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke, which has led to US recommendations to reduce cholesterol intake [32]. The triacylglycerol (TAG) are the main constituent of fats in both plants and animals; their presence in the blood enable the bidirectional transference of adipose fats and blood glucose from the liver [18, 29]. A lipoprotein is a compound containing both lipids (fat) and protein [49]. They include LDL (low density lipoprotein) often referred to as bad cholesterol that conveys cholesterol from the liver to the extra-hepatic tissues, while HDL (high density lipoprotein) is often referred to as good or happy cholesterol, which is responsible for the reverse cholesterol transport—a process of absorbing cholesterol from the extra-hepatic tissues back to the liver where it under goes catabolic processes thus preventing cholesterol accumulations in those tissues [49, 50].

Alcohol ingestion resulted in elevated levels of TAG (only in the serum) and cholesterol in the serum and VLDL + LDL when compared to the control. Chronic alcohol consumption increases the hepatic NADH/NAD+ ratio, which in turn suppresses mitochondrial β-oxidation of fatty acids and stimulates de novo lipogenesis, thus causing lipid accumulation in hepatocytes which in turn is transported into the blood stream and may be responsible for the observed elevation in TAG and cholesterol in serum and VLDL + LDL [11, 14]. Co-treatment with *A. barbadensis* gel extract reverted the elevated lipids back to normal as compared to alcohol group (group B). This reduction in the lipid level by *A. barbadensis* extracts might be as a result of up-regulation of AMP dependent protein kinase (AMPK-α2) gene expression and down-regulation of sterol regulatory binding protein -1c (SREBP-1c) as reported by Cui et al. [11]. AMPK-α2, a key regulator of lipid metabolism in the liver, is responsible for the inactivation of acetyl-CoA carboxylase (ACC), which results in increased fatty acid oxidation in liver, while SREBP-1c particularly stimulates fatty acid synthesis through up-regulation in the transcription of lipogenic enzymes [2]. This suggests that *A. barbadensis* might possess anti-hyperlipidemic effects which may be attributed to the presence of aloe vera gel polysaccharides (AVGP) as reported by Cui et al. [11] that supplementation of AVGP significantly restored the decreased AMPK-α2 gene expression to a near-normal level.

Many bioactive compounds with different types of molecules and diverse structures from aloe vera gel have been isolated and reported to be responsible for the beneficial effects of the plant by previous researchers [10, 11, 26, 28, 44, 45]; to mention a few are oleic acid, 11,14-eicosadienoic acid—methyl ester, n-hexadecanoic acid, 1,2-benzenedicarboxylic acid—butyloctyl ester, hexadecanoic acid—methyl ester, tetradecanoic acid, 1,2-benzenedicarboxylic acid — diisooctyl ester, squalene, anthraquinones and polysaccharides (primarily acemannan). Debnath et al. [12] also reported that high levels of vanillic acid detected in aloe vera might be responsible for their strong antioxidant activities against DPPH (1,1-diphenyl-2-picryl-hydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), hydroxyl, and superoxide radicals. This study further confirmed the *in vivo* beneficial and pharmacological effects of lyophilized *A. barbadensis* with its less toxicity level at the tested dosages. However, the mechanisms by which this extract confined their beneficial and pharmacological effects are still not established at present and will be subjected to further investigations.

CONCLUSIONS

The results obtained in this study indicate that *A. barbadensis* gel extracts attenuated the alcohol–induced oxidative stress in various organs by enhancing their antioxidative status and also possess the ability to reverse dyslipidemia induced by alcohol. It is of note that the two doses investigated have different way they modulate the parameters and the effects were not much different from each other; although 125 $mg.kg^{-1}$ body weight of *A. barbadensis* appeared to be the therapeutic dose as far as this study was concerned. Therefore, *Aloe barbadensis* probably possesses the ability to alleviate alcohol-induced oxidative stress and dyslipidemia in experimental rats.

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