



## INVESTIGATION OF THE TOXICITY OF AQUEOUS AND METHANOL EXTRACTS OF *Dacryodes edulis* SEED ON WISTAR RATS

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### Abstract

The aim of this study is to investigate the toxicity of aqueous and methanol extracts of *Dacryodes edulis* seed on Wistar Rats. The seed of *Dacryodes edulis* were locally sourced from a farmer in Olobo area of Benin City, Edo State, Nigeria and were identified by a botanist. Forty-five Wistar rats were divided into 9 groups of 5 rats each. Group A served as control, Groups B, C, D and E were administered 250, 500, 1000 and 2000 mg/kg of aqueous extract of *D. edulis* seed respectively while groups F, G, H and I were administered 250, 500, 1000 and 2000 mg/kg bw of methanol extract of *D. edulis* seed respectively via oral route for 28 days. The result of the acute toxicity of *Dacryodes edulis* seed showed that the LD<sub>50</sub> of its aqueous and methanolic extract 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 250, 500 and 1000 mg/kg of aqueous and methanol extracts of *D. edulis* seed 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* seed significantly ( $p < 0.05$ ) perturbed hepatic, protein, renal, lipid profile and oxidative stress biomarkers when respectively compared with those in control animals. The result of this study showed that both aqueous and methanol extracts of *D. edulis* seed 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* seed, Hepatotoxicity, High dosage, Nephrotoxicity.

### INTRODUCTION

Xenobiotics are absorbed in the body as gaseous substances and corpuscular-insoluble substrates (e.g. heavy metals) and may be present in water-soluble (hydrophilic) or in fat-soluble (lipophilic) form but on arriving to the liver, it must be rendered inactive and harmless thus, capable of being excreted. Change in metabolism is not only that foreign substance is detoxified by the liver but toxic substances after undergoing metabolism in the liver can become toxic. Therefore, toxic metabolites may be produced as a result of dysregulation of cellular metabolic processes which are not metabolites of the parent compound. Moreover, therapeutic active substances are converted in the liver into inactive metabolites (“inactivation”) and some inactive such as prodrugs (e.g. cyclophosphamide), into active metabolites (“activation”). The enzymes responsible for the actions are primarily produced in hepatocytes and mainly divided into two groups phase I and phase II. The phase I enzymes are predominantly from the P-450 family of genes, whose general function is to add polar groups, such as hydroxyl groups, to lipophilic molecules thus rendering them more hydrophilic (Park *et al.*, 1995). The main function of the phase II enzymes are to covalently attach a water-soluble moiety to the polar group added by the phase I enzymes. Usually, such molecules are sugars or peptides, such as glucuronic acid or glutathione. This usually renders the compound less reactive. If the phase II reaction is impaired for some reasons or the phase I reaction is induced, the organism will have excess of reactive molecules from the phase I reaction, which can be harmful. This can take place in the case of drug induced hepatotoxicity, when reactive metabolites of the parent compound are formed, which afterward negatively affects cellular functions (Liu *et al.*, 2004).

*Dacryodes edulis* is the most popular species under the genus *Dacryodes*, which derived its name from the term “edible” emphasizing its nutritional importance (Ajibesin, 2011). The common names include African pear, Bush butter, Bush fruit, Native pear in English; and Ube and Eleme in Southeast and Western Nigeria, respectively (Orwa *et al.*, 2009). In Nigeria, it is widely claimed to have originated from the southeastern part of Nigeria where it is called “ube.” Traditionally, the leaves are chewed with kolanut as an antiemetic, the leaf sap is used as ear drop to treat ear infections, while the decoction is prepared as a remedy for fever and headache (Omonhinmin, 2012). A decoction of the leaves with other plant leaves has been claimed to ameliorate high blood sugar, hypertension, and labor pains (Erukainure *et al.*, 2017). Moise *et al.* (2012) and Longo-Mbenza *et al.* (2012) in 2 different case-control cross-sectional studies reported that intake of the fruits reduced the odds of retinopathy and visual impairment in diabetes, respectively, in the studied populations. Uhunmwangho and Omeregbe (2017) reported that oil extracted from the fruit exerted significant antioxidant activity in Wistar rats challenged with sodium arsenate. Oboh *et al.* (2015) examined comparatively the *in vitro* antioxidant and inhibitory activity of the fruits (roasted vs hot water treatment) against carbohydrate digesting enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase), and lately, Erukainure *et al.* (2017) reported that solvent extracts of the leaves inhibited  $\alpha$ -glucosidase, pancreatic lipases, pancreatic ATPase, and glucose-6-phosphatase activities and exerted significant antioxidant effect on Fe<sup>2+</sup>-challenged (oxidatively) pancreatic and liver tissues. Okolo *et al.* (2016) have also reported that the hexane extract of the fruits reduced hyperglycemia in alloxan challenged Wistar rats. The studies thus far suggest that the plant has antioxidant and hypoglycemic potential; however, there are still some knowledge gaps. Till date, there is still a dearth of extensive study on the seed of this plant. This present study therefore sought to investigate the toxicity of both aqueous and methanol extracts of *D. edulis* seed in Wistar rats.

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## MATERIALS AND METHODS

**Collections and Identification of Plant Materials:** The seed of *Dacryodes edulis* were locally sourced from a farmer in Olobo area of Benin City, Edo State, Nigeria and were identified by a botanist. 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 72 hours with regular stirring, followed by sieving through a cheese cloth and concentration using a freeze drier.

**Acute Toxicity Study:** Lorke (1983) 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* seed, while those in groups D, E and F were administered 10, 100 and 1000 mg/kg methanol extract of *D. edulis* seed 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, 2000 and 5000 mg/kg aqueous extract of *D. edulis* seed, while those in groups D, E and F were administered 1600, 2000 and 5000 mg/kg methanol extract of *D. edulis* seed 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 (2008) which encourages the use of few numbers of animals by limiting the use of experimental groups. Forty-five (45) 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 humane 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 nine (9) groups of five (5) rats each. Group A served as control in which rats were given only feed and water. Groups B, C and D were administered 250, 500, 1000 and 2000 mg/kg body weight of aqueous extract of *D. edulis* seed respectively while groups E, F and G were administered 250, 500, 1000 and 2000 mg/kg bw of methanol extract of *D. edulis* seed 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 (1957). Alkaline Phosphatase (ALP) activity was determined by

Phenolphthalein Monophosphate method described by Babson *et al.* (1966). Total bilirubin concentration was determined by diazo method described by Royden and Alfred (1962).

**Determination of Plasma Protein:** Total protein concentration was determined by the method of Tietz (1995). Albumin concentration was determined by the method of Grant *et al.* (1987). 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 (2002). Urea concentration was determined using a Randox Commercial Kit based on the methods of Fesus *et al.* (1983).

**Determination of Lipids:** Lipids were extracted and determined according to previously described methods (Owoade *et al.*, 2018a, b).

**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.* (2019a).

### Statistical Analysis

Results are expressed as mean  $\pm$  standard error of the mean (SEM). The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 6.00 and P values  $< 0.05$  were considered statistically significant.

## RESULTS

**Acute Toxicity of *D. edulis* Seed Extracts:** The result of the acute toxicity of *Dacryodes edulis* seed in this study showed that the LD<sub>50</sub> of its aqueous extract of seed (AQSD) and methanolic extract of seed (MTSD) 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* Seed on Animal Body Weight after 28 days of Treatment:** Both aqueous and methanol extracts of *D. edulis* seed were observed to significantly reduce the weight gained by animals after 28 days of treatment when compared with those in the control group as presented in table 2. The results of the extracts on relative organ weight is presented in Table 3.

**Effect of Extracts of *D. edulis* Seed 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* Seed 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* seed 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 (Tables 4-10).

Table 1. Acute Toxicity of Extracts of *D. edulis* Seed

Study Phase/ (Animal)	Dosage of Extract (mg/kg) b.w	No of Rate per Group	No. of Death Recorded	% Mortality
<b>PHASE ONE</b>				
A	10 mg/kg AQSD	3	0	0
B	100 mg/kg AQSD	3	0	0
C	1000 mg/kg AQSD	3	0	0
D	10 mg/kg MTSD	3	0	0
E	100 mg/kg MTSD	3	0	0
F	1000 mg/kg MTSD	3	0	0
<b>PHASE TWO</b>				
A	1600 mg/kg AQSD	3	0	0
B	2000 mg/kg AQSD	3	0	0
C	5000 mg/kg AQSD	3	0	0
D	1600 mg/kg MTSD	3	0	0
E	2000 mg/kg MTSD	3	0	0
F	5000 mg/kg MTSD	3	0	0

Table 2. Effect of Extracts of *Dacryodes edulis* Seed on Animal Body Weight after 28 days of Treatment

Groups	Initial Weight (g)	Final Weight (g)	Weight Gain (g)
Control	145.10±8.82	170.30±11.48	25.20±2.65 <sup>b</sup>
AQSD 250 mg/kg	146.44±8.19	168.40±6.60	21.96±1.64 <sup>a</sup>
AQSD 500 mg/kg	147.28±8.19	165.13±9.15	17.85±0.98 <sup>a</sup>
AQSD 1000 mg/kg	145.33±9.19	162.29±11.28	16.96±2.08 <sup>a</sup>
AQSD 2000 mg/kg	149.23±3.01	165.35±8.83	16.12±5.81 <sup>a</sup>
MTSD 250 mg/kg	148.23±8.19	170.30±6.60	22.07±1.04 <sup>a</sup>
MTSD 500 mg/kg	145.18±8.19	168.53±9.15	23.35±0.95 <sup>a</sup>
MTSD 1000 mg/kg	146.22±9.19	167.09±11.28	20.87±2.08 <sup>a</sup>
MTSD 2000 mg/kg	149.43±3.01	170.05±8.83	20.62±5.83 <sup>a</sup>

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:** AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed

Table 3. Effect of Extracts of *Dacryodes edulis* Seed on Relative Organ Weight (%) of Animals after 28 days of Treatment

Groups	Heart (%)	Liver (%)	Kidney (%)
Control	0.0038±0.0003 <sup>a</sup>	0.029±0.009 <sup>a</sup>	0.0030±0.0001 <sup>a</sup>
AQSD 250 mg/kg	0.0039±0.0008 <sup>a</sup>	0.030±0.0012 <sup>a</sup>	0.0034±0.0005 <sup>b</sup>
AQSD 500 mg/kg	0.0038±0.0005 <sup>a</sup>	0.036±0.005 <sup>b</sup>	0.0035±0.0004 <sup>bc</sup>
AQSD 1000 mg/kg	0.0037±0.0004 <sup>a</sup>	0.031±0.0006 <sup>a</sup>	0.0035±0.0007 <sup>bc</sup>
AQSD 2000 mg/kg	0.0040±0.0004 <sup>a</sup>	0.034 ±0.003 <sup>ab</sup>	0.0033±0.0006 <sup>b</sup>
MTSD 250 mg/kg	0.0033±0.0003 <sup>a</sup>	0.034±0.0022 <sup>ab</sup>	0.0032±0.0002 <sup>ab</sup>
MTSD 500 mg/kg	0.0035±0.0005 <sup>a</sup>	0.036±0.005 <sup>b</sup>	0.0035±0.0004 <sup>bc</sup>
MTSD 1000 mg/kg	0.0036±0.0004 <sup>a</sup>	0.039±0.0006 <sup>c</sup>	0.0037±0.0003 <sup>cd</sup>
MTSD 2000 mg/kg	0.0038±0.00033 <sup>a</sup>	0.040 ±0.0042 <sup>c</sup>	0.0039±0.0004 <sup>d</sup>

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:** AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed

Table 4. Effect of Extracts of *D. edulis* Seed on Plasma Hepatic Biomarkers of Rats after 28 Days of Treatment

Groups	AST(U/L)	ALT (U/T)	ALP (U/L)	GGT (U/L)	LDH	T. BIL
Control	33.02 ± 1.85 <sup>ab</sup>	20.53 ± 2.09 <sup>a</sup>	45.67±2.07 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	17.23± 1.39 <sup>a</sup>	0.58±0.016 <sup>a</sup>
AQSD 250 mg/kg	31.78±0.98 <sup>a</sup>	21.07±1.06 <sup>a</sup>	45.09±1.14 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	17.73± 2.30 <sup>a</sup>	0.57±0.036 <sup>a</sup>
AQSD 500 mg/kg	34.83 ± 1.12 <sup>b</sup>	21.54± 3.01 <sup>a</sup>	46.59± 3.02 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	17.40 ± 3.37 <sup>a</sup>	0.61±0.022 <sup>a</sup>
AQSD 1000 mg/kg	31.27± 0.70 <sup>a</sup>	21.42 ± 3.20 <sup>a</sup>	44.96±1.38 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	17.80 ± 2.07 <sup>a</sup>	0.54±0.019 <sup>a</sup>
AQSD 2000 mg/kg	44.63± 1.16 <sup>c</sup>	36.44 ± 1.82 <sup>b</sup>	54.02 ± 1.24 <sup>b</sup>	2.32 ± 0.01 <sup>b</sup>	32.16 ± 0.85 <sup>b</sup>	0.93±0.084 <sup>b</sup>
MTSD 250 mg/kg	32.11±0.98 <sup>a</sup>	22.56±1.15 <sup>a</sup>	43.98±1.07 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	17.87± 3.95 <sup>a</sup>	0.50±0.018 <sup>a</sup>
MTSD 500 mg/kg	34.33 ±0.9 <sup>b</sup>	22.88± 2.05 <sup>a</sup>	44.40± 2.35 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	17.17± 3.15 <sup>a</sup>	0.53±0.019 <sup>a</sup>
MTSD 1000 mg/kg	33.75 ± 1.26 <sup>b</sup>	23.00 ± 2.31 <sup>a</sup>	45.23 ± 2.54 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	18.07± 2.85 <sup>a</sup>	0.51±0.020 <sup>a</sup>
MTSD 2000 mg/kg	42.75± 2.02 <sup>c</sup>	35.46± 0.68 <sup>b</sup>	55.76 ± 2.09 <sup>b</sup>	2.32 ± 0.01 <sup>b</sup>	30.31±0.86 <sup>b</sup>	1.00±0.107 <sup>b</sup>

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:** AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, 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* Seed on Plasma Protein of Rats after 28 Days of Treatment

Treatment Group	Total Protein (mg/dL)	Albumin (mg/dL)	Globulin(mg/dL)	Albumin/Globulin Ratio
Control	6.36±0.27 <sup>a</sup>	3.80±0.044 <sup>b</sup>	2.56±0.02 <sup>a</sup>	1.48±0.32 <sup>bc</sup>
AQSD 250 mg/kg	6.26±0.19 <sup>a</sup>	3.78±0.26 <sup>b</sup>	2.48±0.03 <sup>a</sup>	1.52±0.66 <sup>c</sup>
AQSD 500 mg/kg	6.55±0.39 <sup>a</sup>	3.94±0.33 <sup>b</sup>	2.61±0.05 <sup>a</sup>	1.51±0.41 <sup>c</sup>
AQSD 1000 mg/kg	6.89±0.29 <sup>a</sup>	3.89±0.31 <sup>b</sup>	3.00±0.02 <sup>a</sup>	1.30±0.82 <sup>b</sup>
AQSD 2000 mg/kg	10.09±0.44 <sup>b</sup>	2.00±0.47 <sup>a</sup>	8.09±0.06 <sup>b</sup>	0.25±0.61 <sup>a</sup>
MTSD 250 mg/kg	6.37±0.31 <sup>a</sup>	3.93±0.38 <sup>b</sup>	2.44±0.05 <sup>a</sup>	1.61±0.52 <sup>cd</sup>
MTSD 500 mg/kg	6.47±0.24 <sup>a</sup>	3.88±0.60 <sup>b</sup>	2.59±0.04 <sup>a</sup>	1.50±0.52 <sup>c</sup>
MTSD 1000 mg/kg	6.94±0.14 <sup>a</sup>	3.90±0.32 <sup>b</sup>	3.04±0.04 <sup>a</sup>	1.28±0.40 <sup>b</sup>
MTSD 2000 mg/kg	10.12±0.64 <sup>b</sup>	1.96±0.75 <sup>a</sup>	8.16±0.02 <sup>b</sup>	0.24±1.02 <sup>a</sup>

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:** AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed.

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

Groups	Urea (mg/dL)	Creatinine (mg/dL)
Control	25.94±2.85 <sup>ab</sup>	0.68±0.057 <sup>a</sup>
AQSD 250 mg/kg	28.19±1.58 <sup>b</sup>	0.73±0.040 <sup>a</sup>
AQSD 500 mg/kg	26.78±1.88 <sup>b</sup>	0.70±0.074 <sup>a</sup>
AQSD 1000 mg/kg	22.00±2.51 <sup>a</sup>	0.71±0.050 <sup>a</sup>
AQSD 2000 mg/kg	41.65±2.74 <sup>c</sup>	1.19±0.020 <sup>b</sup>
MTSD 250 mg/kg	26.22±1.34 <sup>b</sup>	0.70±0.083 <sup>a</sup>
MTSD 500 mg/kg	24.94±3.26 <sup>ab</sup>	0.69±0.043 <sup>a</sup>
MTSD 1000 mg/kg	25.37±1.45 <sup>ab</sup>	0.66±0.04 <sup>a</sup>
MTSD 2000 mg/kg	39.57±2.15 <sup>c</sup>	1.16±0.19 <sup>b</sup>

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:** AQSD = Aqueous extract of *D. edulis* seed, MTSD = Methanol extract of *D. edulis* seed.

**Table 7. Effect of Extracts of *D. edulis* Seed 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	57.15±3.93 <sup>ab</sup>	59.90±5.45 <sup>a</sup>	24.92±2.55 <sup>b</sup>	35.39±1.97 <sup>ab</sup>	11.98±0.65 <sup>ab</sup>	1.42±0.5 <sup>b</sup>
AQSD 250 mg/kg	53.23±2.76 <sup>a</sup>	54.42±3.24 <sup>a</sup>	20.74± 1.64 <sup>b</sup>	34.45±2.72 <sup>a</sup>	10.88±0.32 <sup>a</sup>	1.66±0.86 <sup>bc</sup>
AQSD 500 mg/kg	51.48±1.18 <sup>a</sup>	57.95±5.61 <sup>a</sup>	24.77±4.19 <sup>b</sup>	36.76±0.52 <sup>a</sup>	11.59±0.87 <sup>a</sup>	1.48±0.5 <sup>b</sup>
AQSD 1000 mg/kg	54.60±8.55 <sup>a</sup>	59.58±5.11 <sup>a</sup>	24.29±3.21 <sup>b</sup>	36.12±1.69 <sup>c</sup>	11.92±1.13 <sup>ab</sup>	1.49±0.71 <sup>b</sup>
AQSD 2000 mg/kg	82.83±2.59 <sup>c</sup>	111.14±2.66 <sup>b</sup>	39.76±1.34 <sup>a</sup>	26.28±0.61 <sup>d</sup>	22.23±2.45 <sup>d</sup>	0.66±0.85 <sup>a</sup>
MTSD 250 mg/kg	56.23±3.65 <sup>ab</sup>	60.36±3.25 <sup>a</sup>	23.38±2.38 <sup>b</sup>	35.22±3.56 <sup>a</sup>	12.07±0.86 <sup>ab</sup>	1.51±0.60 <sup>b</sup>
MTSD 500 mg/kg	59.15±3.21 <sup>ab</sup>	61.17±5.41 <sup>a</sup>	23.97±3.80 <sup>b</sup>	35.98±0.59 <sup>b</sup>	12.23±1.08 <sup>bc</sup>	1.50±0.65 <sup>b</sup>
MTSD 1000 mg/kg	58.61±6.45 <sup>ab</sup>	64.74±5.38 <sup>a</sup>	21.21±2.61 <sup>b</sup>	33.62±0.63 <sup>b</sup>	12.95±0.21 <sup>bc</sup>	1.58±0.83 <sup>bc</sup>
MTSD 2000 mg/kg	83.66±4.62 <sup>c</sup>	116.28±1.39 <sup>b</sup>	38.64±1.25 <sup>a</sup>	25.67±1.48 <sup>d</sup>	23.26±0.9 <sup>d</sup>	0.66±0.9 <sup>a</sup>

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:** AQSD = aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, 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* Seed on Oxidative Stress Biomarkers in the Liver of Rats after 28 Days of Treatment**

Treatment Groups	GSH (units/gwet tissue)	TP (units/g wet tissue)	CAT (units/g wet tissue)	SOD (units/g wet tissue)	MDA (units/g wet tissue)	GPx (units/g wet tissue)
Control	0.33±0.002 <sup>b</sup>	66.26±4.79 <sup>ab</sup>	0.0021±0.0000 <sup>a</sup>	0.042±0.0008 <sup>a</sup>	0.0253±0.0008 <sup>a</sup>	0.0221±0.0008 <sup>a</sup>
AQSD 250 mg/kg	0.31±0.009 <sup>b</sup>	63.04±3.44 <sup>b</sup>	0.002±0.0002 <sup>a</sup>	0.041±0.0007 <sup>a</sup>	0.0251±0.004 <sup>a</sup>	0.0223±0.002 <sup>a</sup>
AQSD 500 mg/kg	0.31±0.008 <sup>b</sup>	62.04±3.54 <sup>b</sup>	0.0023±0.0002 <sup>a</sup>	0.043±0.0008 <sup>a</sup>	0.0252±0.002 <sup>a</sup>	0.0223±0.001 <sup>a</sup>
AQSD 1000 mg/kg	0.32±0.008 <sup>b</sup>	66.53±5.47 <sup>ab</sup>	0.0024±0.0004 <sup>a</sup>	0.043±0.0006 <sup>a</sup>	0.0254±0.0007 <sup>a</sup>	0.0222±0.0007 <sup>a</sup>
AQSD 2000 mg/kg	0.20±0.006 <sup>a</sup>	93.14±3.09 <sup>c</sup>	0.0042±0.00008 <sup>b</sup>	0.072±0.001 <sup>b</sup>	0.0353±0.002 <sup>b</sup>	0.0249±0.0008 <sup>b</sup>
MTSD 250 mg/kg	0.33±0.02 <sup>b</sup>	65.74±1.78 <sup>b</sup>	0.002±0.00005 <sup>a</sup>	0.044±0.003 <sup>a</sup>	0.0251±0.0005 <sup>a</sup>	0.0220±0.003 <sup>a</sup>
MTSD 500 mg/kg	0.32±0.02 <sup>b</sup>	68.74±1.88 <sup>b</sup>	0.002±0.0006 <sup>a</sup>	0.043±0.002 <sup>a</sup>	0.0251±0.0006 <sup>a</sup>	0.0222±0.002 <sup>a</sup>
MTSD 1000 mg/kg	0.34±0.007 <sup>b</sup>	61.04±5.98 <sup>a</sup>	0.0024±0.00008 <sup>a</sup>	0.043±0.002 <sup>a</sup>	0.0253±0.0008 <sup>a</sup>	0.0223±0.001 <sup>a</sup>
MTSD 2000 mg/kg	0.24±0.02 <sup>a</sup>	97.49±2.57 <sup>c</sup>	0.0045±0.00010 <sup>b</sup>	0.074±0.002 <sup>b</sup>	0.0350±0.0007 <sup>b</sup>	0.0252±0.0006 <sup>b</sup>

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:** AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, GSH = Glutathione, TP = Total Protein, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase

**Table 9. Effect of Extracts of *D. edulis* Seed on Oxidative Stress Biomarkers in Kidney of Rats after 28 Days of Treatment**

Treatment Groups	GSH (units/gwet tissue)	TP (units/gwet tissue)	CAT (units/gwet tissue)	SOD (units/gwet tissue)	MDA (units/gwet tissue)	GP (units/gwet tissue)
Control	0.38±0.01 <sup>b</sup>	70.97±5.15 <sup>a</sup>	0.0025±0.00016 <sup>b</sup>	0.0221±0.002 <sup>a</sup>	0.0281±0.001 <sup>a</sup>	0.0331±0.002 <sup>a</sup>
AQSD 250 mg/kg	0.36±0.02 <sup>b</sup>	74.83±4.34 <sup>a</sup>	0.0026±0.00007 <sup>b</sup>	0.0221±0.001 <sup>a</sup>	0.0282±0.0005 <sup>a</sup>	0.0332±0.0004 <sup>a</sup>
AQSD 500 mg/kg	0.34±0.02 <sup>b</sup>	72.73±5.44 <sup>a</sup>	0.0027±0.00008 <sup>b</sup>	0.0223±0.002 <sup>a</sup>	0.0282±0.0006 <sup>a</sup>	0.0333±0.0006 <sup>a</sup>
AQSD 1000 mg/kg	0.35±0.007 <sup>b</sup>	70.65±5.46 <sup>a</sup>	0.0027±0.00011 <sup>b</sup>	0.0222±0.001 <sup>a</sup>	0.0281±0.002 <sup>a</sup>	0.0331±0.0005 <sup>a</sup>
AQSD 2000 mg/kg	0.26±0.007 <sup>a</sup>	92.91±3.91 <sup>b</sup>	0.0039±0.0002 <sup>a</sup>	0.0248±0.002 <sup>b</sup>	0.0356±0.002 <sup>b</sup>	0.0372±0.0008 <sup>b</sup>
MTSD 250 mg/kg	0.34±0.04 <sup>b</sup>	71.83±6.44 <sup>a</sup>	0.0024±0.00006 <sup>b</sup>	0.0223±0.003 <sup>a</sup>	0.0282±0.0004 <sup>a</sup>	0.0334±0.0005 <sup>a</sup>
MTSD 500 mg/kg	0.33±0.006 <sup>b</sup>	73.60±3.44 <sup>a</sup>	0.0025±0.00006 <sup>b</sup>	0.0221±0.004 <sup>a</sup>	0.0283±0.0006 <sup>a</sup>	0.0333±0.001 <sup>a</sup>
MTSD 1000 mg/kg	0.37±0.02 <sup>b</sup>	69.53±7.00 <sup>a</sup>	0.0026±0.00012 <sup>b</sup>	0.0222±0.006 <sup>a</sup>	0.0281±0.0007 <sup>a</sup>	0.0331±0.0008 <sup>a</sup>
MTSD 2000 mg/kg	0.24±0.006 <sup>a</sup>	90.66±7.80 <sup>b</sup>	0.0041±0.00018 <sup>a</sup>	0.0251±0.004 <sup>b</sup>	0.0370±0.0007 <sup>b</sup>	0.0375±0.002 <sup>b</sup>

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

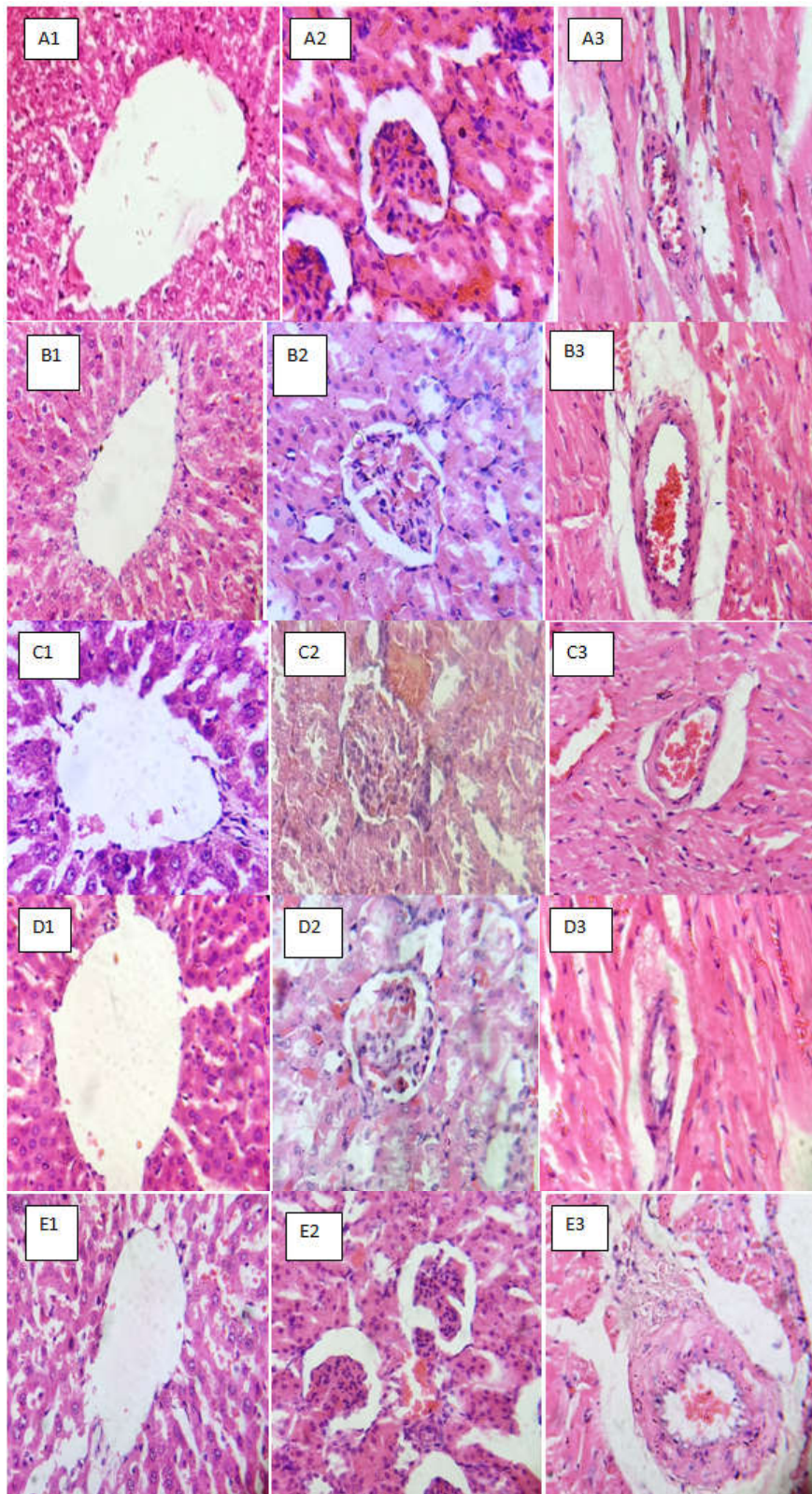
**Legend:** AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, GSH = Glutathione, TP = Total Protein, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase

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

Treatment Groups	GSH (units/gwet tissue)	TP (units/gwet tissue)	CAT (units/gwet tissue)	SOD (units/gwet tissue)	MDA (units/gwet tissue)	GPx (units/gwet tissue)
Control	0.33±0.00 <sup>b</sup>	74.25±2.64 <sup>a</sup>	0.0039±0.00012 <sup>a</sup>	0.0272±0.0008 <sup>a</sup>	0.0340±0.001 <sup>a</sup>	0.0286±0.0006 <sup>a</sup>
AQSD 250 mg/kg	0.34±0.009 <sup>b</sup>	76.56±4.36 <sup>a</sup>	0.0038±0.00006 <sup>a</sup>	0.0271±0.0005 <sup>a</sup>	0.0341±0.0007 <sup>a</sup>	0.0284±0.0006 <sup>a</sup>
AQSD 500 mg/kg	0.34±0.008 <sup>b</sup>	76.56±8.35 <sup>a</sup>	0.0036±0.00008 <sup>a</sup>	0.0273±0.0004 <sup>a</sup>	0.0342±0.0008 <sup>a</sup>	0.0287±0.0008 <sup>a</sup>
AQSD 1000 mg/kg	0.35±0.008 <sup>b</sup>	78.46±3.85 <sup>a</sup>	0.0038±0.00012 <sup>a</sup>	0.0272±0.0002 <sup>a</sup>	0.0341±0.0006 <sup>a</sup>	0.0285±0.001 <sup>a</sup>
AQSD 2000 mg/kg	0.18±0.01 <sup>a</sup>	110.71±4.52 <sup>b</sup>	0.0055±0.00006 <sup>b</sup>	0.0308±0.0006 <sup>b</sup>	0.0394±0.001 <sup>b</sup>	0.0342±0.001 <sup>b</sup>
MTSD 250 mg/kg	0.32±0.005 <sup>b</sup>	79.79±3.24 <sup>a</sup>	0.0036±0.00004 <sup>a</sup>	0.0273±0.0005 <sup>a</sup>	0.0341±0.0006 <sup>a</sup>	0.0287±0.0007 <sup>a</sup>
MTSD 500 mg/kg	0.33±0.006 <sup>b</sup>	77.89±5.20 <sup>a</sup>	0.0037±0.00006 <sup>a</sup>	0.0274±0.0004 <sup>a</sup>	0.0343±0.0005 <sup>a</sup>	0.0286±0.0008 <sup>a</sup>
MTSD 1000 mg/kg	0.34±0.02 <sup>b</sup>	76.65±3.42 <sup>a</sup>	0.0037±0.00006 <sup>a</sup>	0.0272±0.0002 <sup>a</sup>	0.0342±0.001 <sup>a</sup>	0.0288±0.0006 <sup>a</sup>
MTSD 2000 mg/kg	0.17±0.008 <sup>a</sup>	113.59±7.03 <sup>b</sup>	0.0056±0.00017 <sup>b</sup>	0.0311±0.0008 <sup>b</sup>	0.0392±0.0008 <sup>b</sup>	0.0350±0.0008 <sup>b</sup>

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

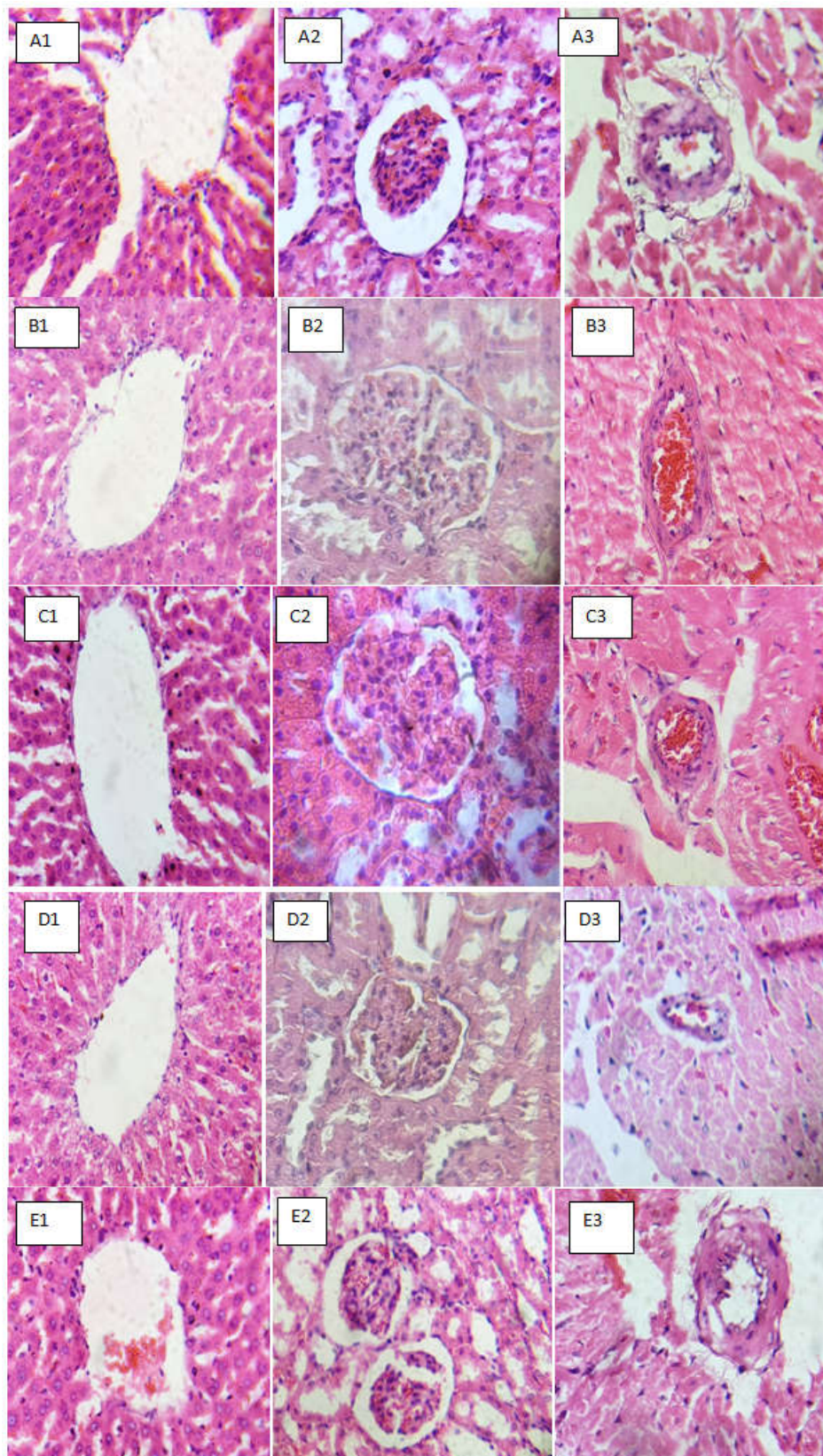
**Legend:** AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, GSH = Glutathione, TP = Total Protein, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase



**Plate 1 Sections of Rats' Liver, Kidney and Heart.**

(A1-A3) = Control liver, kidney and heart rat respectively, (B1-B3) = rat liver, kidney and heart respectively of animals administered 250 mg/kg b.w aqueous extract of *Dacoydes edulis* seed, (C1-C3) = rat liver, kidney and heart respectively of animals administered 500 mg/kg b.w aqueous extract of *Dacoydes edulis* seed, (D1-D3) = rat liver, kidney and heart respectively of animals administered 1000 mg/kg b.w aqueous extract of *Dacoydes edulis* seed, (E1-E3) = rat liver, kidney and heart respectively of animals administered 2000 mg/kg b.w aqueous extract of *Dacoydes edulis* seed.

Sections were stained with heamatoxylin and eosin (Mag 100x). Rats in groups A, B, C and D administered with control, 250, 500, and 1000 mg/kg b.w reveals liver visible centriole with the hepatocytes and nucleus and a well fenestrated sinusoidal, kidney visible renal corpuscles and renal tubules and Heart composed of bundles of myocardial fibres cells, interstitial space and visible coronary artery while Rat group E1-E3 administered with 2000 mg/kg b.w reveals; Liver histology reveals prominent centriole and well fenestrated hepatocytes with mild steatosis and slightly pyknotic nucleus, Kidney histology reveals a mild atrophied renal corpuscles and renal tubules with diffuse mononuclear infiltrates and Heart histology reveals large coronary artery with mild fatty changes and bundles of myocardial fibres and interstitial space.



**Plate 2. Sections of rats' Liver, Kidney and Heart**

(A1-A3) = Control liver, kidney and heart rat respectively, (B1-B3) = rat liver, kidney and heart respectively of animals administered 250 mg/kg b.w methanol extract of *Dacoydes edulis* seed, (C1-C3) = rat liver, kidney and heart respectively of animals administered 500 mg/kg b.w methanol extract of *Dacoydes edulis* seed, (D1-D3) = rat liver, kidney and heart respectively of animals administered 1000 mg/kg b.w methanol extract of *Dacoydes edulis* seed, (E1-E3) = rat liver, kidney and heart respectively of animals administered 2000 mg/kg b.w methanol extract of *Dacoydes edulis* seed.

Sections were stained with hematoxylin and eosin (Mag 100x). Rat group A,B, C and D administered with control, 250, 500, 1000 and 2000 mg/kg b.w reveals Liver visible centriole with the hepatocytes and nucleus and a well fenestrated sinusoidal, kidney distinct renal corpuscles and renal tubules and Heart visible coronary artery and bundles of myocardial fibres and interstitial space. while Rat group E1-E3 administered with 2000 mg/kg b.w reveals; Liver histology reveals prominent centriole with mild mononuclear cells surrounding it and well fenestrated hepatocytes with slightly pyknotic nucleus with mild steatosis, Kidney histology reveals prominent large renal corpuscles and renal tubules and Heart histology reveals visible coronary artery with visible mild fatty changes and bundles of myocardial fibres and interstitial space.

## DISCUSSION

Lethal dose (LD<sub>50</sub>) 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 (Ping *et al.*, 2011). It provides information on the nature of toxicity and the basis for the classification and dosage design of a substance or drug (Zakaria *et al.*, 2016). The acute toxicity of *Dacryodes edulis* in this study showed that the LD<sub>50</sub> of its aqueous extract of seed (AQSD) and methanolic extract of seed (MTSD) 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.* (2020) who reported that the LD<sub>50</sub> of methanolic extract of *D. edulis* is greater than 5000 mg/kg. Yelwaet *al.* (2017) has also reported that the LD<sub>50</sub> of methanolic extract of *D. edulis* is greater than 4000 mg/kg. According to Kennedy *et al.* (1986), any substance with LD<sub>50</sub> 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. Evaluation of hepatic biochemical parameters including enzymes (aspartate transaminase, alanine transaminase, and alkaline phosphatase) and metabolites (total proteins and albumin) are very useful in assessing the functional integrity of liver during subacute exposure of chemical substances or natural products/plant extracts (Airaodion *et al.*, 2019b). The transaminase (ALT and AST) are enzymes of carbohydrate and amino acid metabolism while alkaline phosphatase is involved in hydrolysis of phosphate bonds. They are often used in assessing the functional integrity of liver, plasma membrane and endoplasmic reticulum (Airaodion *et al.*, 2019a).

In this study, no significant difference was observed in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in animals treated with 250, 500 and 1000 mg/kg of both aqueous and methanol extracts of *D. edulis* seed when compared with those of the control animals at P<0.05. It has been reported that an increase in the enzymatic activities of ALT and AST in the serum directly reflects hepatocellular damage (Airaodion *et al.*, 2019c; Ogbuagu *et al.*, 2019a). Results of this study therefore suggest that extract of *D. edulis* seed is not hepatotoxic at these doses. ALT is considered most reliable marker of hepatocellular injury because it is solely confined to the liver, unlike AST which is also abundantly present in other body organs such as the kidneys, brain, and hearts (Airaodion *et al.*, 2020a; Ogbuagu *et al.*, 2019b). However, both aqueous and methanol extracts of *D. edulis* seed significantly increased the activities of AST, ALT, GGT and LDH when animals treated with 2000 mg/kg were compared with those in the control group. This suggests that the extract might have compromised the functional integrity of the liver and thus might be hepatotoxic. Alkaline phosphatase (ALP) is involved in the hydrolysis of a wide range of phosphomonoester substrates. In this study, a significant (p<0.05) increase in the activity of ALP was observed in animals treated with 2000 mg/kg body weight of *D. edulis* seed when compared with those in the control group. ALP is a marker enzyme for the plasma membrane and

endoplasmic reticulum of the tissues (Airaodion *et al.*, 2019d). It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli in the bile canaliculi, located in the plasma membrane (Airaodion *et al.*, 2019b; 2020a), thus, its significant elevation at this dose might not be primarily related to hepatotoxicity. Since ALP hydrolyses phosphate monoesters, its significant increase in animals exposed to 2000 mg/kg of *D. edulis* seed could constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver (Akanji *et al.*, 1993). Consequently this may adversely affect the facilitation of the transfer of metabolites across the cell membrane of animals exposed to high doses *D. edulis* seed. This effect might be due to the high content of tannins in *D. edulis* seed.

In this study, no significant difference was observed in the concentrations of total protein and albumin in animals treated with 250, 500 and 1000 mg/kg of extracts of *D. edulis* seed when compared with those in control animals. This might suggest that *D. edulis* seed did not affect the synthetic ability of protein by the liver. It is also an indication that *D. edulis* seed did not distort the functional activity of the liver by interfering with the equilibrium in the rate of synthesis and destruction, removal or clearance of total protein and albumin from the system of the animals (Bashir *et al.*, 2015). Increase in total protein has been reported to lead to dehydration which is detrimental to cellular homeostasis (Ogbuagu *et al.*, 2021a) which negatively affects the metabolic activities of the liver and consequently the health of the animals. Albumin binds and transports metal ions, bilirubin, and drugs. Its level is used to assess the synthetic function of the liver. Serum protein levels are regulated via synthesis in the liver and its levels thus reflect the synthetic ability of the liver. Therefore, the elevation of protein in animals exposed to 2000 mg/kg of extracts of *D. edulis* seed in this study is an indication that the extracts perturbed the integrity of the liver at this dose.

The result of this study showed that 250, 500 and 1000 mg/kg of aqueous and methanol extracts of *D. edulis* seed had no significant effect on the concentration of bilirubin but 2000 mg/kg significantly perturbed bilirubin level when compared with those in the control group. Bilirubin is the breakdown product of heme moiety of hemeoglobin; other hemeoproteins include cytochromes, catalase, peroxidase, tryptophan pyrrolase and a small pool of free heme (Airaodion *et al.*, 2019d; Ogbuagu *et al.*, 2021b). Increase in concentration of direct reacting bilirubin in blood causes hyperbilirubinaemia, which is toxic under certain conditions inducing jaundice, hyperbilirubinemia-induced auditory dysfunction and neurotoxicity resulting in brain damage (Shapiro, 2003; Airaodion *et al.*, 2020c). On the other hand, mild unconjugated hyperbilirubinaemia behaves as mild antioxidant and might offer protection against cardiovascular diseases and tumour development (Airaodion *et al.*, 2020d). Recent research survey has reported that low concentration of direct reacting bilirubin induces stroke in body and sometimes causes cardiac problems too. Serum bilirubin levels are often enhanced under a variety of clinical conditions. In the circulation of blood, bilirubin is bound to serum albumin, which prevents its potential toxicity thought to be caused by free bilirubin (Perlstein *et al.*, 2008). Despite its high affinity of binding to albumin, bilirubin is rapidly and selectively taken up by the liver, biotransformed upon conjugation with glucuronate, and

secreted into bile (Airaodion *et al.*, 2020e). Thus bilirubin is converted into bilirubin glucuronic acid in the liver and excreted along with bile. Thus the significant increase observed in the concentrations of bilirubin in animals administered 2000 mg/kg in this study suggests that the extract affected liver function. Studies on the tissue biomarker alterations might reflect the metabolic abnormalities and cellular injuries in some organs. The kidney has extremely important function in detoxification and excretion of metabolic wastes and xenobiotics (Airaodion 2019e). Exposure to toxic chemicals causes alterations in some tissue enzyme activities (Airaodion *et al.*, 2020d). The kidneys control the excretion of urea, creatinine, and reabsorption of electrolytes into the blood. Defeat in activities of kidney will result in accumulation of electrolytes, urea, and creatinine in the biological fluid (Shittu *et al.*, 2015).

Administration of 250, 500 and 1000 mg/kg *D. edulis* seed was observed to have no significant effect in the plasma concentrations of urea and creatinine while 2000 mg/kg significantly ( $p < 0.05$ ) increase their concentrations when compared with those in control animals as presented table 6. Airaodion *et al.* (2019d), reported that the relationship between high renal resistive index (RI) and cardiovascular and renal outcomes is significant and persisted after multivariate Cox regression analysis, including traditional risk factors. The serum creatinine concentration is widely interpreted as a measure of the glomerular filtration rate (GFR) and it is used as an index of renal function in clinical practice (Airaodion *et al.*, 2020c). Glomerular filtration of creatinine, however, is only one of the variables that determine its concentration in serum. Alterations in renal handling and metabolism of creatinine and methodological interferences in its measurement may have a profound impact on the serum concentration of creatinine metabolism and is constant among individuals and over time, with the creatinine production rate being equal to the renal excretion rate (Ogbuagu *et al.*, 2021b). In the theoretical situation where both criteria are satisfied, the serum creatinine is inversely proportional to the GFR, so that each halving of the GFR results in a doubling of the serum creatinine concentration (Kassirer, 2001). Secretion of creatinine was observed even in early studies of the clearance of exogenously administered creatinine (Airaodion *et al.*, 2020d). Mandell *et al.*, (2003), reported that the exogenous creatinine clearance decreased as the concentration of creatinine in the blood was acutely increased 10-fold by creatinine infusion. This decrease was thought to be due to saturation of the tubular secretory mechanism, because the inulin clearance was not affected by this exogenous increase of the creatinine concentration in the blood (Airaodion *et al.*, 2020c). Creatinine reabsorption during low rates of urine flow is thought to result from its passive back-diffusion from the lumen to the blood (Ogbuagu *et al.*, 2020b). Thus, when urine flow rate is very low, passive reabsorption of creatinine might result in a lower creatinine clearance and a higher concentration of serum creatinine than what one would expect solely on the basis of the Glomerular Filtration Rate (GFR) (Airaodion *et al.*, 2019d; 2020e). Dietary protein deficiency leads to negative nitrogen balance and loss of muscle mass, thereby decreasing creatinine production. Less severe alterations in the diet, however, also may have important effects on the size of the creatine pool and creatinine excretion, which are independent of nitrogen balance and muscle mass. The significant increase observed in the concentrations of urea and creatinine in animals treated with 2000 mg/kg of *D. edulis*

seed extracts when compared with those in control animals might be suggestive that the extracts compromise renal functional capacity and thus, nephrotoxic at this dose. There was a significant ( $p < 0.05$ ) perturbation in the lipid profile of animals treated with 2000 mg/kg of seed extracts of *D. edulis* 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 (Airaodion and Ogbua 2020; Megwaset *et al.*, 2021). Thus, administration of 2000 mg/kg of the extract might enhance dyslipidemia. Several researchers had reported that lower doses of stem bark and seed extracts of *D. edulis* possess hypolipidemic potential (Okolo *et al.*, 2016; Ndemet *et al.*, 2017). Results from this study showed that 2000 mg/kg of aqueous and methanolic extracts of *D. edulis* seed 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 (Airaodion *et al.*, 2019c; 2020e). 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 (Airaodion *et al.*, 2020f). 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.

## Conclusion

Both aqueous and methanol extracts of *D. edulis* seed 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.

## Ethical consideration

Animal ethic Committee approval has been collected and preserved by the author.

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