International Journal of Clinical Biology and Biochemistry
www.biochemistryjournal.net
Online ISSN: 2664-6196, Print ISSN: 2664-6188
Received: 25-12-2020, Accepted: 10-01-2021, Published: 25-01-2021
Volume 3, Issue 1, 2021, Page No. 01-10

Toxicological evaluation of aqueous and methanol extracts of *Dacryodes edulis* stem bark

PN Akunne[1*], NEJ Orhue[1], PA Obarisiagbon[2]
[1] Department of Biochemistry, University of Benin, Edo State, Nigeria
[2] Department of Pharmacology and Toxicology, University of Benin, Edo state, Nigeria

DOI: https://doi.org/10.33545/26646188.2021.v3.i1a.16

Abstract
Aim: This study is aimed at evaluating the toxicological effect of aqueous and methanol extracts of *Dacryodes edulis* stem bark on Wistar Rats
Methodology: The stem bark of *Dacryodes edulis* were locally sourced from a farmer in Ologbo area of Edo State, Nigeria and were identified by a botanist. The stem bark were chopped in pieces, air dried and ground to fine powder using milling machine and extracted using water and methanol respectively. Acute toxicity was determined using Lorke's method. In the main study, 35 adult male Wistar were acclimatized for seven days and were divided into 7 groups of 5 rats each. Group A served as normal control in which rats were given only feed and water. Groups B, C and D were administered 500, 1000 and 2000 mg/kg of aqueous extract of *D. edulis* stem bark respectively while groups E, F and G were administered 500, 1000 and 2000 mg/kg bw of methanol extract of *D. edulis* stem bark respectively via oral route for 28 days. At the end of the 28 day, animals were sacrificed and blood was collected for biochemical assays. Liver, kidney and heart of animals were harvested for enzymes assays and histopathology.
Results: The result of the acute toxicity of *Dacryodes edulis* in this study showed that the LD_{50} of its aqueous and methanolic extract of stem bark is greater than 5000 mg/kg as no mortality was recorded at this dose except for a decline in the normal activity of the animals administered 5000 mg/kg dose. Administration of 500 and 1000 mg/kg of aqueous and methanol extracts of *D. edulis* Stem bark was observed to have no significant effect on the parameters determined when compared with those in the control animals. However, administration of 2000 mg/kg of aqueous and methanol extracts of *D. edulis* stem bark significantly ($p<0.05$) perturbed hepatic, protein, renal, lipid profile and oxidative stress biomarkers when respectively compared with those in the animals in the control group.
Conclusion: The result of this study showed that both aqueous and methanol extracts of *D. edulis* stem bark are not toxic at a dose of 1000 mg/kg and below. However, a dose of 2000 mg/kg elicited toxic effects on the measured parameters. Thus, its consumption at high dose should be discouraged.

Keywords: *Dacryodes edulis*, hepatotoxicity, high dosage, nephrotoxicity

Introduction

The Liver is an essential organ that plays a vital role in protecting the body from harmful substances and toxic metabolic byproduct [1]. This function is done by metabolism, extraction and detoxification of various xenobiotic that could harm the body. Excessive exposure of this organ to harmful substances via physiological process leads to hepatic damage [2] which generates highly reactive radicals that attacks the membrane lipid causing lipid peroxidation and oxidative stress which in turn alters membrane integrity [3]. Hepatic tissue damage has deleterious effect on human health and the management of this disease is still a major challenge to scientists. However, the use of antioxidant has been projected as therapeutic agent against liver damage [4]. Since most renal disease in human are incurable when kidney is severely damage, it has become a focus point for medical research. Kidney is an important organ, it is a primary target in preclinical studies where by drug-induced nephrotoxicity is a recurrent discovery. To a greatest extent, the damage tubular epithelium in acute renal failure is repaired via repopulation and tubular function recovers. The tubular epithelium is very sensitive to toxic compounds due to water and solute absorption and active transport systems, which led to concentration of toxicants in tubular cells [5].

Dacryodes edulis is African pear that belongs to the family of Burseracaea. The leaves shades and are dioecious plant species found in the humid tropical zone of non-flooded forest [6]. The fruit tree is grown in Africa countries. The plant is known in English as "Bush butter tree" and "Native pear". Numerous species of *Dacryodes* have been use in traditional medicine. Diverse parts of *D. edulis* have antibiotic, immunostimulating, anti-inflammatory, antioxidant, hypoglycemic and hypolipidemic potentials [7-9]. An array of chemical constitutes have been isolated from the plant such as terpenes, flavonoids, tannins, alkaloids and saponins. The stem exudates contain tannin, saponin and alkaloids with implications in the treatment of variety of skin disease and inflammation [10]. A pervious finding by Chimaobi *et al.*, [11] showed that, *D. edulis* leaves have a high capacity to scavenge free radicals (DPPH, superoxide, and hydroxyl) as well as high reducing power as depicted by the ferric and molybdate. The capacity was linked to the presence of flavonoids and phenolic content which denotes electron or proton to oxidants/ radicals and neutralized them.

Materials and Methods

Collections and Identification of Plant Materials

The seed and stem bark of *Dacryodes edulis* were gotten from the same plant in Ologbo area, Edo State, Nigeria. For the avoidance of doubt, the seeds were gotten from the ripe fruits of *D. edulis*. They were chopped in pieces, air dried and ground to fine powder using milling machine. Aqueous and methanol extracts were made by soaking 500 g of the plant powder in 4 litres of distilled water or 4 litres of methanol respectively for 48 and 72 hours with regular stirring, followed by sieving through a cheese cloth and concentration using a freeze drier.

Acute Toxicity Study

Lorke [12] method was applied in this study. It is usually done in two phases. In phase I a total of 18 rats were divided into 6 groups of 3 rats each. Animals in groups A, B and C were administered 10, 100 and 1000 mg/kg aqueous extract of *D. edulis* stem bark, while those in groups D, E and F were administered 10, 100 and 1000 mg/kg methanol extract of *D. edulis* stem bark via oral. Animals were monitored regularly for clinical signs as well as mortality. At the end of day 14th, recovery and survival of acute intoxication was observed and experiment was terminated.

In phase II experiment, 18 rats were also divided into 6 groups of 3 rats each. Animals in groups A, B and C were administered 1600, 2900 and 5000 mg/kg aqueous extract of *D. edulis* stem bark, while those in groups D, E and F were administered 1600, 2900 and 5000 mg/kg methanol extract of *D. edulis* stem bark via oral route. Animals were monitored regularly for clinical signs as well as mortality. At the end of day 14th, recovery and survival of acute intoxication was observed and experiment was terminated.

Experimental Design and Animals Treatment

This study was based on the "limited study" described in OECD guidelines [13] which encourages the use of few numbers of animals by limiting the use of experimental groups. Thirty-five (35) adult male Wistar rats with body weight between 145 and 170 g were used for the experiment. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received human care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health (NAS, 2011). They were divided into 7 groups of 5 rats each. Group A served as control in which rats were given only feed and water. Groups B, C and D were administered 500, 1000 and 2000 mg/kg body weight of aqueous extract of *D. edulis* stem bark respectively while groups E, F and G were administered 500, 1000 and 2000 mg/kg bw of methanol extract of *D. edulis* stem bark respectively via oral route for 28 days. These doses were based on the survival of the acute administration. Their weights were taken weekly and the animals were monitored closely throughout the period of this experiment. At the end of the 28 day, animals were sacrificed and blood was collected for biochemical assays. Liver, kidney and heart of animals were harvested for enzymes assays and histopathology.

Determination of Hepatic Biomarkers

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities were determined using Randox commercial Enzyme kits according to the method of Reitman and Frankel [15]. Alkaline Phosphatase (ALP) activity was determined by Phenolphthalein Monophosphate method described by Babson *et al* [16]. Total bilirubin concentration was determined by diazo method described by Royden and Alfred [17].

Determination of Plasma Protein

Total protein concentration was determined by the method of Tietz [18]. Albumin concentration was determined by the method of Grant *et al.* [19]. Globulin concentration was determined by subtracting albumin from total protein.

Determination of Renal Indices

Creatinine concentration was determined using Jaffe reaction described by Toora and Rejagopal [20]. Urea concentration was determined using a Randox Commercial Kit based on the methods of Fesus*et al.* [21].

Determination of Lipids

Lipids were extracted and determined according to previously described methods [22, 23].

Determination of Oxidative Stress Biomarkers

Blood concentrations of Lipid Peroxidation (LPO), Reduced Glutathione (GSH), activities of Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione peroxidase (GPx) were determined following the methods of Airaodion *et al*. [24].

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Dunca Pos Hoc test. All analyses were done using SPSS Software Version 16.0 and P values < 0.05 were considered statistically significant.

Results, Discussion and Conclusion

Results

Acute Toxicity of *D. edulis* Extracts

The result of the acute toxicity of *Dacryodes edulis* in this study showed that the LD_{50} of its aqueous extract of stem bark (AQSB) and methanolic extract of stem bark (MTSB) is greater than 5000 mg/kg as no mortality was recorded at this dose except for a decline in the normal activity of the animals administered 5000 mg/kg dose.

Effect of Extracts of *Dacryodes edulis* Stem bark on Animal Body Weight after 28 days of Treatment

Both aqueous and methanol extracts of *D. edulis* stem bark were observed not to have significant effect on weight gained by animals after 28 days of treatment when compared with those in the control group as presented in table 2.

Effect of Extracts of *Dacryodes edulis* Stem bark on Relative Organ Weight (%) of Animals after 28 days of Treatment

Administration of aqueous and methanol extracts of *D. edulis* stem bark to animals for 28 days at all doses showed no significant effect in the relative weight of the heart of animals when compared with those in the control group. Similarly, 500 mg/kg of both extracts had no significant effect in the relative liver weight of animals when compared with those in the control group. However, increasing the doses of both extracts to 1000 and 2000 mg/kg respectively, the relative liver weight of animals were significantly (p<0.05) increased when compared with those in the control group. In the same vein, both extracts only showed significant increase in the relative weight of the kidneys when animals treated with 2000 mg/kg of aqueous and methanol extracts of *D. edulis* stem bark were compared with those in the control group (Table 3).

Effect of Extracts of *D. edulis* Stembark on Plasma Hepatic, Protein, Renal, Lipid Profile and Oxidative Stress Biomarkers of Rats after 28 Days of Treatment

Administration of 500 and 1000 mg/kg of aqueous and methanol extracts of *D. edulis* Stem bark was observed to have no significant effect on the parameters determined when compared with those in the control animals. However, administration of 2000 mg/kg of aqueous and methanol extracts of *D. edulis* stem bark significantly (p<0.05) altered hepatic, protein, renal, lipid profile and oxidative stress biomarkers when respectively compared with those in the animals in the control group (Tables 4-10).

Table 1: Acute Toxicity of Extracts of *D. edulis* Stem bark

Study Phase/ (Animal)	Dosage of Extract (mg/kg) b.w	No of Rats per Group	No. of Death Recorded	% Mortality
Phase one				
A	10 mg/kg AQSB	3	0	0
B	100 mg/kg AQSB	3	0	0
C	1000 mg/kg AQSB	3	0	0
D	10 mg/kg MTSB	3	0	0
E	100 mg/kg MTSB	3	0	0
F	1000 mg/kg MTSB	3	0	0
Phase two				
A	1600 mg/kg AQSB	3	0	0
B	2000 mg/kg AQSB	3	0	0
C	5000 mg/kg AQSB	3	0	0
D	1600 mg/kg MTSB	3	0	0
E	2000 mg/kg MTSB	3	0	0
F	5000 mg/kg MTSB	3	0	0

Table 2: Effect of Extracts of *Dacryodes edulis* Stem bark on Animal Body Weight after 28 days of Treatment

Groups	Initial Weight (g)	Final Weight (g)	Weight Gain (g)
Control	147.11±11.62	170.19±8.22	23.08±3.4[a]
AQSB 500 mg/kg	146.18±11.09	170.11±7.22	23.93±3.87[a]
AQSB 1000 mg/kg	145.12±10.3	169.02±15.20	23.90±4.78[a]
AQSB 2000 mg/kg	146.23±9.02	169.23±15.11	23.00±6.07[a]
MTSB 500 mg/kg	145.25±7.11	168.31±9.05	23.06±1.94[a]
MTSB 1000 mg/kg	147.32±8.4	170.14±4.20	22.82±4.2[a]
MTSB 2000 mg/kg	147.34±12.22	169.00±17.13	21.66±4.91[a]

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = Aqueous extract of *D. edulis* Stembark, MTSB = Methanol extract of *D. edulis* Stembark,

Table 3: Effect of Extracts of *Dacryodes edulis* Stembarkon Relative Organ Weight (%) of Animals after 28 days of Treatment

Groups	Heart (%)	Liver (%)	Kidneys (%)
Control	0.0036±0.0003[a]	0.030±0.006[a]	0.0031±0.0002[a]
AQSB 500 mg/kg	0.0037±0.0005[a]	0.033±0.005[a]	0.0032±0.0004[a]
AQSB 1000 mg/kg	0.0038±0.0004[a]	0.066±0.0003[b]	0.0034±0.0005[a]
AQSB 2000 mg/kg	0.0039±0.0004[a]	0.075±0.003[c]	0.0085±0.0003[b]
MTSB 500 mg/kg	0.0032±0.0002[a]	0.033±0.002[a]	0.0031±0.0002[a]
MTSB 1000 mg/kg	0.0034±0.0001[a]	0.036±0.0005[a]	0.0033±0.0003[a]
MTSB 2000 mg/kg	0.0037±0.00033[a]	0.089 ±0.002[d]	0.0098±0.0003[c]

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = Aqueous extract of *D. edulis* Stembark, MTSB = Methanol extract of *D. edulis* Stembark,

Table 4: Effect of *D. edulis* Stembark on Plasma Liver Biomarkers of Rats after 28 Days of Treatment

Treatment Groups	AST(U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	T. BIL (mg/dL)
Control	34.01 ± 0.78[a]	19.78 ± 0.34[a]	43.58 ±1.38[ab]	1.16 ± 0.00[a]	16.51 ± 0.00[a]	0.607±0.014[a]
AQSB 500 mg/kg	35.06 ± 0.11[a]	17.89 ± 0.80[a]	47.72 ± 2.65[b]	1.16 ± 0.00[a]	16.51 ± 0.00[a]	0.640±0.117[a]
AQSB 1000 mg/kg	33.26 ± 0.82[a]	18.93 ± 0.70[a]	43.58 ±4.73[ab]	1.16 ± 0.00[a]	16.51 ± 0.00[a]	0.591±0.042[a]
AQSB 2000 mg/kg	62.33 ± 1.65[b]	46.92 ± 0.53[b]	87.90 ± 1.94[d]	4.32 ± 0.01[b]	33.33 ± 0.01[b]	3.589±0.073[b]
MTSB 500 mg/kg	34.38 ±0.46[a]	19.92± 4.07[a]	40.81 ± 3.48[a]	1.16 ± 0.00[a]	16.51 ± 0.00[a]	0.581±0.108[a]
MTSB 1000 mg/kg	32.09 ± 0.82[a]	19.15 ± 0.36[a]	42.89 ±3.57[ab]	1.16 ± 0.00[a]	16.51 ± 0.00[a]	0.551±0.0624[a]
MTSB 2000 mg/kg	71.50 ± 1.67[c]	48.97 ± 0.53[b]	76.60 ± 4.36[c]	5.32 ± 0.01[c]	30.33 ±0.01[b]	4.467±0.091[c]

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = Aqueous extract of *D. edulis* Stembark, MTSB = Methanol extract of *D. edulis* Stembark, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase, GGT = Gamma-Glutamyl Transferase, LDH = Lactate Dehydrogenase, T. BIL = Total Bilirubin.

Table 5: Effect of Extracts of *D. edulis* Stembark on Plasma Protein of Rats after 28 Days of Treatment.

GROUP	Total Protein (mg/dL)	Albumin(mg/dL)	Globulin(mg/dL)	Albumin/ Globulin Ratio
Control	**7.41±0.83^{a}**	**3. 79±0.028^{a}**	**3.62±0.80^{a}**	**1.05±0.60^{c}**
AQSB 500 mg/kg	7.49±0.58a	3.99±0.026^{a}	3.5±0.44^{a}	1.14±0.32^{cd}
AQSB 1000 mg/kg	7.40±0.58^{a}	3. 70±0.035^{a}	3.71±0.10^{a}	1.00±0.43^{c}
AQSB 2000 mg/kg	17.55±0.75^{b}	8.44±0.040^{c}	9.11±0.15^{b}	0.92±0.8^{b}
MTSB 500 mg/kg	7.33±0.66^{a}	3. 55±0.025^{a}	3.78±0.18^{a}	0.94±0.22^{b}
MTSB 1000 mg/kg	7.27±0.44^{a}	3.68±0.055^{a}	3. 59±0.54^{a}	1.03±0.31^{c}
MTSB 2000 mg/kg	18.10±1.13^{c}	7.65±0.047^{b}	10.45±0.04^{c}	0.73±0.54^{a}

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = Aqueous extract of *D. edulis* stem bark, MTSB = Methanol extract of *D. edulis* stem bark.

Table 6: Effect of Extracts *D. edulis* Stem bark on Plasma Renal Biomarkers of Rats after 28 Days of Treatment

Treatment Groups	Urea (mg/dL)	Creatinine (mg/dL)
Control	26.73±2.61^{a}	0.70±0.11^{a}
AQSB 500 mg/kg	24.99±1.17^{a}	0.82±0.06^{a}
AQSB 1000 mg/kg	25.74±3.77^{a}	0.74±0.15^{a}
AQSB 2000 mg/kg	41.48±1.33^{b}	1.94±0.084^{b}
MTSB 500 mg/kg	24.43±3.10^{a}	0.74±0.10^{a}
MTSB 1000 mg/kg	27.77±1.05^{a}	0.80±0.15^{a}
MTSB 2000 mg/kg	57.03±0.41^{c}	1.93±0.19^{b}

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = aqueous extract of *D. edulis* stem bark, MTSB = Methanol extract of *D. edulis* stem bark.

Table 7: Effect of Extracts of *D. edulis* Stem bark on Plasma Lipid Profile of Rats after 28 Days of Treatment.

Treatment Groups	TC (mg/dL)	TAG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	HDL/LDL RATIO
Control	54.33±5.58^{a}	59.84±5.90^{a}	23.25±1.42^{a}	33.88±2.90^{b}	11.97±0.33^{a}	1.46±0.004^{cd}
AQSB 500	54.96±6.66^{a}	57.71±7.62^{a}	24.19±2.05^{a}	36.66±2.76^{b}	11.54±0.23^{a}	1.52±0.007^{d}
AQSB 1000	51.27±3.09^{a}	62.79±3.71^{ab}	22.97±0.74^{a}	35.28±1.09^{b}	12.96±0.75^{ab}	1.54±0.003^{d}
AQSB 2000	79.24±2.56^{b}	91.97±2.48^{c}	58.60±1.18^{b}	13.91±1.62^{a}	18.39±0.56^{c}	0.24±0.001^{ab}
MTSB 500	57.33±4.41^{a}	59.92±4.84^{a}	24.87±1.28^{a}	35.12±2.91^{b}	11.98±0.43^{a}	1.41±0.009^{cd}
MTSB 1000	57.10±4.32^{a}	60.34±4.92^{ab}	23.01±2.14^{a}	35.37±2.77^{b}	12.07±1.05^{a}	1.54±0.002^{d}
MTSB 2000	77.26±2.80^{b}	98.18±2.13^{c}	61.40±1.54^{c}	11.70±1.40^{a}	19.64±0.29^{d}	0.19±0.005^{a}

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = aqueous extract of *D. edulis* stem bark, MTSB = Methanol extract of *D. edulis* stem bark, TC = Total Cholesterol, TAG = Triacylglycerol, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, VLDL = Very Low Density Lipoprotein.

Table 8: Effect of Extracts of *D. edulis* Stem bark on Oxidative Stress Biomarkers in the Liver of Rats after 28 Days of Treatment.

Treatment Groups	GSH (units/gwet tissue)	CAT (units/gwet tissue)	SOD (units/gwet tissue)	MDA (units/gwet tissue)	GPx (units/gwet tissue)
Control	0.31±0.04^{c}	0.002±0.0002^{a}	0.040±0.002^{a}	0.0251±0.004^{a}	0.0219±0.005^{ab}
AQSB 500 mg/kg	0.33±0.007^{c}	0.002±0.0003^{a}	0.041±0.002^{a}	0.0252±0.003^{a}	0.0219±0.01^{a}
AQSB 1000 mg/kg	0.31±0.005^{c}	0.002±0.0003^{a}	0.041±0.002^{a}	0.0253±0.002^{a}	0.0222±0.002^{b}
AQSB 2000 mg/kg	0.18±0.04^{b}	0.004±0.0004^{b}	0.62±0.0005^{b}	0.0282±0.003^{b}	0.0246±0.003^{c}
MTSB 500 mg/kg	0.32±0.04^{c}	0.002±0.0002^{a}	0.040±0.003^{a}	0.0250±0.004^{a}	0.0218±0.003^{a}
MTSB 1000 mg/kg	0.30±0.05^{c}	0.002±0.0004^{a}	0.040±0.001^{a}	0.0251±0.002^{a}	0.0220±0.00^{b}
MTSB 2000 mg/kg	0.13±0.02^{a}	0.004±0.0003^{b}	0.061±0.002^{b}	0.0283±0.003^{b}	0.0245±0.005^{c}

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = aqueous extract of *D. edulis* stem bark, MTSB = Methanol extract of *D. edulis* stem bark, GSH = Glutathione, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase.

Table 9: Effect of Extracts *D. edulis* Stembark on Oxidative Stress Biomarkers in the Kidneys of Rats after 28 Days of Treatment.

Treatment Groups	GSH (units/gwet tissue)	CAT (units/gwet tissue)	SOD (units/gwet tissue)	MDA (units/gwet tissue)	GPx (units/gwet tissue)
Control	0.31 ± 0.02^{b}	0.0024 ± 0.0004^{a}	0.0222 ± 0.002^{a}	0.0284 ± 0.004^{a}	0.0334 ± 0.002^{a}
AQSB 500 mg/kg	0.33 ± 0.005^{b}	0.0025 ± 0.0003^{a}	0.0223 ± 0.001^{a}	$0.0285\pm.0006^{a}$	0.0331 ± 0.003^{a}
AQSB 1000 mg/kg	0.34 ± 0.007^{b}	0.0027 ± 0.00005^{a}	0.0222 ± 0.004^{a}	0.0286 ± 0.003^{ab}	0.0331 ± 0.0005^{a}
AQSB 2000 mg/kg	0.11 ± 0.03^{a}	0.0038 ± 0.0004^{b}	0.0246 ± 0.004^{b}	0.0322 ± 0.002^{c}	0.0368 ± 0.003^{b}
MTSB 500 mg/kg	0.35 ± 0.005^{b}	0.0026 ± 0.0003^{a}	0.0224 ± 0.003^{a}	0.0281 ± 0.003^{a}	0.0332 ± 0.002^{a}
MTSB 1000 mg/kg	0.31 ± 0.02^{b}	0.0022 ± 0.0003^{a}	0.0223 ± 0.002^{a}	0.0287 ± 0.002^{ab}	0.0334 ± 0.003^{a}
MTSB 2000 mg/kg	0.14 ± 0.02^{a}	0.00391 ± 0.0001^{b}	0.0247 ± 0.002^{b}	0.0325 ± 0.005^{c}	0.0369 ± 0.002^{b}

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

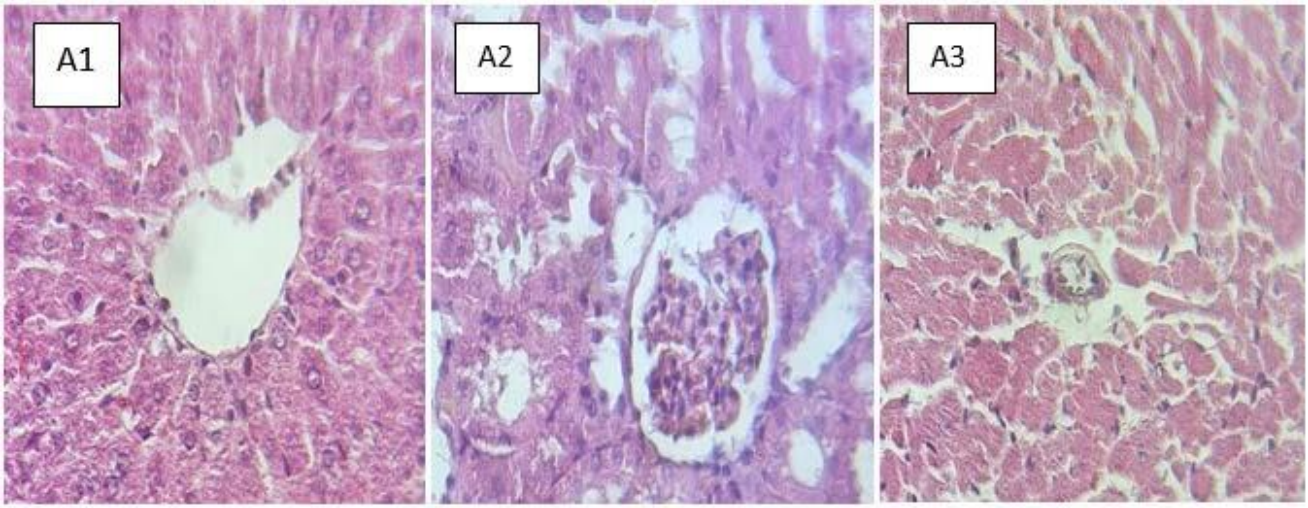
Legend: AQSB = aqueous extract of *D. edulis* stem bark, MTSB = Methanol extract of *D. edulis* stem bark, GSH = Glutathione, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase.

Table 10: Effect of Extracts of *D. edulis* Stem bark on Oxidative Stress Biomarkers in the Heart of Rats after 28 Days of Treatment.

Treatment Groups	GSH (units/gwet tissue)	CAT (units/gwet tissue)	SOD (units/gwet tissue)	MDA (units/gwet tissue)	GPx (units/gwet tissue)
Control	0.34 ± 0.04^{b}	0.0033 ± 0.0003^{a}	0.0270 ± 0.0006^{a}	0.0342 ± 0.004^{a}	0.0282 ± 0.002^{a}
AQSB 500 mg/kg	0.33 ± 0.006^{b}	0.0035 ± 0.0001^{a}	0.0272 ± 0.004^{a}	$0.0340\pm.003^{a}$	0.0283 ± 0.004^{a}
AQSB 1000 mg/kg	0.32 ± 0.002^{b}	0.0036 ± 0.0002^{a}	0.0271 ± 0.003^{a}	0.0343 ± 0.002^{a}	0.0280 ± 0.001^{a}
AQSB 2000 mg/kg	0.12 ± 0.04^{a}	0.0056 ± 0.00005^{b}	0.0331 ± 0.003^{b}	0.0382 ± 0.003^{b}	0.0330 ± 0.002^{c}
MTSB 500 mg/kg	0.32 ± 0.006^{b}	0.0034 ± 0.0003^{a}	0.0269 ± 0.004^{a}	0.0341 ± 0.004^{a}	0.0280 ± 0.004^{a}
MTSB 1000 mg/kg	0.35 ± 0.007^{b}	0.0035 ± 0.0002^{a}	0.0272 ± 0.0005^{a}	0.0344 ± 0.0005^{a}	0.0284 ± 0.0006^{ab}
MTSB 2000 mg/kg	0.15 ± 0.005^{a}	0.0052 ± 0.0004^{b}	0.0328 ± 0.002^{b}	0.0385 ± 0.0005^{b}	0.0332 ± 0.002^{c}

Results are presented as mean ± SEM with n = 5. Values with different superscripts along the same column are significantly different at p<0.05.

Legend: AQSB = aqueous extract of *D. edulis* stem bark, MTSB = Methanol extract of *D. edulis* stem bark, GSH = Glutathione, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase.



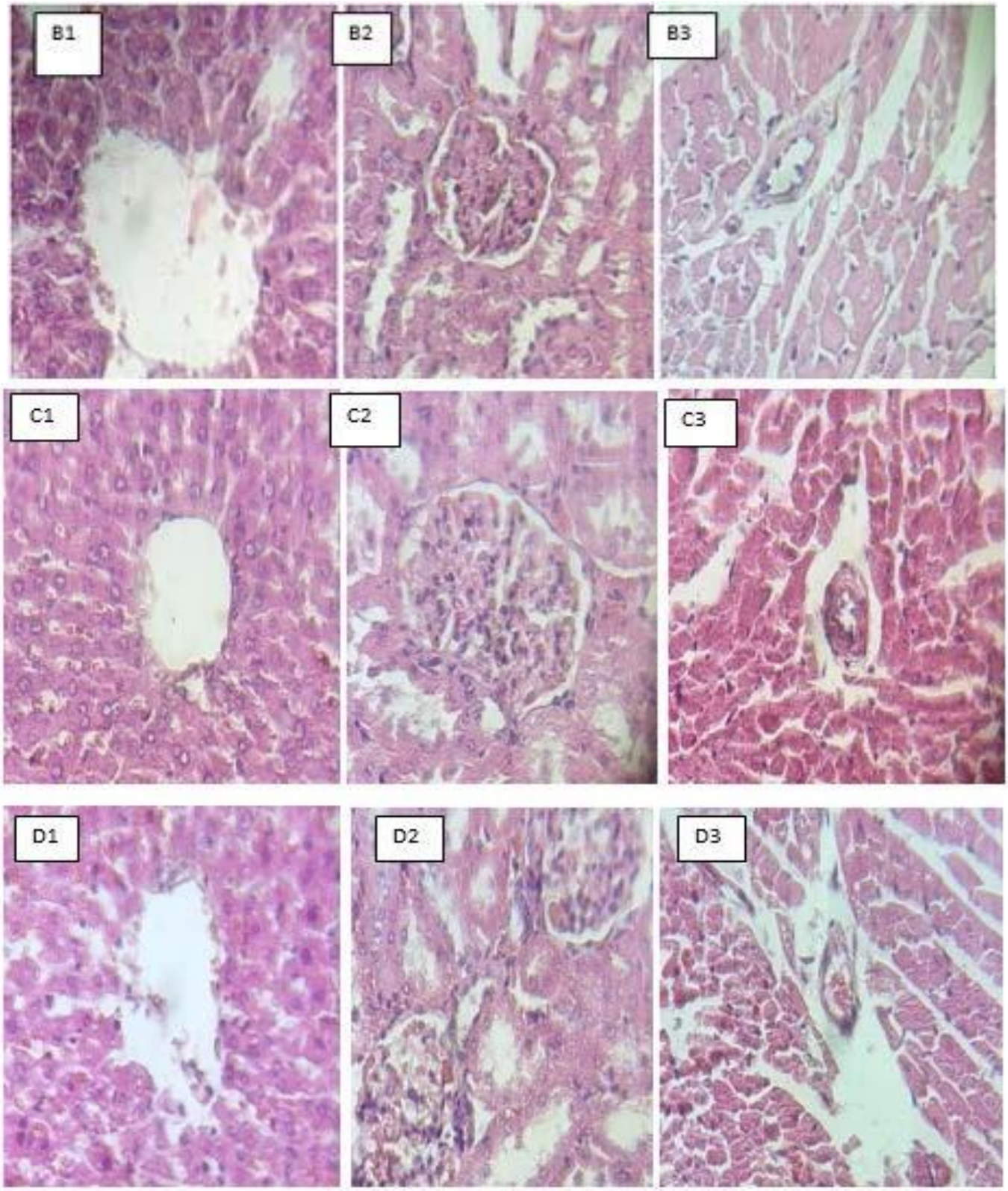


Plate 4.1: Sections of rat's liver, kidney and heart (A1-A3) Control liver, kidney and heart rat respectively, (B1-B3) rat liver, kidney and heart respectively administered 500 mg/kg b.w aqueous extract of *D edulis* stem bark, (C1-C3) rat liver, kidney and heart respectively administered 1000 mg/kg b.w aqueous extract of *Dacoydes edulis* stem bark, (D1-D3) rat liver, kidney and heart respectively administered 2000 mg/kg b.w aqueous extract of *Dacoydes edulis* stem bark (Mag 100x).

Sections of the tissues were stained with heamatoxylin and eosin and viewed under microscope. Rat group A,B and C administered with control, 500 and 1000 mg/kg b.w respectively reveals liver visible centriole with the hepatocytes and nucleus and a well fenestrated sinusoidal. Kidney visible renal corpuscle and interstitial space and tubules and Heart composed of bundles of

myocardial fibres cells, interstitial space and visible coronary artery while Rat group D1-D3 administered with 2000 mg/kg b.w reveals; liver visible centriole with the hepatocytes revealing visible mild vacuolated nucleus and mild steatosis, Kidney visible distorted renal corpuscle and interstitial space and mildly necrosed tubules and Heart composed of bundles of lose myocardial fibres cells, interstitial space and visible not so prominent coronary artery.

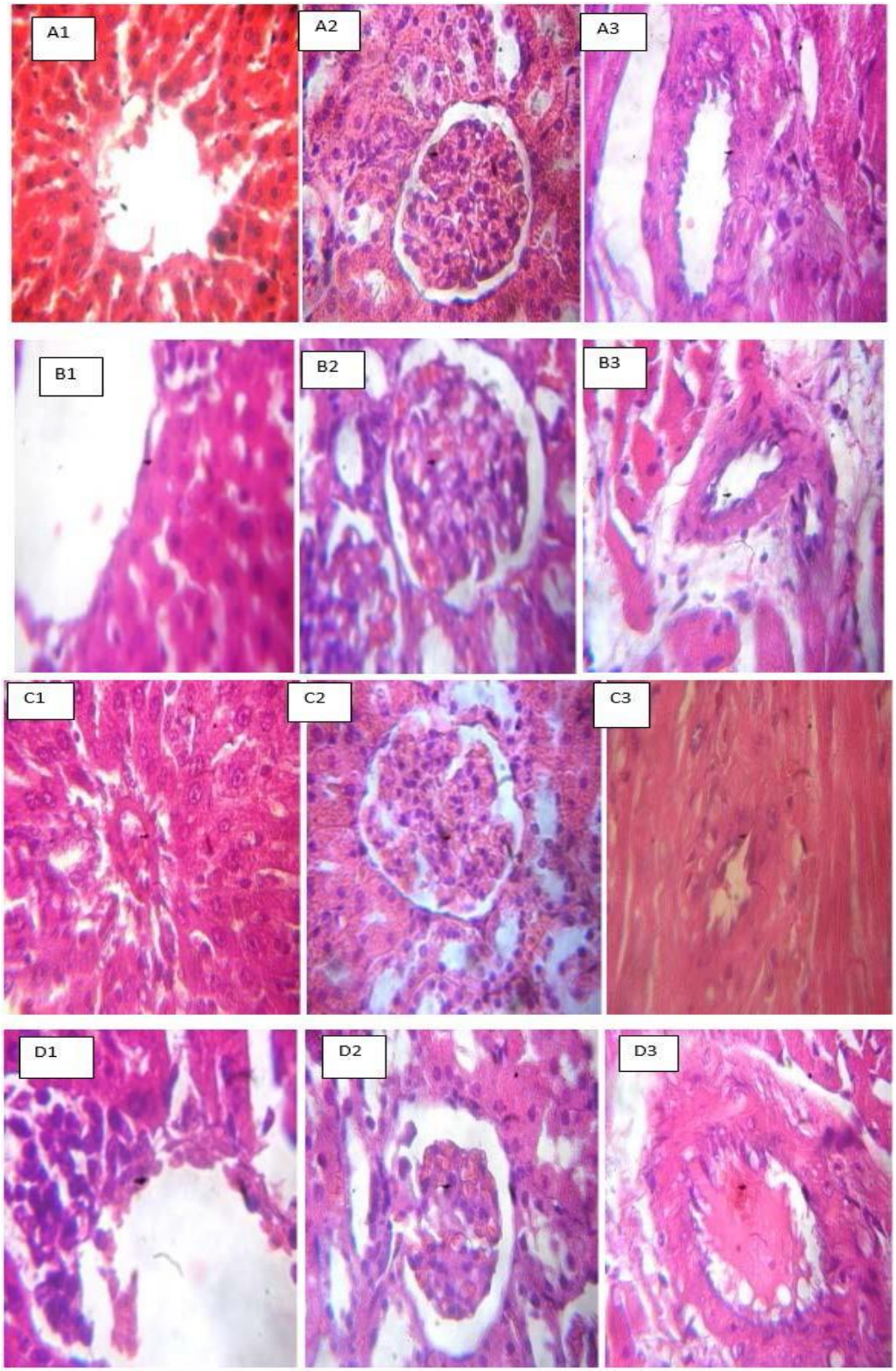


Plate 4.2: Sections of rat's liver, kidney and heart (A1-A3) Control liver, kidney and heart rat respectively, (B1-B3) rat liver, kidney and heart respectively administered 500 mg/kg b.w methanol extract of *D edulis* stembark, (C1-C3) rat liver, kidney and heart respectively administered 1000 mg/kg b.w methanol extract of *Dacoydes edulis* stembark, (D1-D3) rat liver, kidney and heart respectively administered 2000 mg/kg b.w methanol extract of *Dacoydes edulis* stembark (Mag 100x).

Sections of the tissues were stained with heamatoxylin and eosin and viewed under microscope. Rat group A, B and C administered with control, 500 and 1000 mg/kg b.w respectively reveals liver visible centriole with the hepatocytes and nucleus and a well fenestrated sinusoidal. Kidney visible renal corpuscle and interstitial space and tubules and Heart composed of bundles of myocardial fibres cells, interstitial space and visible coronary artery while Rat group D1-D3 administered with 2000 mg/kg b.w reveals; liver visible centriole with the hepatocytes revealing visible mild vacuolated nucleus and mild steatosis, Kidney visible distorted renal corpuscle and interstitial space and mildly necrosed tubules and Heart composed of bundles of lose myocardial fibres cells, interstitial space and visible not so prominent coronary artery.

Discussion

Lethal dose (LD_{50}) is a quantitative index of acute toxicity, which is usually determined in the preliminary step of evaluating the safety/toxicity of drugs, compounds, and medicinal plants [25]. It provides information on the nature of toxicity and the basis for the classification and dosage design of a substance or drug [26]. The acute toxicity of *Dacryodes edulis* in this study showed that the LD_{50} of its aqueous extract of stem bark (AQSB) and methanolic extract of stem bark (MTSB) is greater than 5000 mg/kg as no mortality was recorded at this dose except for a decline in the normal activity of the animals administered 5000 mg/kg dose (Table 1). This might implies that the plant extract is relatively safe and harmless. This is consistent with the study of Ononamadu *et al*., [27] who reported that the LD_{50} of methanolic leaves extract of *D. edulis* is greater than 5000 mg/kg. Yelwa *et al*., [28] has also reported that the LD_{50} of methanolic leaves extract of *D. edulis* is greater than 4000 mg/kg. According to Kennedy *et al.* [29], any substance with LD_{50} greater than 5000 mg/kg by the oral route is regarded as safe and practically harmless. The administered graded doses of the aqueous and methanolic extracts of *D. edulis* did not result in lethality over the 24-hour period. No death and latent toxicity was observed in the animals after keeping them for extra 14 days. Hence, the acute toxicological results showed that the plant is relatively safe. Liver is the main target for every xenobiotic that gains entry to the system. It is an organ charged with metabolism and biotransformation of foreign compounds in animals. Some of these foreign compounds may trigger injury and releases liver enzymes and proteins in magnitude higher than basal level [30]. An indicative injury on hepatocytes is a significant alterations (particularly increase) in the activities of serum liver function enzymes (ALT, AST, and ALP), particularly ALT [30]. Serum proteins such as albumin are synthesized by the liver and the relative level could give information on the synthetic capability of the liver and a decline in serum level of albumin usually indicates a perturbation in the capacity of the liver to synthesize proteins [31]. Any rise in this level shows anomalies in glomerular filtration or kidney function [26]. Creatinine level is a good index of glomerular filtration rate while urea level is linked with kidney diseases and urinary tract problems. In this study, it was observed that both the aqueous and methanol stembark extracts at 250, 500 and 1000 mg/kg body weight for all parameters measured had no significant effect when compared with those in animals of the normal control group (Tables 4-10). This is similar to the findings of Ononamadu *et al*., [27] who reported a nonsignificant difference in hepatic and renal indices in animals administered up to 600 mg/kg body weight of *D. edulis*. It is also consistent with the findings of Efosa *et al*., [50] who reported a nonsignificant difference in hepatic and renal indices in animals administered 1000 mg/kg body weight of *D. edulis* seed oil. This is suggestive that the extract is not toxic to the tissues at a dose up to 1000 mg/kg. However, at a higher dose of 2000 mg/kg body weight, there was a significant (p<0.05) increase in hepatic and renal indices when compared with those in the control group (Tables 4 and 6 respectively). This might be an indication that the extracts cause an alteration in hepatic and renal biomarkers thus compromising the integrity of the liver and kidney at this dose. Previous studies have reported that elevated levels of hepatic and renal biomarkers in the blood results from injury or damage of these tissues [33-37].

Administration of 2000 mg/kg of extract to animals resulted in a significant difference in total protein, albumin and globulin when compared with those in the control group (Table 5). This implies that there is abnormal accumulation of protein in the blood. This might also mean that the extracts at this dose increased the efflux of protein from the liver into the blood since it is mainly synthesized in the liver. It is also possible that loss of liver proteins and the need for enzyme involved with the metabolism of the components of the extract may also have contribute to increase protein synthesis which ultimately leaked out of the liver due to liver damage. Therefore, the elevation of protein in animals exposed to 2000mg/kg of extract of *D. edulis* in this study is an indication that the extracts interfere with the interity of the liver. Furthermore, there was a significant (p<0.05) increase in the lipid profile of animals treated with 2000 mg/kg when compared with those in the control group. This is suggestive that the extract at this dose might induce dyslipidemia. Increase in lipids has been implicated in cardiovascular diseases [38, 39]. Thus, administration of 2000 mg/kg of the extract might enhance dyslipidemia. Several researchers had reported that lower doses of stem bark extracts of *D. edulis* possess hypolipidemic potential [40, 41].

Results from this study showed that 2000 mg/kg of aqueous and methanolic extracts of *D. edulis* stem bark caused a significant decrease in the hepatic, renal and heart concentrations of glutathione (GSH) when compared with those in the normal control group. The extract at this dose might have generated free radical which glutathione tends to combat thereby reducing its concentration [42-45]. Similarly, the extracts at this dose was observed to increase lipid peroxidation and activities of antioxidant enzymes (CAT, SOD and GPx) in experimental animals when compared with the untreated group. This is an indication of the toxicity of the extract at 2000 mg/kg body weight in the treated animals. The extract at this dose might have increased the generation of free radical in treated animals which the antioxidant enzymes tend to combat, thus increasing their activities. The significant increase in the activity of GPx at 2000 mg/kg extract might not be unconnected with the significant reduction in the concentration of GSH at this dose. The oxidation of GSH to GSSG is catalyzed by GPx [46]. Therefore, the reduced level of GSH in this study is an indication that its conversion to GSSG has increased due to toxicity, thus increasing the activity of GPx. Previous studies have reported that leaves and fruits of *D. edulis* possess antioxidant potential [47, 48].

Conclusion

The result of this study showed that both aqueous and methanol extracts of *D. edulis* stem bark are not toxic at a dose of 1000 mg/kg and below. However, a dose of 2000 mg/kg elicited toxic effects on the measured parameters. Thus, its consumption at high dose should be discouraged.

Consent

It is not applicable.

Ethical Disclaimer

Animal ethic is according with animal ethic University of Benin, Benin City. Edo State. Nigeria.

References

1. Ashoush IS, El-Batawy OI, El-Shourbagy GA. Antioxidant activity and hepatoprotective effect of pomegranate peel and whey powders in rats. Ann Agri Sci,2013:58:27-32.
2. Huo HZ, Wang B, Liang YK, Bao YY, Gu Y. Hepatoprotective and antioxidant effects of licorice extract against CCl_4-induced oxidative damage in rats. Int J Mol Sci,2011:12:6529-6543.
3. Airaodion AI, Ogbuagu EO, Ekenjoku JA, Ogbuagu U, Airaodion EO. Therapeutic effect of methanolic extract of *Telfairia occidentalis* leaves against acute ethanol-induced oxidative stress in Wistar rats. International Journal of Bio-Science and Bio-Technology,2019:11(7):179-189.
4. Madrigal-Santillan E, Madrigal-Bujaidar E, Alvarez-Gonzalez I, Sumaya-Martinez MT, Gutierrez-Salinas J. Review of natural products with hepatoprotective effects. World Journal of Gastroenterology,2014:20:14787-14804.
5. Pazhayattil GS, Shirali AC. Drug-induced impairment of renal function. Int J NephrolRenovasc Dis,2014:12:457-468.
6. Leakey R, Atangana E, Kengnni A. Characterization of genetic variation and Domestication of *Dacryodes edulis* in West and Central Africa. Journal of Tree livelihoods,2002:12:57-71.
7. Johns T. Dietary, diversity, global change and human health. Proceeding of the symposium Managing Biodiversity in Agricultural Ecosystems, Nov. 8-10, Montreal, Canada, 2001, 1-11.
8. Agbor GA, Kuate D, Oben JE. Medicinal plants can be good source of antioxidants: Case study in Cameroon. Pak. J. Biol. Sci,2007:10:537-544.
9. Koudou J, Edou P, Obame LC, Bassole IH, Figueredo G. Volatile components, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis* G. don from gabon, J Applied Sci,2008:8:3532-3535.
10. Okwu DE, Nnamdi FU. Evaluation of the chemical composition of *Dacryodes edulis* and Raphiahookerimann and wendlexudates used in herbal medicine in south eastern Nigeria. Afr J Trad CAM,2008:5:194-200.
11. Chimaobi J, Ononamadua B, Alhassan BJ, Aminu IB, Abdullahi A, Imam B *et al*. Toxicological study of aqueous-methanol solvent fraction of methanol extract of *Dacryodes edulis* leaves. Science *Direct* Journal, 2020, 909-918
12. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol,1983:54:275-287.
13. OECD. Organization for Economic Co-operation and Development. Guidelines for the testing of chemicals. Repeated dose 28-day oral toxicity study in rodents, 2008, 407.
14. NAS. Guide for the Care and Use of Laboratory Animals. Eighth Edition, 2011.
15. Reitman S, Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. American Journal of Clinical Pathology,1957:28:56-58.
16. Babson AL, Grecley SJ, Coleman CM, Phillips GE. The use of phenolphthalein in monophosphate as a substrate for serum alkaline phosphatase. Clinical Chemistry,1966:12:482.
17. Royden NR, Alfred P. A New Diazo Method for the Determination of Bilirubin. Clinical Chemistry,1962:8(6):570-578.
18. Tietz NW. Clinical Guide to Laboratory Tests, 3rd Ed. Philadelphia; W.B. Saunders, 1995.
19. Grant GH, Silverman RH. Amino Acids and proteins: fundamentals of clinical chemistry. 3rd edition. WB Saunders, Philadelphia, 1987.
20. Toora BD, Rejagopal G. Measurement of creatinine concentration by Jaffe's Reaction. Indian Journal of Experimental Biology,2002:40(3):352-354.
21. Fesus PD, Pressac M, Braconnier F, Aymard P. Automated determination of urinary Na, K, inorganic phosphate, urea, and creatunine without sample dilution, with the RA-XT. Cliii Chem,1983:35:481-483.
22. Owoade AO, Adetutu A, Airaodion AI, Ogundipe OO. Toxicological assessment of the methanolic leaf extract of *Brideliaferrugelia*. The Journal of Phytopharmacology,2018:7(5):419-424.
23. Owoade AO, Airaodion AI, Adetutu A, Akinyomi OD. Levofloxacin-induced dyslipidemia in male albino rats. Asian Journal of Pharmacy and Pharmacology,2018:4(5):620-629.
24. Airaodion AI, Ogbuagu EO, Ogbuagu U, Adeniji AR, Agunbiade AP, Airaodion EO *et al*. Hepatoprotective effect of *Parkiabiglobosa*on acute ethanol-induced oxidative stress in wistar rats. International Research Journal of Gastroenterology and Hepatology,2019:4(6):1-10.
25. Ping XW, Akowuah GA, Chin JH. Acute oral toxicity study of *Clinacanthusnutans*in mice, Int J Pharm Sci Res,2012:3(11):4202-4205.
26. Zakaria ZA, Rahim HFA, Mohtarrudin N, Kadir AA, Cheema MS, Ahmad Z *et al*. Acute and sub-chronic oral toxicity studies of methanol extract of *Clinacanthusnutans*in mice, Afr. J. Tradit. Complement. Altern. Med,2016:13(2):210-222.
27. Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe AT *et al*. Toxicological study of aqueous-methanol solvent fraction of methanol extract of *Dacryodes edulis* leaves. Toxicology Reports,2020:7:909-918.
28. Yelwa AS, Mshelia HE, Cyril O, Lawal SI, Bature HB, Shamsiya A *et al*. Phytochemical screening, acute toxicity study and evaluation of *in vitro* antimicrobial activities of the fractions of *Dacryodes edulis* against selected clinical bacterial isolates. Journal of Pharmacognosy and Phytochemistry,2017:6(4):1910-1915.

29. Kennedy RC, Henkel RD, Pauletti D, Allan JS, Lee TH, Essex M *et al*. Antiserum to a synthetic peptide recognizes the HTLV-III envelope glycoprotein. Science,1986:231(4745):1556-1559.
30. Ogunmoyole T, Adeyeye RI, Olatilu BO, Akande OA, Agunbiade OJ. Multiple organ toxicity of *Datura stramonium*seed extracts, Toxicology Report,2019:6:983-989.
31. Rasekh HR, Nazari P, Kamli-Nejad M, Hosseinzadeh L. Acute and subchronic oral toxicity of *Galega officinalis* in rats. J. Ethnopharmacol,2008:166:(1) 21-26.
32. Mukhtar M, Muhammad H, Ibrahim S, Ahmad G, Muhammad Y, Abubakar S. Antioxidant and acute toxicity of stem extracts of the *Ficus iteophylla*. Int. J Adv. Chem. Res. 2020;2(1):17-19. DOI: 10.33545/26646781.2020.v2.i1a.18
33. Airaodion AI, Ngwogu KO, Ngwogu AC, Megwas AU, Ekenjoku JA. Nephrotoxicity of Monosodium Glutamate (MSG) in Wistar Rats. International Journal of Advances in Nephrology Research,2020:3(1):1-10.
34. Dr. Kiran Kumar Akka, Dr. Pampa Reddy. Lipid profile in patients of type 2 diabetes mellitus with myocardial infarction. Int J Adv Biochem Res 2021;5(1):14-19. DOI: 10.33545/26174693.2021.v5.i1a.59
35. Airaodion AI, Ene AC, Ogbuagu EO, Okoroukwu VN, Ekenjoku JA, Ogbuagu U *et al*. Biochemical changes associated with consumption (by rats) of "garri" processed by traditional and instant mechanical methods. Asian Journal of Biochemistry, Genetics and Molecular Biology,2019:2(4):1-11.
36. Airaodion AI, Alabi OJ, Ogbuagu EO, Atiba FA, Olawoyin DS. Nephro and hepatotoxic effect of air-freshener in Wistar rats. Asian Journal of Research in Nephrology,2020:3(2):1-9.
37. Airaodion AI, Megwas AU, Ekenjoku JA, Ngwogu KO, Ngwogu AC. Nephro- and Hepato-toxicity of common household insecticides used in Nigeria. International Research Journal of Gastroenterology and Hepatology,2020:3(2):21-28.
38. Airaodion AI, Ogbuagu EO, Ogbuagu U, Awosanya OO, Airaodion EO. Effect of Methanolic extract of *Corchorusolitorius* leaves on hypoglycemic and hypolipidaemic activities in albino rats. Asian Plant Research Journal,2019:2(4):1-13.
39. Airaodion AI, Ogbuagu EO, Airaodion EO, Ogbuagu U, Ekenjoku JA. Antidiabetic effect of ethanolic extract of *Carica papaya* leaves in alloxan-induced diabetic rats. American Journal of Biomedical Science & Research,2019:11(8):93-109.
40. Okolo CA, Ejere VC, Chukwuka CO, Ezeigbo II, Nwibo DD, Okorie AN *et al*. Hexane extract of *Dacryodes edulis* fruits possesses anti-diabetic and hypolipidaemic potentials in alloxan diabetes of rats. African Journal of Traditinal and Complementary Alternative Medicine,2016:13(4):132-144.
41. Ndem JI, Bassey UE, Etim OE. Hypolipidemic potential of raw seeds of *Dacryodes edulis* on Wistar rats. European Journal of Biomedical and Pharmaceutical Sciences,2017:4(6):111-115.
42. Airaodion AI, Ogbuagu EO, Ewa O, Ogbuagu U, Awosanya OO, Adekale OA *et al*. Ameliorative efficacy of phytochemical content of *Corchorusolitorius*leaves against acute ethanol-induced oxidative stress in Wistar rats. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2019:2(2):1-10.
43. Airaodion AI, Ngwogu KO, Ngwogu AC, Megwas AU, Ekenjoku JA, Awosanya OO *et al*. Common household insecticides used in Nigeria induced oxidative stress in Wistar rats. Asian Journal of Immunology,2020:3(2):39-45.
44. Airaodion AI, Olawoyin DS, Alabi OJ, Atiba FA, Ogbuagu EO. Air freshener induced oxidative stress and its adverse effects on immunity. International Journal of Health, Safety and Environment,2020:6(05):579-587.
45. Megwas AU, Akunne PN, Oladosu NO, Njoku OC, Airaodion AI. Ameliorative potential of Bambara nuts against acute ethanol-induced oxidative stress in Wistar rats. International Journal of Environmental Health and Safety,2021:12(2):1-12.
46. Airaodion AI, Ngwogu AC, Ekenjoku JA, Ngwogu KO. Hepatoprotective potency of ethanolic extract of *Garcinia kola* (Heckel) seed against acute ethanol-induced oxidative stress in Wistar rats. International Research Journal of Gastroenterology and Hepatology,2020:3(2):1-10.
47. Moise MM, Benjamin LM, Etienne M, Thierry G, Ndembe-Dalida K. Intake of GnetumAfricanum and Dacryodes Edulis, Imbalance of Oxidant/Antioxidant Status and Prevalence of Diabetic Retinopathy in Central Africans. *PLoS ONE*,2012:7(12):1-10.
48. Omonhinmin AC, Agbara IU. Assessment of *In vivo* antioxidant properties of Dacryodes edulis and Ficus exasperate as anti-malaria plants. Asian Pacific Journal of Tropical Disease,2013:3(4):294-300.
49. Airaodion AI, Ogbuagu EO, Ewa O, Ogbuagu U, Awosanya OO, Adekale OA, *et al*. Ameliorative efficacy of methanolic extract of Corchorusolitorius leaves against acute ethanol-induced oxidative stress in Wistar rats. Asian Journal of Biochemistry, Genetics and Molecular Biology,2019:7(6):1-9.
50. Efosa JO, Egielewa SJ, Ojei JU, Udoji NJ. Toxicological and biochemical evaluation of oil from the seed of *Dacroydesedulis*. Global Scientific Journal. 2019:7(1):847-863.