Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2014; 2(4B):1290-1294 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sjams.2014.v02i04.027

Research Article

Liver Status and Oxidative Stress Activity of Wistar Albino Rat Fed *Parkia Biglobosa* Seeds

Omeh Y. Saidu.*, Ojukwu C. Barnabas., Ejiofor U. Emmanuel.
Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

***Corresponding author**
Omeh Y. Saidu
Email: nduk41@yahoo.com

Abstract: The liver as a very important organ plays a central role in energy metabolism in the body. If the liver is impaired it could lead to serious clinical integrations. The study was designed to possibly investigate the effect of boiled *Parkia biglobosa* at different level of dietary incorporations on the liver and oxidative stress activity using Wistar Albino Rats. Twenty Wistar Albino Rats' age and sex matched were randomized into four groups. The control group was fed with growers mash, the group labelled A had 5% of *Parkia biglobosa* incorporated in their diet, the group labelled B had 10% *Parkia biglobosa* incorporated in their diet while the group labelled C had 20% *Parkia biglobosa* incorporated in their diet. The animals were fed for a period of 28 days after which they were sacrificed and serum collected from the blood for biochemical estimation. The result from liver function tests shows that there is a significant increase ($p<0.05$) of ALT, AST, ALP, TB, DB, protein, in the 5%, 10% and 20% as compared to the control. Result from oxidative stress reveals that there is a high level MDA and CATALASE in the 5%, 10% and 20% ($p<0.05$) as compared to the control. The increase in high level of serum liver enzyme markers observed in the test rats may indicate that *Parkia biglobosa* exhibited some hepatotoxic effect.
Keywords: Parkia Biglobosa, AST, ALT, ALP, Catalase, MDA

INTRODUCTION

Parkia biglobosa belongs to the large family *Leguminoseceae.* The pods are flat, large, irregular dusters from which the locust bean seeds are obtained [1].

The seeds of *P. biglobosa* are fermented for production of food condiments in Nigeria and other West African Countries. The locust bean is rich and provides valuable protein in the dry season [2]. Medicinaly it is used as mouth wash to relieve toothache. Many food products are prepared by fermentation process with the action of micro-organism [3].

The study was designed to possibly investigate the effect of boiled *Parkia biglobosa* at different level of dietary incorporations on the liver and oxidative stress activity using Wistar Albino Rats.

MATERIALS AND METHOD

Collection and Identification Of Plant Materials

The *P. biglobosa* were collected from Apeh Ezeocha herbal garden at Awokwuru, Olido in Enugu- Ezike, Igbo-Eze North L.G.A of Enugu state and was identified by Mr. A. Ozioko of Bioresources Development and Conservation Programme, (BDCP), Aku Road, Nsukka, Enugu state.

Animals

Mature Wistar Albino Rats sex and age matched weighing between 100g to 165g obtained from the laboratory animal units of the faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the experiment. The animals were kept in a well-ventilated stainless steel cages at room temperature of about 28^0C. Normal feed and clean drinking water was provided to the animals until the time of the experiment. The animals were allowed 1 week for acclimatization in the animal house of the department of biochemistry, Michael Okpara University of Agriculture, Umudike, before the experiment and ethical rules guiding the use of laboratory animals according to Zimmerman [4] strictly followed.

Preparation of *P. Biglobosa* Seed Meal for Animal Feeding

The *Parkia biglobosa* seeds were oven dried at the temperature of 40^0C for 60 minutes, after the seeds were de-hauled to separate the seed coat from the inner seed. The seed coat was discarded and the inner seed boiled before grinding into powdery form also with the

aid of a mechanical grinder and stored in a refrigerator at 15^0C until ready for use.

***Parkia Biglobosa* Seed Meal Inclusion Diet Preparations**

The milled parkia seeds were incorporated at different percentages (5, 10 and 20%) with the normal feed for the different group of test animals while the control groups had normal feed only. The animals were fed for a period of 28days.

Preparation of Sample for Biochemical Assay

Collection of blood sample

The wistar albino rats were scarified by dazing and the blood collected through cardiac puncture. The pooled blood from each group was centrifuged at 300rpm for 30 minutes after which the serum was collected and kept in the refrigerator for biochemical analysis.

Chemicals

All the chemicals used in this study were of analytical grade.

Biochemical Analysis

Alanine Amino Transferase

The activities of ALT were determined using Reitman and Frankel [5] method.

Aspartate Amino Transfarse

The activities of AST were determined using Reitman and Frankel [5] method.

Alkaline Phosphatase Activity

The activity of Alkaline phosphatase was determined using method described by King and King [6].

Conjugated Bilirubin (CB) and Total Bilirubin (TB)

The conjugated and total bilirubin were determined using the Max Discovery ^{Tm} Total bilirubin Assay Kit and Diazyme's Direct Bilirubin Vanadate Oxidation assay by the colorimetric method described by Jendrassik and Grof [7].

Determination of Total Protein

The serum should be free from hemolysis and if not to be used immediately should be stored at 2-8◦C.The principle of total protein determination is that at alkaline pH value, proteins form a stable complex with Cu^{2+} ions, which is photometrically measured as contained in the QCA Test kit [8].

Test for Malonaldehyde

The (NWLSS NWK-MDA01) North West live science specialities assay for MAD was used and this is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); forming a MDA-TBA_2 adduct that absorbs strongly at 532 nm [9].

Catalase Assay

The Beers and Sizer [10] method was used. And the disappearance of peroxide is followed spectro-photometrically at 240 nm

Statistical Analysis

The data gotten were analysed by one way anova (LSD) using SPSS Ver. 16.0. The mean were reported with standard deviation. Significant difference was accepted at 0.05 level of probability.

RESULTS AND DISCUSSION

Alanine Amino Transferase

The result of serum ALT concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 2. The result showed ALT significant increase ($p<0.05$) in ALT concentration of 20, 10 and 5% group when compared with the control group.

Thus giving the value of control group as 44.00±3.26, 20% group as 114.00±1.63, 10% group as 110.00±1.63 and 5% group as 94.00±3.26 as the concentration of ALT in the serum.

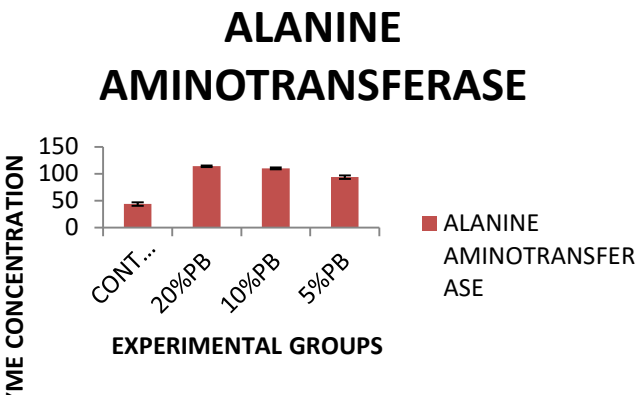


Fig. 1: ALT concentration in test and control animals

Aspartate Amino Transferase

The result of serum AST concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 1. The result showed ALT significant increase ($p<0.05$) in ALT concentration of 20, 10 and 5% group when compared with the control group.

Thus giving the value of control group as 80.00±1.63, 20% group as 126.00±3.26, 10% group as 110.00±1.63 and 5% group as 90.00 ± 3.26 as the concentration of AST in the serum

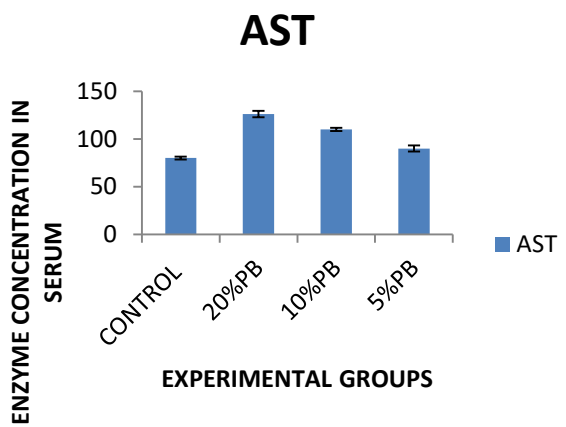


Fig. 2: AST concentrations in test and control animals

Alkaline Phosphatase

The result of serum ALP concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 3. The result showed ALP significant decrease ($p<0.05$) in ALP concentration of 20, 10 and 5% group when compared with the control group.

Thus giving the value of control group as 60.00±3.26, 20% group as 45.00±1.63, 10% group as 45.00±3.26 and 5% group as 43.00±1.63 as the concentration of ALP in the serum

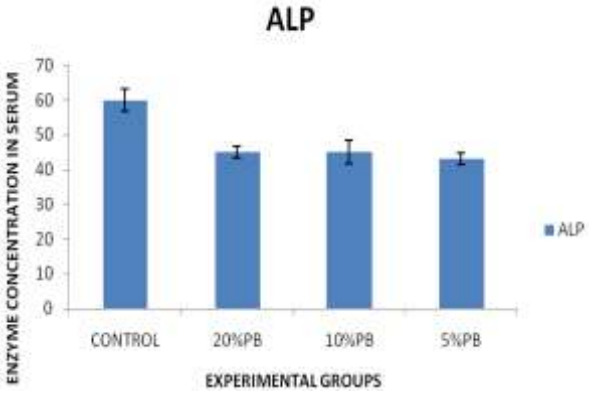


Fig. 3: ALP concentrations in test and control animals

Total Bilirubin

The result of serum TB concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 4. The result showed TB significant increase ($p<0.05$) in TB concentration of 10 and 5% group when compared with the control group.

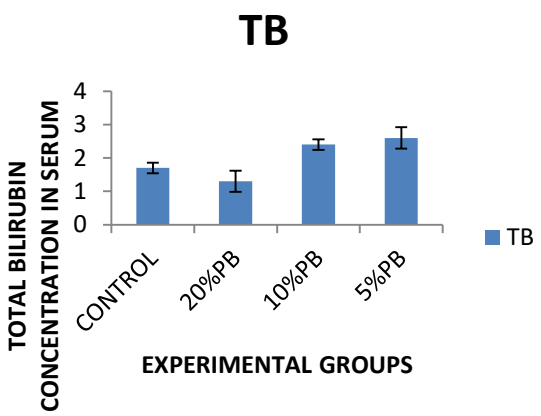


Fig. 4: TB concentration in test and control animals

Direct Bilirubin

The result of serum DB concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 5. The result showed TB significant increase ($p<0.05$) in DB concentration of 20% group when compared with the control group.

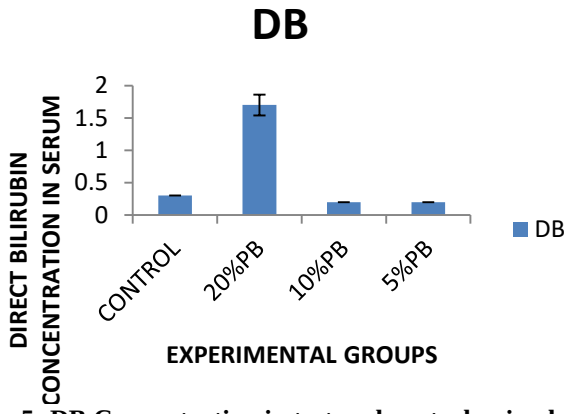


Fig. 5: DB Concentration in test and control animals

Serum Protein

The result of serum protein concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 4. The result showed TB significant increase ($p<0.05$) in protein concentration of 20% group when compared with the control group.

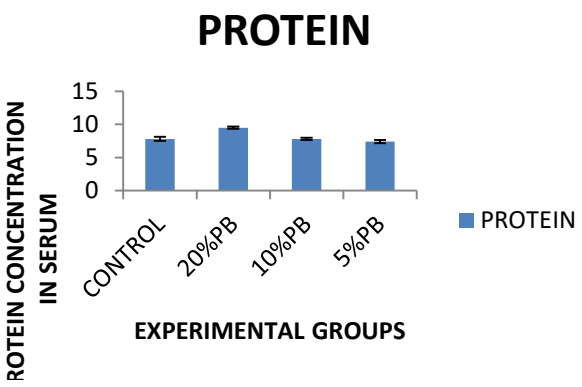


Fig. 6: Protein Concentration in test and control animals

Serum Protein

The result of serum MDA concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 7. The result showed MDA significant decrease ($p<0.05$) in MDA concentration of 20 and 10% group when compared with the control group.

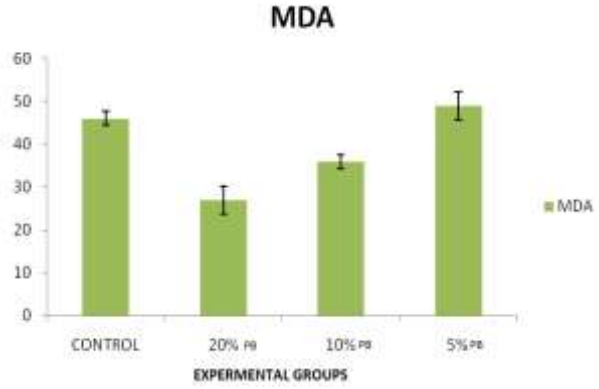
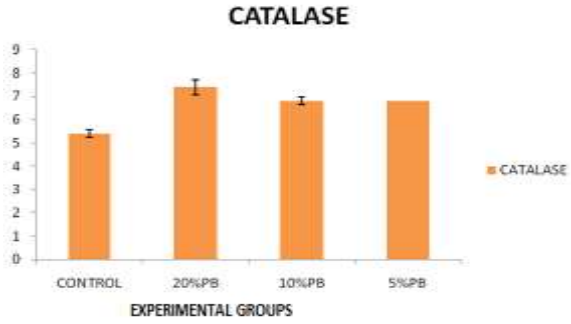


Fig. 7: Malondialdehye concentration in test and control animals

Catalase

The result of serum catalase concentration level of rat fed with diet containing grounded *Parkia biglobosa* at different percentage (%) incorporation in diet is presented in Fig. 8. The result showed MDA significant increase ($p<0.05$) in catalase concentration of 20, 10 and 5% group when compared with the control group.



DISCUSSION

Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase are periodically produced by hepatic and other cells. These enzymes are capable of leaking out into the blood stream in the situation of liver cirrohosis or loss of liver integrity. The result reveals there was immense damage produced by the dietary incorporation on the liver. It can be inferred that serum ALT level significantly increased against as well as serum AST level.

ALT and AST catalyses the transfer of amino group into ketoacid, that can be used in the biosynthesis of certain macromolecules, viz DNA protein etc.

In increase in Liver Enzyme concentration as it appeared in blood serum revealed they were leaked into the blood. ALT and AST are significantly ($p<0.05$) higher and this can result to liver disease, hepatitis and liver malfunction.

The result obtained from the liver function test assay shows that the liver function and serum protein analysis carried out on test group had an high value than the control. According to Roschester [11] any rise in serum bilirubin and conjugated bilirubin above 10mg/dl is abnormal and an indication of liver damages and disease. Then increase in ALT, AST, ALP and serum protein is an indication of liver damages and diseases ranging from viral, hepatitis, cirrohosis, nephritic syndrome and bile duct obstruction.

The results obtained from the serum lipid peroxidation and antioxidants level analysis shows that Malondialdehyde is formed by the degradation of polyunsaturated lipids by reactive oxygen species. It is an end product of enzymatic and oxygen radical-induced lipid peroxidation [12]. This reactive species occurs naturally and is an unstable maker for oxidation stress and decomposes to form complex, reactive byproducts that cause cellular damages to the membrane. The increase level of MDA in experimental animals is an indication that the oil is prone to perodixation which could be due to the high amount of polyunsaturated fatty acid present in the oil that are liable to attacks by free radicals.

The result obtained from serum catalase analysis shows that catalase which is an anti-oxidation enzyme which is used to mop up free radicals produce from lipid peroxidation of MDA. The increase level of catalase in the experimental rats indicates that there was high activity of catalase in the experimental rats due to peroxidation of the polyunsaturated fatty acid present in *Parkia biglobosa*.

This study demonstrates that dietary incorporation of boiled a *Parkia biglobosa* at 5%, 10% and 20% decrease the concentration of liver enzymes in the liver, and thus it is not liver friendly and its use should not be encouraged.

The findings of this study suggested that there is evidence of liver damages as there is an increase in the concentration of liver enzyme in the serum, these effect can lead to edema, cirrohosis and vasculation of the liver tissues.

REFERENCES

1. Omafuvbe BO, Falade OS, Osuntogun BA, Adewusi SRA; Chemical and biochemical compositon changes in African locust beans *Parkia biglobosa* and melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pak J Nutr., 2004; 3(3): 140-145.
2. Odunfa SA; Legume-based Fermented Foods. CRC Press Inc., Boca Raton, FL., 1986: 173-189.
3. Tweyongyrere R, Katonogole I; Cyanogenic potential of cassava peels and thrie detoxification for utilization as life stock feeds. Vet Hum Toxicol., 2002; 44(6): 366-369.
4. Zimmermann M; Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 1983; 16(2): 109-110.
5. Reitman S, Reitman S, Frankel SA; Colorimetric determination of serum glutam oxalo-acetatic and glutamic pyruvic transaminase. Am J Clin Pathol., 1957; 28(1): 56-63.
6. Kind PR, King EJ; Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol., 1954; 7(4): 322–326.
7. Jendrassik L, Grof P; Colorimetric Method of Determination of bilirubin. Biochem Z., 1938; 297: 81-82.
8. Weichselbaum CTE; An accurate and rapid method for determination of protein in small amounts of blood serum and plasma. Am J Clin Pathol., 1946; 16: 40.
9. Analytical methods for resolving data from TBA2-MDA reaction mixtures. Available from https://www.nwlifescience.com/products/assaykit/tba-mdaanalysis.htm
10. Beers RF Jr, Sizer IW; A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem., 1952; 195(1):133-140.
11. Rochester M; Laboratory reference values. Foundation for Medical Education and Research, 2010.
12. Marnelt LJ; Lipid Peroxidation – DNA damage by malondialdfehyde. Mutation Research, 1997; K424 (1-2): 83-95.