

## Antioxidant Properties Associated with the Biochemical Changes in the Development of African Pear (*Dacryodes edulis*) Fruit

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### Original Research Article

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**Abstract:** The antioxidant properties of oil and fruit extracts of *Dacryodes edulis* (African pear) from four weeks after anthesis (WAA) to fruit maturation were assessed in this study. The oils from the fruits were extracted with chloroform-methanol 1:2 (v/v) using standard procedures. The *in vitro* antioxidant capacity of the oil extracts was determined by spectrophotometric methods using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, H<sub>2</sub>O<sub>2</sub> and malondialdehyde (MDA) assays. Results from the study indicated that DPPH and H<sub>2</sub>O<sub>2</sub> scavenging capacity as well as percentage inhibition of MDA were significantly ( $P < 0.05$ ) higher in oil at 20 WAA (45.47%, 45.1%, 18.3%), but with significant ( $P < 0.05$ ) lesser percentage of inhibition at 4-12 WAA. The hepatoprotective effects of the extracts were examined *in vivo* in male wistar rats challenged with sodium arsenate. Results showed that the rats fed with the oil WAA had significant reduction ( $p < 0.05$ ) in lipid peroxidation, increase in superoxide dismutase (SOD) and catalase (CAT) activities especially relative to control values. African walnut extracts treated rats also showed similar decrease in serum AST ( $70.3 \pm 2.54$  U/L), ALT ( $63.7 \pm 2.34$  U/L), ALP ( $59.3 \pm 0.60$  U/L) and GGT ( $230.8 \pm 1.12$  U/L) levels at 20 WAA when compared to 4 WAA extracts treated rats, AST ( $105.2 \pm 1.38$  U/L), ALT ( $73.0 \pm 1.12$  U/L), ALP ( $72.0 \pm 2.26$  U/L) and GGT ( $299.7 \pm 1.88$  U/L). The results suggest that the various fruit extracts possess varied degrees of potent antioxidant activity both *in vitro* and *in vivo* and may serve as important sources of antioxidants in food, cosmetics and pharmaceutical industries.

**Keywords:** *Dacryodes edulis*, antioxidants, sodium arsenate, hepatoprotective.

## INTRODUCTION

*Dacryodes edulis* (also called African plum, African pear or Safou) is an indigenous fruit tree in the humid low lands and plateau regions of West, Central African and Gulf of Guinea countries. *Dacryodes edulis* belongs to the *Burseraceae* family. It is an evergreen tree indigenous to the central Africa and Gulf of Guinea regions. The genus name is derived from the Greek word 'Dakruon' (a tear) in reference to the resin droplets that appears on the bark surface of its species. The species specific name *edulis* means edible [1]. The genus *Dacryodes* comprises about 40 species, occurring in the American, Asian and African tropics. In Africa, about 20 species have been described [2]. In south-east Nigeria, the trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October [3, 4]. The contribution of fruits to a healthy and nutritious diet, the world over is a well-established fact. *D. edulis* is a tree cultivated widely for its edible and nutritious fruits. Generally, the fruit may be cooked in hot water, or roasted/baked in an oven at about 50°C. The cooked

fruit can be eaten with maize, plantain, cassava, cocoyam, bread, etc.

The entire plant of *D. edulis* has pharmaceutical properties that are variously exploited by many African communities [5]. Oral treatment against leprosy and it is also gargled as mouth-wash for the treatment of tonsillitis. In the western parts of Cameroon, the bark is crushed and used in concoctions against dysenteries while in central Cameroon the bark is used to treat toothache. The leaves are boiled in combination with *Lantana camara*, *Cymbopogon citratus* and *Persea americana* yielding a steam bath taken to treat fever/headaches and malaria in Republic of Congo. The leaves made into a plaster have been recently reported to treat snake bites in South West Cameroon [6]. The leaves are also crushed and the resultant juice used to treat skin diseases such as scabies, ring worm, rashes, while twigs from branches are sometimes used as chewing sticks [7, 8, 9]. The leaves and seed are used in Nigeria for animal feed [10].

The resin from the bark has long been reported to treat parasitic skin diseases and jiggers in Nigeria, whereas when applied in lotions and body creams it smoothenes the skin. The resin is also used in some communities as incense and is believed to send off evil spirits in Nigeria [11].

## MATERIALS AND METHODS

### Plant materials:

Matured fruits from *Dacryodes edulis* were collected from private farm land in Okada Town of Ovia North-East LGA of Edo State, Nigeria. These fruits were authenticated by the Department of Botany, University of Medical Sciences, Ondo City. A voucher specimen of each plant was there after deposited in the herbarium of the same Department.

### Preparation of plant extracts

Methanol: chloroform extracts of each plant fruits was prepared according to previous method of Bligh and Dyer [12] as reported by Bafor and Osagie [13]. The sterilised mesocarp was macerated three times in 500ml of  $\text{CHCl}_3/\text{MeOH}$  (1:2 v/v) using MSE homogeniser. The homogenate was filtered each time and the filtrate residue re-blended with the  $\text{CHCl}_3/\text{MeOH}$  solution. Appropriate volume of chloroform and 0.72% NaCl solution were added to the combine filtrate to form a biphasic system chloroform/methanol/water in volume 1:1:0.9. The chloroform layer was withdrawn, and concentrated to dryness on a rotary evaporator at  $30^\circ\text{C}$  under reduced pressure and then stored at  $40^\circ\text{C}$  for subsequent analysis.

### Animals and treatment

30 male rats of Wister strain weighing between (100-120) g were obtained from the animal house unit of the Department of Biochemistry, University of Medical Sciences, Ondo City, Nigeria.

The animals were divided into three sub-groups having five animals each. Animals in sub-group 1 were fed with 50mg/kg of the extract for seven days, while sub-group 2 and 3 was given normal saline. At the 8<sup>th</sup> day, sub-group 1 and 2 rat were injected intraperitoneally with 1mg/kg of Sodium Arsenate, while sub-group 3 was given distilled water. Twenty-four hours later, the animals were sacrificed by cervical dislocation. The livers were removed at once, blotted dry, weighed and stored at  $4^\circ\text{C}$ .

### Preparation of homogenates

1g of the liver tissue was homogenised in 10ml of ice-cold physiological saline to obtain 10% (w/v) homogenates. The resulting homogenates were centrifuged at 5000g for ten minutes and the supernatants obtained were used for determination of superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation.

## Chemicals

DPPH (2, 2-diphenyl-2-picrylhydrazyl), Gallic acid, folin-ciocalteau's reagent were obtained from Sigma Chemical company, USA. All other reagents and chemicals were of analytical grade and obtained locally from BDH and Aldrich in Nigeria.

## Determination of *in vitro* antioxidant activities

### TBARS

TBARS (Thiobarbituric acid reactive substances) modified assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532nm [14].

Inhibition of lipid peroxidation (%) by concentrates was calculated with formula:  $(C-E) / C \times 100$  where C is the absorbance value of the fully oxidized control and E is  $(\text{Abs}_{532\text{TBA}} - \text{Abs}_{532\text{-TBA}})$

### DPPH Radical Scavenging Activity

The radical scavenging activity of the fruit extracts against 2, 2-diphenyl-2-picryl-hydrazyl radical was determined by measuring UV absorbance at 532nm. Radical scavenging activity was measured according to the method of Blois [15]. The following 0.02, 0.04, 0.06, 0.08 and 0.1mg/ml concentration of samples were placed into test tubes and 0.5ml of 1mM DPPH solution in methanol was added after. The experiment was carried out in triplicate. The test was incubated for 15 minutes at room temperature and the absorbance was read at 517nm. A blank solution was prepared and measured containing the same amount of methanol and DPPH. Lower absorbance of reaction of the reaction mixture indicates higher radical scavenging activity. The radical scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity \%} = \frac{[AB - AA]}{AB} \times 100$$

Where, AB = Absorption of blank sample and AA is the absorption of tested extract solution.

### Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) Determination:

The hydrogen peroxide scavenging assay was carried out following the procedure [16] as described [17]. Crude extract at  $20\mu\text{g/ml}$  concentration in 3.4ml phosphate buffer (0.1M, pH 7.4) was added to 0.6ml of  $\text{H}_2\text{O}_2$  solution (43Mm). The absorbance was taken at 230nm. Blank solution contain phosphor buffer without  $\text{H}_2\text{O}_2$ . The concentration of hydrogen peroxide (mM) in the assay medium was determined using a standard curve.

### Calculations

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A<sub>0</sub> - Absorbance of control

A<sub>1</sub> - Absorbance in the presence of plant extract

### Determination of in vivo antioxidant activity

#### Superoxide dismutase (SOD) assay

SOD was assayed in the liver according to the method of Misra and Fridovich [18] based on the rapid auto-oxidation of adrenalin due to the presence of superoxide anions. This is measured spectrophotometrically at 420nm and SOD concentration is expressed as units/g tissue.

#### Catalase activity

Catalase activity in the liver was determined as residual H<sub>2</sub>O<sub>2</sub> after incubation with the enzyme according to the method described in [19].

#### Estimation of Lipid Peroxidation

Lipid peroxidation in the tissue involves the determination of thiobarbituric acid reactive species (TBARS) which is the indicator of membrane lipid peroxidation. Values for TBARS were reported as Malondialdehyde (MDA) and quantified using a Molar extinction coefficient of  $1.5 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  and expressed as mmole MDA g<sup>-1</sup> of tissue [20].

#### Statistical Analysis

Data were expressed as mean  $\pm$  standard error of mean (SEM). One way analysis of variance

(ANOVA) was performed to test for differences between the groups mean. Significance differences between the mean were determined by Duncan's multiple range test and P-value <0.05 were regarded as significance [21].

### RESULTS

The antioxidant activity of the fruits were evaluated by determining the percentage inhibition of thiobarbituric acid reactive species (TBARS), 1,1-diphenil-2-picrylhydrazyl (DPPH) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity by the plants whole fruit sample and the extracted oil from the plant. The oil extract were found to possess the highest inhibitive activity of TBARS, DPPH and H<sub>2</sub>O<sub>2</sub> at the matured stage (20 WAA) of the fruit (Table 3.1).

The effects of methanolic extracts of *Tetracarpidium conophorum* fruits on liver function of Wister rats were assessed by evaluating the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl aminotransferase ( $\gamma$ -GT) and alkaline phosphatase (ALP) are showed in (Figure 3.1 – 3.4).

The effects of the methanolic extract of *Tetracarpidium conophorum* fruits on oxidative damage were assessed by estimating the liver superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels. The liver catalase levels in wister rats treated with the methanolic extracts of *Tetracarpidium conophorum* fruits are presented in (Figure 3.5-3.7).

**Table-3.1: Antioxidant activities of *Dacryodes edulis* at Different Stages of Fruit Development**

Week	TBARS % Inhibition	DPPH % Scavenging ability	H <sub>2</sub> O <sub>2</sub> % Scavenging ability
4	11.3 $\pm$ 0.08	42.81 $\pm$ 0.41	1.40 $\pm$ 0.67
6	11.0 $\pm$ 0.12	42.7 $\pm$ 0.86	10.7 $\pm$ 0.78
8	21.1 $\pm$ 0.04	36.4 $\pm$ 0.84	10.1 $\pm$ 0.44
10	28.8 $\pm$ 0.04	49.4 $\pm$ 0.74	10.3 $\pm$ 0.71
12	29.3 $\pm$ 0.08	45.7 $\pm$ 0.82	16.9 $\pm$ 1.21
14	41.6 $\pm$ 0.26	44.4 $\pm$ 0.56	14.9 $\pm$ 0.71
16	38.8 $\pm$ 0.24	45.9 $\pm$ 0.44	16.5 $\pm$ 0.49
18	45.3 $\pm$ 0.31	45.4 $\pm$ 0.71	18.1 $\pm$ 0.72
20	46.3 $\pm$ 0.84	45.8 $\pm$ 0.56	18.9 $\pm$ 0.84

Data are the average of 3 replicates  $\pm$  SE

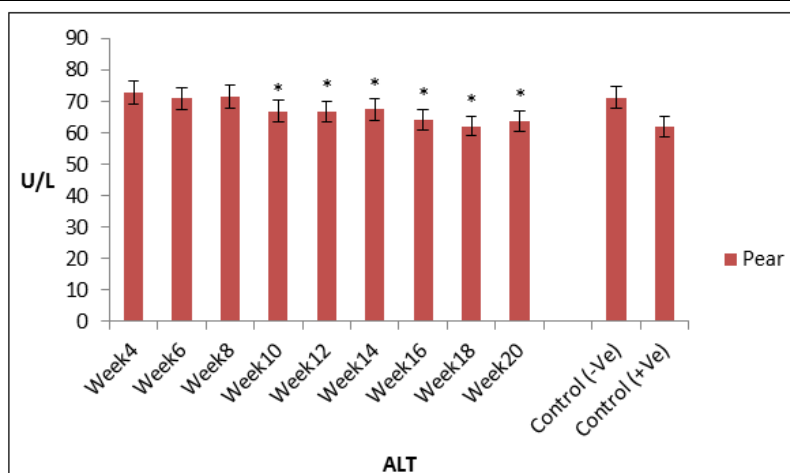


Fig-3.1: Serum Alanine Aminotransferase (ALT) levels in Wister rats administered extracts of *Dacryodes edulis* fruits 4-20 WAA of fruit development. Values are mean  $\pm$  SEM (\* =  $P < 0.05$ ).

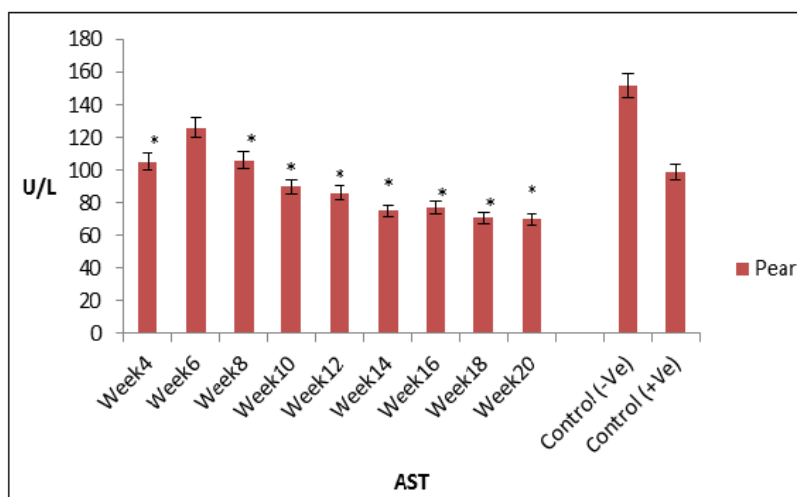


Fig-3.2: Serum Aspartate Aminotransferase (AST) levels in Wister rats administered extracts of *Dacryodes edulis* fruits 4-20 WAA of fruit development. Values are mean  $\pm$  SEM (\* =  $P < 0.05$ ).

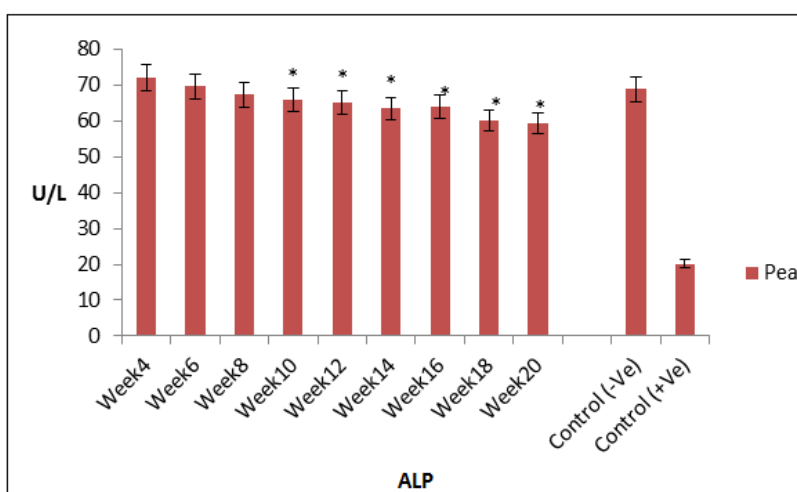


Fig-3.3: Serum Alkaline Phosphate (ALP) Level Level in Wister rats administered extracts of *Dacryodes edulis* fruits 4-20 WAA of fruit development. Values are mean  $\pm$  SEM (\* =  $P < 0.05$ ).

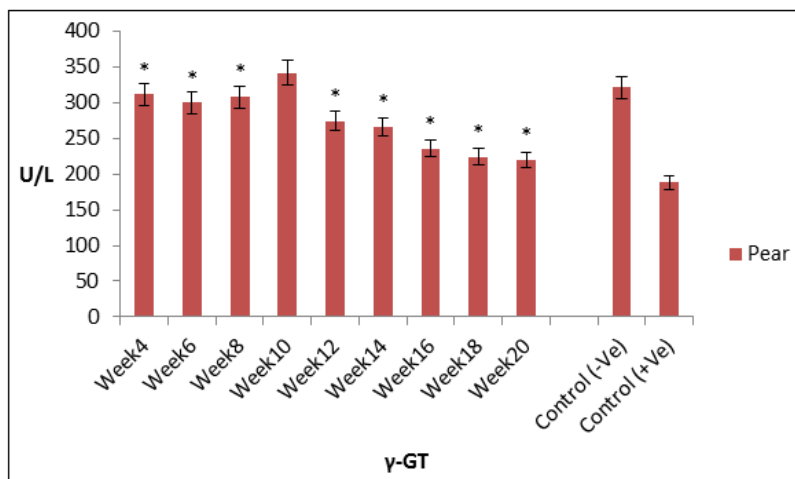


Fig-3.4: Serum L-γ-Glutamyltransferase (GGT) Level in Wister rats administered extracts of *Dacryodes edulis* fruits 4-20 WAA of fruit development. Values are mean  $\pm$  SEM (\* =  $P < 0.05$ ).

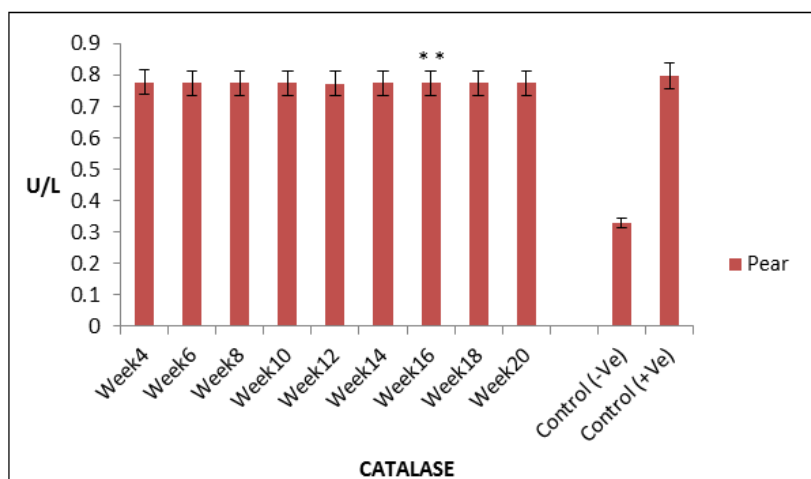


Fig-3.5: Liver Catalase Level Level in Wister rats administered extracts of *Dacryodes edulis* fruits 4-20 WAA of fruit development. Values are mean  $\pm$  SEM (\* =  $P < 0.05$ ).

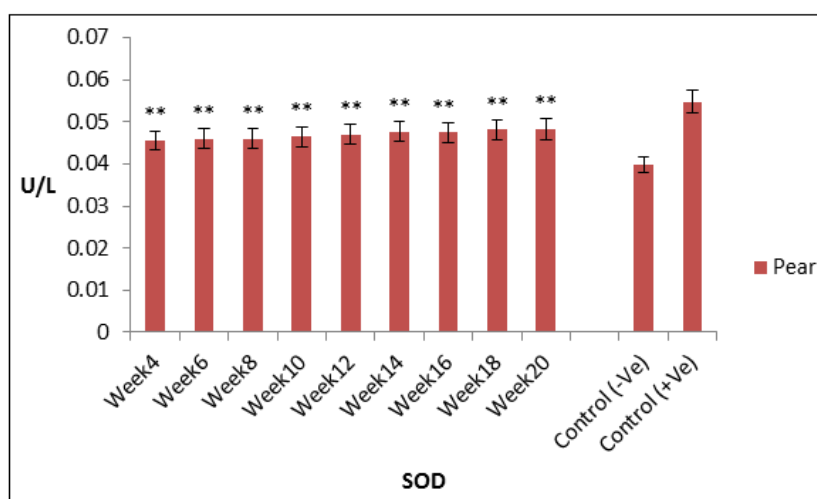
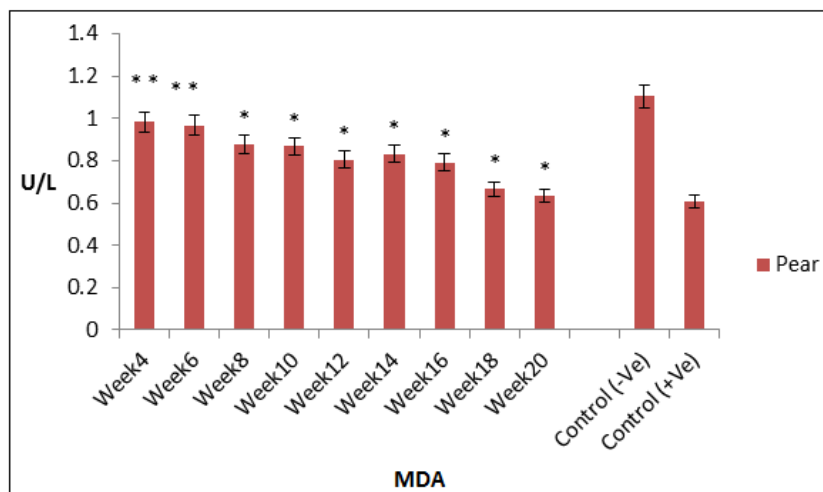
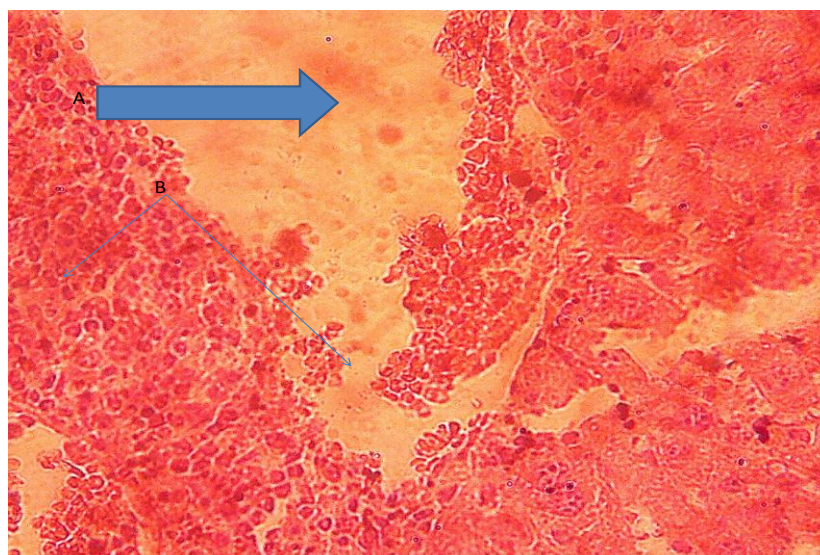


Fig-3.6: Superoxide Dismutase (SOD) level in Wister rats administered extracts of *Dacryodes edulis* fruits at 4-20 WAA of fruit development. Values are mean  $\pm$  SEM (\* =  $P < 0.05$ ).





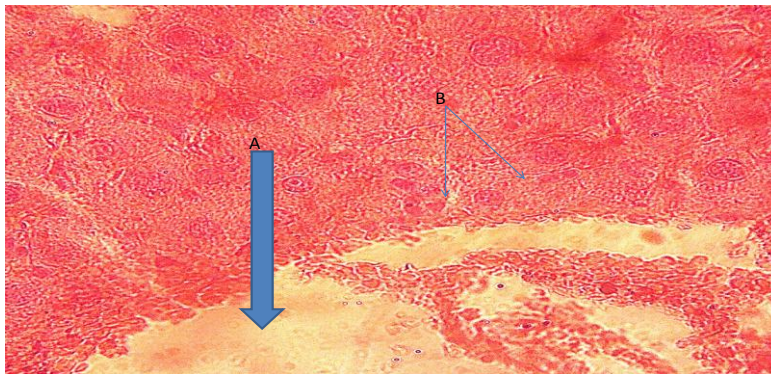
**Fig-3.7: Malondialdehyde (MDA) Level in Wister rats administered extracts of *Dacryodes edulis* fruits at 4-20 WAA of fruit development. Values are mean  $\pm$  SEM (\* =  $P < 0.05$ ).**



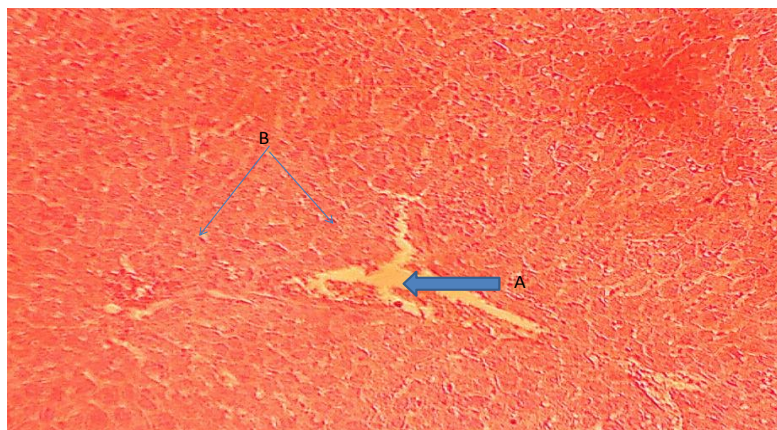
**Plate-3.1: (week 6). Microscopic representation of Liver (X40) section of Wister rats treated with oils extracted from *Dacryodes edulis* fruits of the immature stage of development. The histoarchitecture of the liver tissue shows (A) prominent central vein and (B) hepatocytes prominently visible and no tissue degeneration observed.**

The histoarchitecture of the liver is well organised with the hepatocytes visible and no

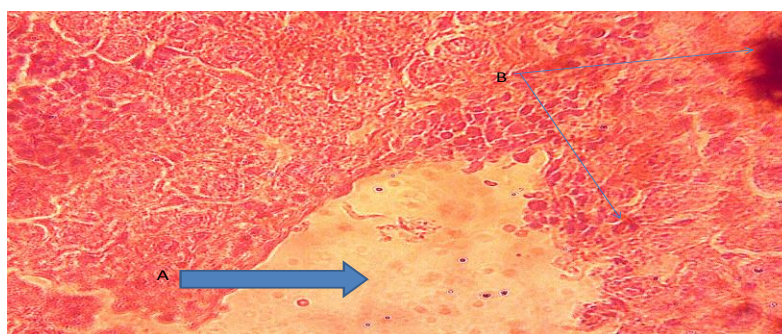
degeneration noticed but the central vein looks elongated.



**Plate 4.1 (week12).** Microscopic representation of Liver (X40) section of Wister rats treated with oils extracted from *Dacryodes edulis* fruits of the premature stage of development. The histoarchitecture of the liver tissue shows (A) prominent central vein and (B) hepatocytes prominently visible and no tissue degeneration observed.

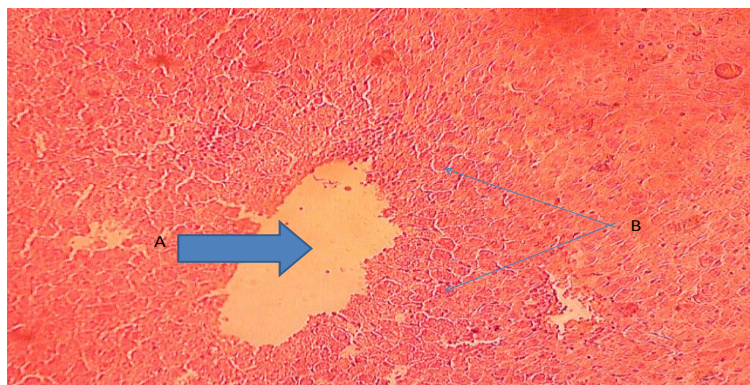


**Plate 4.7 (week 20):** Microscopic representation of liver (x100) section of Wister rats treated with extract of *Dacryodes edulis*. The histoarchitecture of the liver tissue shows (A) prominent central vein and (B) hepatocytes prominently visible and no tissue degeneration observed.



**Plate 3.4 Negative control:** Negative control: Microscopic representation of liver (X40) section of the Wister rats with Sodium Arsenate in distilled water at a dose of 1.0mg/kg. (A) The histoarchitecture of the liver is distorted and the hepatocytes visible and (B) degeneration noticed with patches of blood prominently visible in the central vein.





**Plate 3.5 positive control.** Microscopic representation of liver (x100) section of Wister rats treated with growers mash. The histoarchitecture of the liver is well organized, (A) prominent central vein, (B) hepatocytes visible and no degeneration noticed

## DISCUSSION

Today, there is a geometric increase in demands for vegetable oils in Nigeria for both domestic and industrial purposes. Increasing demand for vegetable oils in Nigeria, for both domestic and industrial purposes, in recent times, has been attributed to the increasing numbers of industries that requires oil and fat as their primary raw material [22]. It is therefore vital to determine the nutritive values of some of these products from tropical plants in Nigeria.

Thus, the efficacies of antioxidants are often associated with their ability to scavenged stable, highly reactive, free radicals. These free radicals have been implicated in the pathology of different numbers of diseases in human such as diabetics mellitus, arteriosclerosis, cancer and other phytochemicals play a vital role in removing free radical and inhibition of lipid peroxidation [17, 23, 24].

In this study, the DPPH scavenging ability of the *Dacryodes edulis* pulp oil had the highest values at the matured fruit stages (within week 18-20) than at the immature stages (within week 4-12). The scavenging ability of these components may be a reflection of the total activities of various components present [25, 26]. Indeed, several studies have reported that the antioxidant activity of most plants with therapeutic properties may be due to the presence of natural substances mainly the phenolic compounds [26].

Egg yolk homogenate as lipid rich media can undergo rapid non-enzymic peroxidation in the presence of ferrous sulphate with concomitant generation of malondialdehyde (MDA) and other aldehydes responsible for the formation of the pink pigments in the presence of thiobarbituric acid (TBA).

*Dacryodes edulis* oil extracts inhibited the lipid peroxidation (Matured fruits) compared to the immature fruits. This suggest that *Dacryodes edulis* oils has ingredients capable of inhibiting lipid peroxidation

[27]. *Dacryodes edulis* scavenged  $H_2O_2$  with higher scavenging abilities at the matured stage and the oil fraction of the fruits. This property may be attributed to their phenolic contents that donate electrons to  $H_2O_2$ , thus reducing it to water [28].

In the immature stages of the fruits development of *Dacryodes edulis*, there were characteristic depression of the antioxidant defend mechanism which attributed to the imbalance between the pro-oxidant load and the antioxidant defend system in the Wister rats. Similarly, the animals treated with the immature fruits extracts (week4 – 10) showed appreciably reduced (inhibition) activities of the antioxidant enzymes- superoxide dismutase (SOD), Catalase (CAT) as well as high level of malondialdehyde (MDA)—an index of lipid peroxidation – in the liver. This might be possible because in the immature state, the concentration of antioxidant status (phytochemicals and essential polyunsaturated fatty acids of the African pear were low, exposing their antioxidant defence system of the rats to damages by the pro-oxidant (Sodium Arsenate). But the concentration of the antioxidant status of the both fruit plants increased there after till fully matured stages (week 14 – 20) and were able to inhibits or prevent oxidation in the animals. Hence, the SOD and CAT increased and the MDA level were drastically reduced.

Malondialdehyde is the major oxidation product of peroxidised polyunsaturated fatty acids (PUFAs) and increase MDA levels an important indicator of lipid peroxidation. Catalase on the other hand, is an enzymatic antioxidant widely distributed in all animal tissues including the red blood cell and the liver. Catalase decomposes  $H_2O_2$  and helps protect the tissues from highly reactive hydroxyl radicals. SOD, another antioxidant enzyme which removes superoxide radicals by converting it to  $H_2O_2$  [23, 29, 30].

The enhanced oxidative stress in the animals treated with immature fruit extracts (week 4 – 10), were



however significantly reduced ( $P < 0.05$ ) in the animals treated with matured fruits (week 16 – 20) when compare with the control. The overall antioxidative capacity of the oil extracted from *Dacryodes edulis* suggest a positive correlation as well as synergistic effects with respect to DPPH scavenging activity, total phenol, flavonoids, anthocyanidins and essential polyunsaturated fatty acids components of the extracts [31]. But there is a slight variation between the two plants (fruits) in their effectiveness as antioxidant.

The serum concentrations of aspartate aminotransferases (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and L-γ-glutamyl transferase (GGT) determines the functionality and cellular integrity of the liver [32].

The enzyme ALT is most prevalent in the liver relative to other tissues or organs, where as AST may be found in heart, skeletal muscle and liver to nearly the same extent [33, 34]. Significance increases in the transaminase are associated with liver diseases such as toxic hepatitis, acute liver necrosis or hepatic cirrhosis. High levels of AST are often seen in haemolytic anaemia, myocardial infarction and cholestatic diseases of the liver [33]. The fractional increase in serum AST and ALT or the ratio of AST: ALT may be a useful tool in assessing the extent of liver damage. The liver cells contain more AST than ALT and with the later confined largely to the cytoplasm in which its concentration is higher than that of the former. With inflammatory or ineffective conditions such as viral hepatitis, the cytoplasmic membrane sustains the greater damage and the relative increase in ALT is higher than that of the AST. The situation is reverse in infiltrative disorders in which both the cytoplasmic and mitochondrial membrane are affected, resulting in a proportionality greater increase in AST relative to ALT [33].

In this study, the AST, ALT, ALP and GGT levels were generally decreased in wistar rats administered with oil extracted from *Dacryodes edulis* fruits revealed reduction in serum AST, ALT, ALP and GGT levels that were significant ( $P < 0.05$ ) at the matured stages of the *Dacryodes edulis*. The presence of the extracts and their metabolism in the experimental animals could cause changes of biochemical processes [35], thus decreasing the indicator of liver injuries [36]. The injuries which have resulted from the pro-oxidant (Sodium Arsenate) as revealed in the histopathological results are implicated in toxic liver injuries. ALP is located in the biliary duct of the liver [37] and obstruction of the duct increases the levels of the enzymes in the serum. The ALP result showed significant reduction of the enzymes in the animal serum with the administration of extracts from the fully matured fruits (Week 18-20). The decrease in ALP levels suggest that the fruit oils of study significantly inhibit damage or obstruction of the duct, as reported by

[38] that ALP increases is noticeable with most liver problems.

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