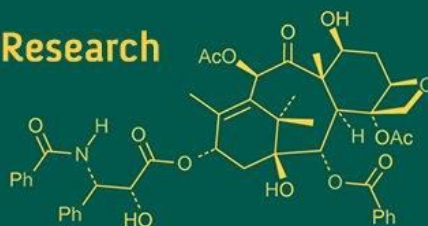


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Effect of African Locust Bean Flour, Wheat Bread and African Locust Bean Composite Wheat Bread on the Histology of Liver in Diabetic and Non Diabetic Wistar Rats

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Abstract

The aim of this research is to investigate the effect of African locust bean flour, wheat flour bread and African locust bean composite wheat flour bread on the histology of the liver in diabetic and non-diabetic wistar rats.

Method: 60 rats were divided into 12 groups of 5 animals per group labeled A to L. Five groups specifically A to F were tag non diabetic group while G to L were tag diabetic group. Group G to L had diabetes induced in them using alloxan at 150mg /kg body weight in overnight fasted rat and the diabetic state was confirmed after 72 hours with blood glucose of 400mg/dl considered diabetic. The rats were fed with African locust bean flour, whole wheat bread and African locust bean composite wheat bread at ratio 5:95, 10:90 and 15:85 respectively for 14 days. On the 14th day, the rat were sacrificed and the liver organ harvested for histological examination.

Result: Group A to F (non diabetic wistar rats) had normal liver morphology and show no sign of liver damage which signifies that the African locust bean flour, wheat bread and African locust bean composite wheat bread had no negative effect on the liver neither did it cause any form of liver damage.

Similarly, Group G to K (diabetic wistar rat) had normal liver morphology and show no sign of liver damage which signifies that the African locust bean flour, wheat bread and African locust bean composite wheat bread had no negative effect on the liver neither did it cause any form of liver damage but rather may have offered hepatoprotective effect that might have resulted from alloxan induced diabetes.

Lastly, Group L (diabetic wistar rat) had liver steatosis which indicate liver damage that might have resulted from alloxan induced diabetes since the rats weren't given any treatment or special feed.

Conclusion: It can therefore be concluded that African locust bean flour, wheat bread and African locust bean composite wheat bread has hepatoprotective effect which suggest the trio as good nutritional feed for diabetic patient. Its therefore recommended that, the effect of the trio on fasting blood sugar and biochemical marker enzymes of the liver be investigated for proper nutritional guide and medical intervention.

Keywords: Wheat, African locust bean, liver, histology, diabetic, non diabetic

Introduction

Diabetes is characterized by impaired carbohydrate, protein and fat metabolism that result from poor insulin secretion, insulin resistance or both. Diabetics mellitus is currently one of the most prevalent and life-threatening metabolic disorder globally (Sara *et al.*, 2021) ^[19].

Diabetes Mellitus is considered as a chronic disease affecting 537 million persons globally and it's expected to rise to 783 million by 2030 (Mohammed *et al.*, 2024). There is a direct link between diabetes and liver disease as the initial is capable of causing damage to the latter. Spectrum of liver diseases is known to occur in type II Diabetes Mellitus (DM). This includes abnormal liver enzymes, Non-Alcoholic Fatty Liver Disease (NAFLD), cirrhosis, hepatocellular carcinoma and acute liver failure. The prevalence of NAFLD in type II DM is 34-74% (Powell *et al.*, 1990; Lee 1989) ^[16, 9] and in diabetes with obesity, virtually 100% (Silverman 1990; Usha *et al.*) ^[20].

As part of the mechanism to reduce the growing trend of diabetics, there is need to look into nutritional means, as several plants possess hypoglycemic ability and feeding on such plant could help reduce the onset of diabetes and liver disease as well as its management.

Parkia biglobosa is commonly known as African locust beans grown in many countries in Sub Saharan Africa including Nigeria. It is native to West Africa and called different local names such as Kinda in Sierra Leone, Kpalugu in Northern Ghana, Nere in Burkina Faso amongst others.

In Nigeria, the seed (Kalwa in Hausa, Iyere in Yoruba) are traditionally used as food condiments (Dawadawa in Hausa, Iru in Yoruba, Ogiri in Eastern Nigeria) (Ayarinde *et al.*, 2023) [4].

Parkia biglobosa is grown in Nigeria as follows. Northern States such as Kano, Katsina, Zamfara, Kebbi, Bauchi, Jigawa, Yobe, Gombe, Taraba. Middle Belt States such as Kwara, Niger, Plateau, Kogi, Benue, Nassarawa, Kaduna and South-West States such as Oyo, Ondo, Osun, Ekiti. It's traditionally use as seasoning, medicinal agent, wound healing agent, treatment of malaria, bronchitis, pneumonia, ulcers, skin infection, diarrhea and hypertension (Airaodion *et al.*, 2019, Peter *et al.*) [1].

Wheat (*Triticum* Species) is one of the most widely cultivated crops in the world today. According to the Food and Agriculture Organisation, the global area harvested under wheat in 2019 accounted for about 216 million hectares and the global wheat production in 2019 accounted for about 766 million metric tons. But its production in Nigeria is plagued with challenges that have limited production area to less than 75 thousand hectares and reduced harvests to a mere 55 thousand metric tons (Boluwade, 2022) [5].

The most important wheat species in Nigeria is the Common wheat or Bread wheat, scientifically known as *Triticum aestivum* and is generally used to make flour-bread. Pasta wheat is mainly used to make Semolina then Spaghetti, Macaroni and Noodles. It is also known scientifically as *Triticum durum* but represents only about 10% of wheat consumption in Nigeria (Tidiane-Sall *et al.*, 2019). Key wheat producing States in Nigeria are Jigawa, Kano, Borno, Adamawa, Plateau and Sokoto grown during the harmattan (cold) season October to January. Wheat is also classified based on its adaptation to different growing climates. Winter wheat requires a cold temperature to induce flowering, over a period of dormancy known as vernalization. While, spring wheat can grow well under higher temperatures. Hence the reason why spring wheat is the only type that can grow optimally in tropical and subtropical regions like Nigeria (Salihu Baba Alkali, 2024) [17].

Materials and Methods

Collection & identification of sample

African locust bean and Wheat grain were purchased from a farm in Kano, and identify and deposited at the Herbarium Unit of the Department of Plant Science and Biotechnology, Faculty of Life Science, University of Benin by Professor Akinnibosun with voucher number UBH -P429 for *Parkia biglobosa* (African locust bean) and UBH-T555 for *Triticum aestivum* (Bread or Common wheat)

Reparation of African locust bean flour

Preparation of Raw African Locust Bean Flour

5kg African locust bean were freed from dirt and cleaned with 5 litres of clean water. It was thereafter sieved and placed in an oven at 60o C for 12 hours. The dried seeds were milled into powder using clean industrial grinding machine which was cleaned before usage to prevent particle contamination from other products. The resulting African locust bean flour was stored in an air tight container Madubuike *et al.*, (2003) and Ezugwu *et al.*, (2018).

Preparation of wheat flour

10kg Wheat grain were freed from dirt and milled into powder using industrial grinding machine which was thoroughly cleaned before usage to prevent particle contamination from other products. The resulting Wheat flour was stored in an air tight container. Madubuike *et al.*, (2003) and Ezugwu *et al.*, (2018).

Production of Bread

Straight dough method will be used to produce the duo bread *viz*: African locust bean composite wheat bread and wheat bread alone as described by Jayeola and Olunlade (2024) [7]. This method involves the addition of all ingredients (flour, salt, water, yeast) at the mixing stage and kneading same to obtain the dough. The different dough samples were placed in baking pans smeared with vegetable oil and covered for the dough to ferment resulting in gas production and gluten development for about 3 hours. The dough was then baked in the oven. The dough was then baked in the oven at 220oC for 30 minutes. The baked loaves were carefully removed from the pans and allowed to cool and packaged in polyethylene bags for usage.

Sensory Evaluation

Bread loaves were cut into slices of uniform thickness in a white coloured plate. A sensory panel of 25 semi trained students of University of Benin familiar with sensory attribute of local bread was employed to evaluate the products. A 5 (five) point hedonic scale was used to rate the bread for crumb appearance, color, taste, texture, flavour and overall acceptability according to Jayeola and Olunlade 2024 [7].

Experimental Design

A total of 60 Wistar rat will be use. 30 diabetic and 30 non-diabetic divided into 6 group with five rat each according to one way ANOVA design for group comparison according to Arifin and Zahiruddin (2017) where;

$$N = nk \text{ and } n_{\max} = (20/k) + 1$$

$$n = \text{number of sample per group}$$

$$N = \text{sample size}$$

$$k = \text{number of group} = 6$$

$$n_{\max} = (20/6) + 1 = 5 \text{ animals}$$

$$N1 \text{ (diabetic rat)} = nk = 5 \times 6 = 30 \text{ rats}$$

$$N2 \text{ (non-diabetic rat)} = nk = 5 \times 6 = 30$$

Groupings

Non-Diabetic Group

Group A - Whole wheat bread (control)

Group B - 100% wheat bread

Group C - 95% wheat + 5% African locust bean bread

Group D - 90% wheat + 10% African locust bean bread

Group E - 85% wheat + 15% African locust bean bread

Group F - African locust bean flour

Diabetic Group

Group G - 100% wheat bread

Group H - 95% wheat + 5% African locust bean bread

Group I - 90% wheat + 10% African locust bean bread

Group J - 85% wheat bread + 15% African locust bean bread

Group K - African locust bean flour

Group L - Group induced with diabetes without any experimental feed. Only rat pellet feed.

Experimental animal handling

60 Wistar rat weighing 150-200g were obtained from animal house of the Department of Biochemistry, University of Benin and kept in cages at room temperature for one week acclimatization and allow access to food and water ad libitum (Free access to food).

The principal of animal handling welfare and safety protocol will be followed according to National Institute of Health, Principle of Animal Care (1985) and Ashmita Debnath *et al* (2024) taking into consideration amongst others, the housing condition and environment factor such as clean and spacious

cages, with adequate ventilation, minimum of 180 square inches of floor spaces per pair, diet and nutrition management, care and monitoring, control of parasitic infection, site for blood collection and the weight of the rat ranging from 150-200gram.

Induction of Diabetes

Diabetes mellitus will be induced in overnight fasted rat by a single intra peritoneal injection (Ip) of 150mg/kg body weight of Alloxan monohydrate according to Salim *et al* (2016) [18] and Etuk, (2010) [6]. Hyperglycaemia will be confirmed by the elevated blood glucose level determined after 72 hours. The rat with elevated glucose level of 400mg/dl and above will be used for the study (Masiello *et al*, 1998) [11].

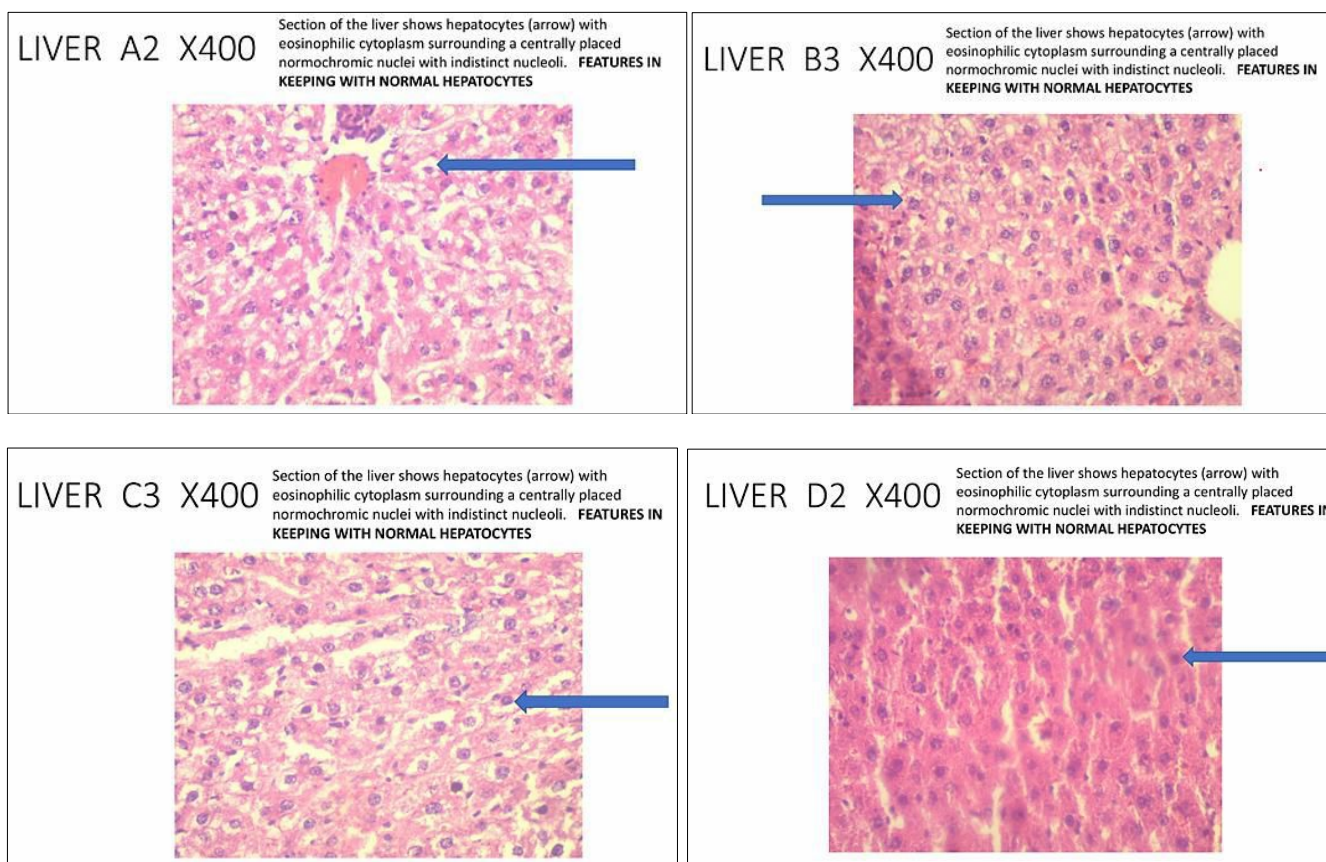
Feeding

50-gram feed (bread) will be administered daily for 14 days after an initial 7 (seven) days acclimatization according to Meite *et al* (2016) [12].

Histological Examination

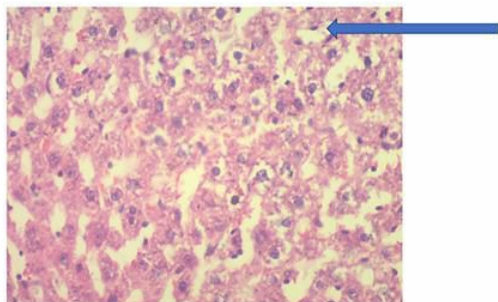
The liver organ were harvested from the wistar rats and fixed with 10% formal saline for histological examination of the morphology. The histological examination was done by Dr Abinokhauno Kingsley, Chairman, Medical Advisory Committee and Consultant Histopathologist at Stella Obasanjo Children and Women Hospital, Benin City, Edo State.

Results

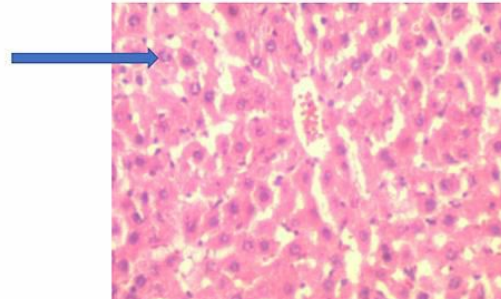


LIVER E1 X400

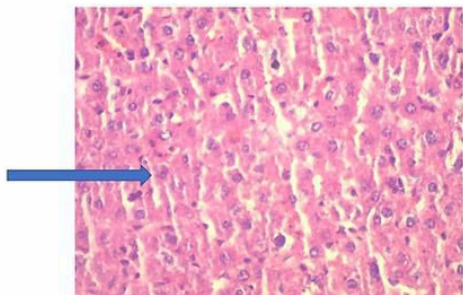
Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

**LIVER F2 X400**

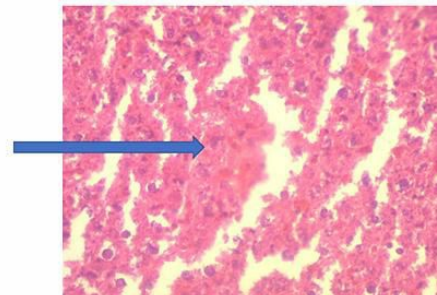
Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

**LIVER G1 X400**

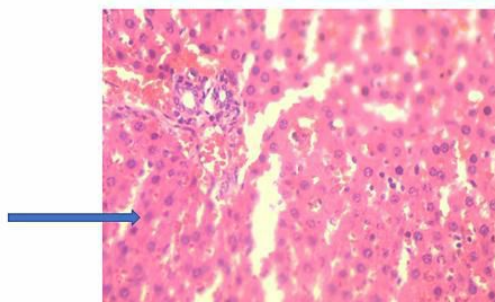
Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

**LIVER H3 X400**

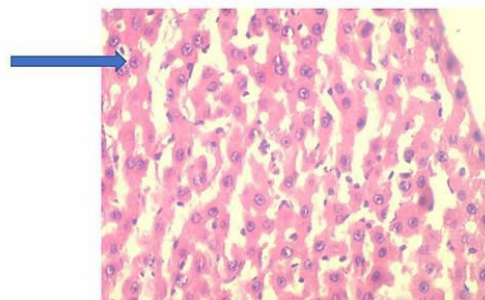
Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

**LIVER I1 X400**

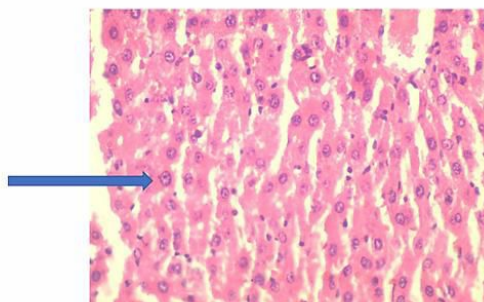
Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

**LIVER J2 X400**

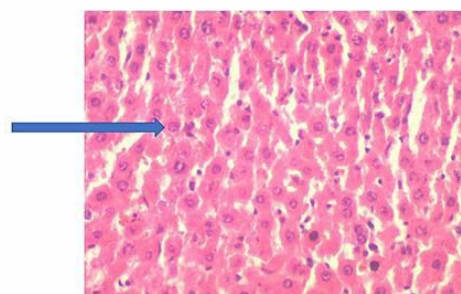
Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

**LIVER K2 X400**

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

**LIVER L3 X400**

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. **FEATURES IN KEEPING WITH STEATOSIS**





Discussion

The composite bread at 5:95%, 10:90% and 15:85% African locust bean: Wheat ratio had an acceptable sensory evaluation which implies that wheat flour could be substituted with 5%, 10% and 15% African locust bean flour for composite bread production.

The histological examination of the non diabetic liver (A to F) morphology basically reveal certain section of the liver showing hepatocytes with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli suggestive of features keeping with normal hepatocytes. Thus, it could be said that group A to F (non diabetic wistar rats) had normal liver morphology and show no sign of liver damage which signifies that the African locust bean flour, wheat bread and African locust bean composite wheat bread had no negative effect on the liver neither did it cause any form of liver damage.

Also, the histological examination of the diabetic liver (G to K) morphology basically reveal certain section of the liver showing hepatocytes with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli suggestive of features keeping with normal hepatocytes. Thus, it could be said that group G to K (diabetic wistar rat) had normal liver morphology and show no sign of liver damage which signifies that the trio feeds had no negative effect on the liver neither did it cause any form of liver damage but rather may have offered hepatoprotective effect that might have resulted from alloxan induced diabetes.

Lastly, the histological examination of the diabetic liver (L) morphology basically reveal certain section of the liver showing hepatocytes with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration) with the cytoplasm surrounding a centrally placed nuclei suggestive of features keeping with steatosis.

This suggest that group L (diabetic wistar rat) had liver steatosis which indicate liver damage that might have resulted from alloxan induced diabetes since the rats weren't given any special feed (negative control group).

Conclusion

it can therefore be concluded that African locust bean flour, wheat bread and African locust bean composite wheat bread has hepatoprotective effect which suggest the trio as good nutritional feed for diabetic patient. This is in line with KC Patrick *et al.*, 2025 which reveal that African locust bean may be protective against liver damage in rat having investigated the hepatoprotective effect of African locust bean and Negro pepper in CCl₄ induced liver damage in wistar rat. Its therefore recommended that, the effect of the trio on fasting blood sugar and biochemical marker enzymes of the liver be investigated for proper nutritional guide and medical intervention.

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