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Research Article
Effects of Fermented *Parkia biglobosa* Seeds at Varied Dietary Inclusion on Liver Function of Albino Wistar Rat

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Abstract
Background and Objective: The popularity of contemporary emerging artificial condiments and their hazards on human health call for more empirical scientific evidences on the health indices of fermented *Parkia biglobosa* seeds. This study determined the effect of fermented *Parkia biglobosa* seeds on the serum levels of Total Bilirubin (TB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and associated histological changes of hepatocytes. **Materials and Methods:** The seeds were purchased at Bagana Market in Kogi state and fermented using traditionally method. Thirty five young adult Albino Wistar rats with average weight of 102 g were purchased from Institute of Veterinary Research, Vom, Plateau State. The rats were separated into 5 groups of 7 and allowed to acclimatize for 7 days before the commencement of the experimental feeding with 10, 20 and 30% of the FSB in the meals and 100% meals of FSB for 672 h in 4 groups while rats in the 5th group were fed with 100% pelletised grower mash for the same number of hours. They were fed *Ad libitum* with the meals and table water throughout the period. The blood and livers were harvested for enzyme assay using enzymatic assay kit obtained from Randox Laboratories Ltd. Co. Antrium, United Kingdom as well as light microscopy after staining the liver tissues with haematoxylin and eosin. **Results:** Analysis of variance showed significant increase ($p \leq 0.05$) in the level of TB. However, hepatocytes, sinusoids and hepatic central vein showed no visible significant effect ($p>0.05$) on ALT and AST levels. The FSB inclusion at all percentages appeared not to show any negative effect on liver function of the Albino Wistar rats. **Conclusion:** Consistent intake of condiment prepared from fermented *Parkia biglobosa* seeds played significant role in the liver health by protecting the architecture of the liver and its functions.

Key words: *Parkia biglobosa*, liver health, alanine aminotransferase, aspartate amino transferase, hepatic central vein, total bilirubin, hepatocytes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The African locust bean tree, *Parkia biglobosa* is a perennial leguminous tree[1] which belongs to the family leguminosae and the subfamily mimosoideae[2,3]. It grows in the Savannah region of West Africa up to the southern edge of the Sahel zone 13°N. In Nigeria, the tree is not normally cultivated but found in the wild in the Savannah region[4]. Amoa-Awua *et al.*[5] reported that the raw *P. biglobosa* seeds are naturally deficient and unpalatable but when fermented into condiment, the physical, chemical and nutritional characteristics of the seeds change.

The FSB serve primarily as a condiment[6]. Its regular consumption as condiment may represent a good dietary alternative for the control of hyperlipidaemia and associated conditions[7]. The brown seeds are ground into a pungent nutritious condiment which is added to soups and stews throughout the Savannah regions of sub-saharan African. The use of these fermented seeds date back many centuries and its use was already described in the 14th century[8]. The production and sale of FSB constitute an important economic activity for women across West-Africa[9]. They are consumed widely especially in developing and under-developed countries where animal protein is limited as a result of economic, social and cultural factors[10]. Hassan and Umar[11] reported that seeds of *P. biglobosa* are good source of protein as well as essential amino acids including sulphur containing amino acid. Fermentation of these seeds enhances protein and lipid enrichment and reduces total carbohydrate present[12]. Souleyman[13] reported that the fermented seed is believed to be good for the treatment of arterial hypertension.

The liver is the largest internal organ of the body and the largest gland tissues. The liver cells have a multiplicity of functions and these functions play a central role in maintaining life[14]. The liver uses enzymes to get rid of the waste produced in the body as a result of body processes and by the breakdown of drugs, alcohol and medications[15]. Liver enzyme test is used to check how well the liver functions. This test measures the liver's synthesis of proteins (Albumin and Platelets), proper breakdown of substances and its ability to metabolise drugs[16]. The liver has the ability to repair itself[17].

The liver bio-transforms food nutrients brought to it from the visceral by the hepatic portal vein into forms that can be assimilated by the body cells. The FSB as condiment is therefore key to the wellness of hepatocytes. Indicators of this wellness include factors such as levels of ALT, AST and TB in the blood as well as the architecture of hepatocytes. Empirical evidence of the effect of FSB on these factors are not available. This research was therefore, aimed at evaluating the effect of diet with varied inclusion levels as well as 100% diet of FSB as condiment on serum levels of ALT, AST, TB and associated histological changes of hepatocytes of the Albino Wistar rat.

MATERIALS AND METHODS

Ethical Approval: Ethical approval was obtained from the Ahmadu Bello University, Animal Use and Care Committee before the commencement of the study.

Sample collection: The study was carried out between May, 2017 and October, 2017. The *P. biglobosa* seeds were purchased from Bagana market in Omala Local Government Area, Kogi state. Traditional method of fermentation was used to ferment the seeds. The seeds were boiled in stream water, using a large metallic pot, with wood fire for 6 h (this period may vary according to the intensity of the wood fire). Water was added at intervals to replace the lost quantity of water through vapourization. This kept the seeds always immersed in boiling water. At the end of the 6 h period, the seeds were pounded lightly using wooden mortar and pestle. This was to separate the seed coat from the cotyledons. The flaps of integument were removed by scrubbing between the palms of the hand and washed with water. The cotyledons were then boiled for 1 h with fresh water after which they were poured into a basket made of oil palm fronds and covered with enough banana leaves to prevent rapid and excessive heat loss. The "incubated" seeds were then kept for 72 h at ambient temperature to obtain fermented seeds. The fermented seeds were then crushed using a milestone, into a brownish paste with characteristics pungent smell. The paste was molded into flakes and small triangular forms and kept in the sun, protected from dust and insects, to hasten drying. The sun drying was done for about 50 h. They were then pulverized using wooden mortar and pestle. The powder was kept in an air-tight plastic container ready for use.

The Albino Wistar rats were purchased from Institute of Veterinary Research Vom, Plateau state. The 35 Albino Wistar rats were separated into 5 groups of 7 each.

Feed preparation: The FSB was included in the feeds at 10, 20 and 30% as follow:

$$X \times \frac{10}{90}$$

$$X \times \frac{20}{80}$$

$$X \times \frac{30}{70}$$

Where:

X = Weight in grams of FSB in the pure feed (100% pelletized growers mash)

× = Multiplication sign

Y = Weight of pure feed in grams (g)

- $(\frac{X \times 10}{90})$g g of FSB+Yg of pure feed = 10% inclusion e.g., 100 g of FSB in 900 g of pure feed
- $(\frac{X \times 20}{80})$g g of FSB+Yg of pure feed = 20% inclusion e.g., 200 g of FSB in 800 g of pure feed
- $(\frac{X \times 30}{70})$g g of FSB+Yg of pure feed = 30% inclusion e.g., 300 g of FSB in 700 g of pure feed

The appropriate weight of FSB in each case was thoroughly mixed with Yg of the pure feed. Some quantity of table water was used to mold the mixture into balls that were dried in the sun protected from dust and insects.

- 100% FSB as feed
- 100% pelletised growers mash as feed (control group, CG)

Feed regimen: The rats in each group were fed with their meals and table water *ad libitum* for 672 h. The animals were sacrificed and the serum was separated from the blood cells by centrifugation for 5 min at 4,000 revolutions per minute (rpm) using centrifuge 5702 model and removing the serum into labeled specimen bottles using a pipette. The bottles were then kept in a refrigerator at 4°C until used. The ALT, AST and TB were assayed as described by Rizk and Ibrahim[18], using diagnostic kits for the assays of ALT, AST and TB obtained from Randox Laboratories Ltd. Co. Antrium, United Kingdom.

The livers were fixed in 10% buffered formalin and stained with haematoxylin and Eosin. They were then observed under light microscope for histological changes using eye piece of magnification ×10 and objective ×40. Photomicrographs of stained tissues were taken using Amscope digital camera for microscope (DCM 500), 5 M pixels, made in Japan.

Statistical analysis: One way analysis of variance (ANOVA) was used to analyze the results which were expressed as the mean of seven replicates±standard error. The $p \leq 0.05$ was regarded as significant. The statistical package for social sciences (SPSS) computer software version 21.0 was used for data analysis.

RESULTS

***P. biglobosa* fed group:** The ALT levels at varying percentage inclusion of *P. biglobosa* revealed highly significant difference ($p \leq 0.01$) with the 100% (66.50 IU L^{-1}) having the highest ALT level. Meanwhile, the lowest ALT level was observed in 20% (34.50 IU L^{-1}) which was not significant ($p > 0.05$) from the control (41.63 IU L^{-1}) and 10% (39.67 IU L^{-1}) (Table 1).

The AST level in Albino Wistar rats was highly significant ($p \leq 0.01$) with the highest at 100% (128.88 IU L^{-1}) which was not significantly different from the control (119.75 IU L^{-1}). Meanwhile, the lowest AST level was at 20% (91.38 IU L^{-1}) which was not significantly different ($p > 0.05$) from the 10% (103.56 IU L^{-1}) and 30% (98.38 IU L^{-1}).

The TB level was highly significant ($p \leq 0.01$) with the highest (14.88 IU L^{-1}) at 100% and the lowest (9.13 IU L^{-1}) in the control group.

Histological analysis: Liver sections of the 5 groups showed the liver architecture: The hepatic Central Vein (CV), Sinusoids(S) and hepatocytes (H) (x400) H and E.

In the Control Group (CG), the liver architecture remain normal with the hepatic Central Vein (CV), Sinusoids (S) and most of the hepatocytes (H) appeared normal (x400) H and E (Fig. 1).

In the 10% FSB group, the liver section architecture appeared normal with slightly dilated hepatic Central Vein (CV) showing most of the hepatocytes (H) and sinusoids (S) appeared normal (×400) H and E (Fig. 2).

In the 20% FSB group, the liver general architecture and hepatocytes appeared normal with slightly dilated hepatic Central Vein (CV) and sinusoids (S) (×400) H and E (Fig. 3).

The 30% FSB group had most of the hepatocytes (H) appeared viable. Although, the terminal hepatic Central Vein (CV) and the sinusoids appeared dilated with notable inflammatory (lymphocytic) cells infiltration (LC) mostly within the hepatic lumen (×400) H and E (Fig. 4).

Table 1: Effect of varying percentage inclusion of FSB on the ALT, AST and TB levels in Albino Wistar rats

Concentration	ALT (IU L^{-1})	AST (IU L^{-1})	Total bilirubin (IU L^{-1})
FSB (10:90)	39.67±2.98^{c}	103.56±3.10^{b}	10.22±0.47^{cd}
FSB (20:80)	34.50±1.07^{c}	91.38±3.75^{b}	10.63±0.50^{bc}
FSB (30:70)	50.75±5.12^{b}	98.38±6.71^{b}	11.88±0.58^{b}
FSB (100:00)	66.50±2.69^{a}	128.88±2.34^{a}	14.88±0.30^{a}
CG	41.63±2.50^{c}	119.75±5.30^{a}	9.13±0.35^{e}
Total	46.44±2.21	108.27±2.88	11.32±0.36
p-value	0.000**	0.000**	0.000**

Means with the same alphabet(s) along column are not significantly different ($p > 0.05$), **Highly significant at $p \leq 0.01$

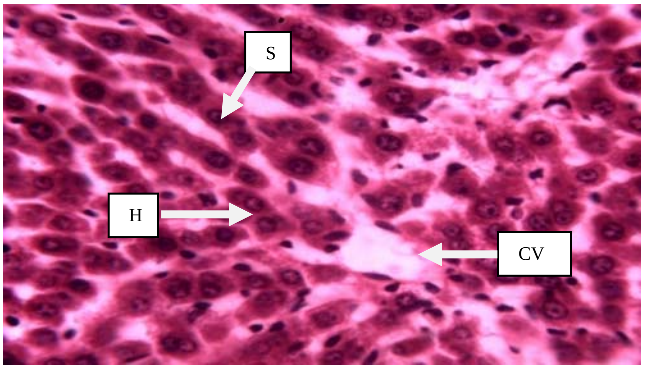

Fig. 1: Liver section of the control group. The hepatic Central Vein (CV), sinusoids (S) and most of the hepatocytes (H) appeared normal (×400) H and E

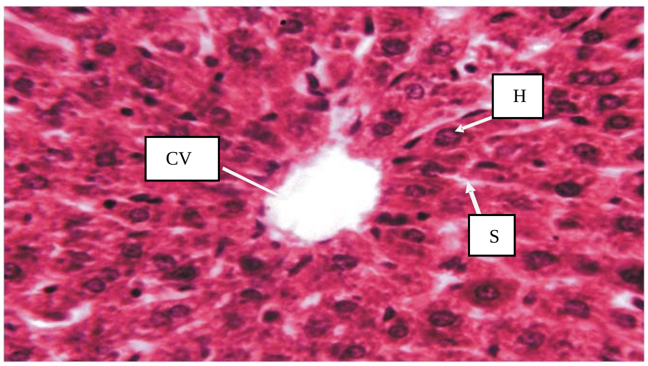

Fig. 2: Liver section of 10% FSB group showing dilated hepatic Central Vein (CV) and normal hepatocytes (H) and sinusoids (S) (×400) H and E

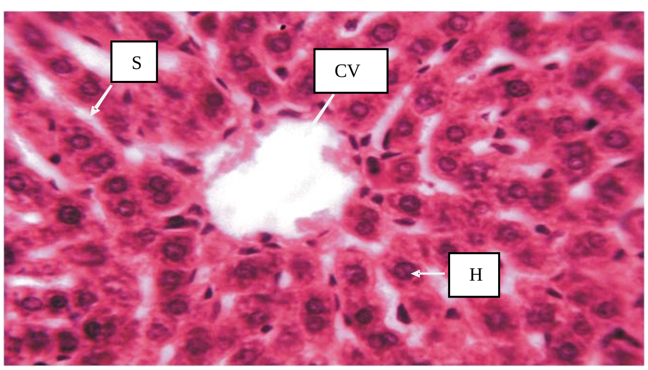

Fig. 3: Liver section of 20% FSB group with normal liver architecture and hepatocytes showing slightly dilated hepatic Central Vein (CV) and sinusoids (S) (×400) H and E

The liver section of 100% FSB group had most of the hepatocytes and sinusoids (S) appeared normal with congested hepatic Central Vein (CV) (×400) H and E (Fig. 5).

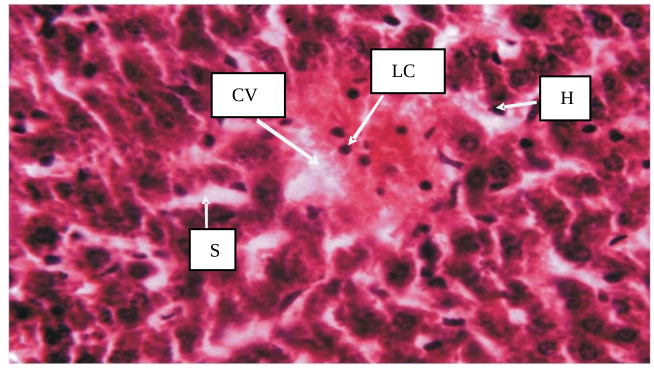

Fig. 4: Liver section of 30% FSB group with terminal hepatic Central Vein (CV) and dilated sinusoids (S). Infiltration of lymphocytic cells (LC) within the hepatic lumen(×400) H and E

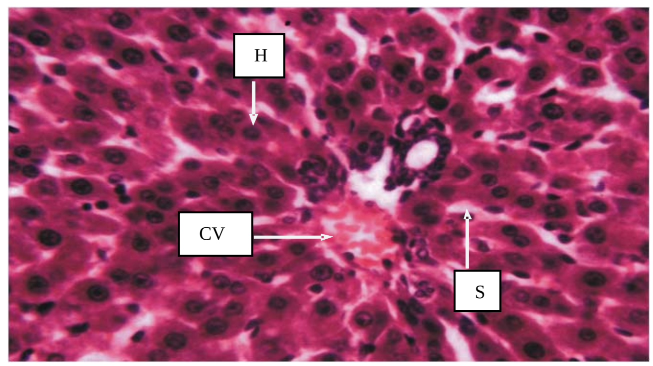

Fig. 5: Liver section of 100% FSB group with most of the hepatocytes and sinusoids (S) appeared normal with congested hepatic central vein (CV) (×400) H and E

DISCUSSION

The FSB incorporated with pure feeds at different inclusion level in this study revealed that ALT and AST decreases in the liver cells with the best obtained when FSB was used at 20% inclusion level with pure feeds while total bilirubin levels did not differ significantly from the control group. Ajiboye *et al.*[19] in their study on the fruit pulp of *P. biglobosa* phenolic extract observed similar decrease in ALT and AST level in both liver and serum of rats. The 100% FSB (without pure feeds) showed higher ALT, AST and total bilirubin than the control group, this signified that too much usage of FSB can increase the production of these enzymes by the liver while moderate use of FSB in combination with pure feeds will decrease the enzymes production. The ALT and AST are well known transaminases used as biomarkers to predict possible toxicity in the blood of sick animals[20]. High quantity of these enzymes can lead to hepatocellular injury or necrosis as reported in the studies of Kim *et al*.[21] and Lee *et al*.[22].

Alanine aminotransferase (ALT) is an enzyme that catalyzes the transfer of amino groups to form the hepatic metabolite oxaloacetate. Kim *et al*.[21] reported that if ALT is found abundantly in the cytosol of the hepatocyte in high quantity, it leads to hepatocellular injury or death as well as increased damage of liver cells. Pratt and Kaplan[23] demonstrated that an elevated ALT is widely used as a marker of liver damage. Yueh *et al*.[24] increased ALT level to future risk of metabolic syndrome components such as obesity and diabetes. Westerbacka *et al*.[25] and Lee *et al*.[22] in their studies reported that elevated ALT is associated with liver fat accumulation which can lead to insulin resistance. Insulin resistance leads to increased free fatty acids that were absorbed by the liver[26]. With the above reports, the decreased ALT and AST levels observed in this study by the use of FSB incorporated with pure feeds showed that FSB usage will help largely in saving and protecting the liver from damage as well as protecting individuals from liver related health issues. The ALT value of greater than or equal to 40 IU L^{-1} is regarded as abnormal by most medical laboratories[22]. Also, the ALT value at 20% inclusion level was 34.50 IU L^{-1} which is below the abnormal level.

The AST catalyse transamination reaction. Higher AST level is an indication of risk to liver diseases such as liver tissue degeneration and necrosis[27]. The low AST level that results on the usage of FSB incorporated with pure feeds is an indication of the significant role of the *Parkia biglobosa* in preventing liver tissue degeneration and necrosis.

Bilirubin is the catabolic product of haemoglobin produced within the reticuloendothelial system, released in unconjugated form which enters into the liver[28] where it is used to produce bile and it served as an important factor in lipid metabolism. Diana[29] stated that the normal serum total bilirubin varies from 2-21 IU L^{-1}. The total bilirubin level observed in this study was within the acceptable limit.

The ALT, AST and TB are traditional biomarkers used widely for detecting drug-induced liver injury[30]. In this study therefore, increase or decrease in serum levels of these biomarkers is defined by comparing the values obtained from the test animals with the values obtained from the control group.

Micrographs of histological changes of the liver tissues appeared to have normal liver architecture, hepatocytes and sinusoids with either slight dilation (10, 20, 30%) or congestion (100%) of hepatic central vein. This could be due to the quality of the FSB as a result of the processing method used. The result corroborated with the study of Ayo-Lawal *et al*.[7] on the investigation of the hypolipidemic potential of FSB by supplementation in feeds of Albino Wistar rats.

CONCLUSION AND RECOMMENDATION

This study revealed that food sources incorporated with appropriate fermented seeds of *Parkia biglobosa* help to maintain the liver architecture as well as improve its functions. The information provided by this study can be used by feed industries as a guide by incorporating the appropriate quantity of fermented seeds of *P. biglobosa* into feeds. Also, future studies on the proximate analysis of the fermented seeds to reveal its nutritional contents will help by providing the necessary information to food nutritionist and consumers. Seminars and workshops on the use of fermented seeds of *P. biglobosa* as alternative to contemporary synthetic condiments should be organized.

SIGNIFICANCE STATEMENT

This study discovered that *Parkia biglobosa* when used moderately alongside with feeds played an important role in reducing ALT, AST and keeping the total bilirubin stable. This study will help researchers and nutritionist formulated the appropriate inclusion level of this condiment needed for human consumption.

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