



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2018; 7(5): 1049-1054  
Received: 01-07-2018  
Accepted: 03-08-2018

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## Studies on the phytochemical, proximate composition and biochemical properties of the combination of *Cyperus esculentus* (tiger nuts) and *Phoenix dactylifera* (date fruit) flour on male albino wistar rats

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### Abstract

This study investigated the effect of the combination of *Cyperus esculentus* (Tiger nuts) flour and *Phoenix dactylifera* (Date palm) flour on selected biochemical parameters in male Wistar albino rats. Thirty male Wistar rats weighing between 100-120g were grouped into six with five rats per group and each group fed with different feed formulation for twenty eight days. Group I rats (control) was given rat feed only. Group II was fed with rat feed and tiger nuts only, Group III was fed with rat feed and Dates only, Group IV was fed with rat feed and a mixture of tiger nut and Dates in ratio 1:1, Group V was fed with rat feed and a combination of tiger nuts and Dates in ratio 1:2 and Group VI rats were fed with rat feed and a combination of tiger nuts and Dates in ratio 2:1. The proximate composition of Tiger nut flour and Date palm flour showed that the Moisture content, crude protein, crude lipid, and energy value were higher in Tiger nuts as compared to Dates fruit. However Dates fruit recorded higher levels of Ash and carbohydrates as compared with Tiger nuts. The phytochemical screening showed that flavonoid, tanins, saponins, glycosides and phenols were present in both samples. The liver enzyme assay showed significant ( $p \leq 0.05$ ) increase in Alanine amino transferase in Group III ( $60.80 \pm 4.6$  IU/L), Aspartate amino transferase in Groups VI ( $232.00 \pm 14.67$  IU/L), V ( $229.80 \pm 7.91$  IU/L) and IV ( $212.00 \pm 2.00$  IU/L) when compared with the control. However, Alkaline phosphatase significantly ( $p \leq 0.05$ ) decreased in Groups III and VI. Albumin level increased significantly in Group VI ( $40.60 \pm 0.67$  g/l) compared with the control ( $34.00 \pm 1.22$  g/l) and total protein increased significantly in Groups III and VI. There was no significant effect on the haematological parameters and the histopathology of the liver. Findings from this study shows that the mixture of both samples is nutritionally adequate and enhanced the functionality of organs.

**Keywords:** Dates, tiger nuts, phytochemicals, blood, liver

### Introduction

Products from plants have been used for a very long time now for medicinal and nutritional purposes [1]. An estimate of 80% individuals in developing countries rely solely on some plants for medicine as a result of the failure of synthetic drugs in the treatment of diseases like hypertension, diabetes, arteriosclerosis etc [2].

Medicinal plants have continued to attract so many people because of its strong speculation and believe of being very safe and effective, which has led to its indiscriminate use especially in developing countries [3]. *Cyperus esculentus* is commonly called Tiger nut. It is a popular tuber plant in Nigeria and other parts of Africa and Europe especially Spain [4]. The cultivation of this plant has been dated to 400 years ago as some graves of pharaohs have been seen containing some jars of tiger nut in Egypt [5]. Till date tiger nut is believed to have some medicinal properties, for example the milk is taken as an energy drink for agility. Studies has shown that this tuber is rich in phosphorus, potassium and also vitamins C and E, and it is cultivated in a grainy sandy soil with mild temperatures [4]. In Nigeria it is known by different name: Akiausa, Aya and Ofio by the Igbo, Yoruba and Hausa tribes respectively [6]. The most preferred of this plant is the yellow variety. This might be because of its bright colour, larger size and ability to produce more milk. And it has also been shown to contain more protein, lower fat and less anti nutrients [7].

The Date palm (*Phoenix dactylifera L.*) has been cultivated since the 6000 years. Date palm is a genus of palm, the most important species of which is the common Date palm, a native of the North half of Africa, the South West of Asia and some parts of India. Dates contain a high percentage of carbohydrates, fats comprising 14 different types of fatty acids,

15 salts and minerals, proteins with 23 different amino acids, 6 vitamins and dietary fibre [8]. These nutritional benefits of dates are for those that take it as food rather than those that just have it as snack.

It is the intention of this research to know the effect of the mixture of tiger nut and Date flour on some biochemical and haematological parameters. Also the proximate and phytochemical analysis was also determined to know the nutrient content and the extent of toxicity of the mixture to the liver.

## Materials and methods

### Experimental Animals

Thirty male albino male Wistar rats weighing about 100g-120 g, were obtained from the Animal house of the University of Port Harcourt, Choba, Rivers State Nigeria. Acclimatization was done for one week prior to experimentation. Water and rat feeds were provided *ad libitum* and the animals kept under room temperature.

### Sample collection and preparation

Tiger nuts (*Cyperus esculentus*) and Date fruits (*Phoenix dactylifera*) were purchased from Slaughter Market in Trans Amadi, Port Harcourt, Rivers State Nigeria. The tubers were taken to the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, for proper identification. The dried Tiger nuts tubers were thoroughly cleaned to remove stones and then sliced with a kitchen knife and subjected to further drying at 50°C in an Oven. After drying it was ground to obtain a flour with a manual engine grinder and then packaged in a transparent air tight container until used for the study.

Dried Date fruits were cleaned and deseeded. This was subjected to oven drying at 50°C, and then ground to flour with a manual engine grinder and also stored in a transparent air tight container until used for the study.

### Experimental Design

Thirty male Albino Wistar rats weighing between 100-120g were used for this study. The animals were separated into six groups of five rats per group and administered different mixtures of tiger nuts and Date flour which was made up to 700g as shown below:

**Table 1:** Summary of experimental design

Groups	Treatment	No of rats
I	Normal feed only	5
II	Normal feed + TNF only	5
III	Normal feed + DF only	5
IV	Normal feed + TNF + DF (1:1)	5
V	Normal feed + TNF + DF (1:2)	5
VI	Normal feed + TNF + DF (2:1)	5

The animals were sacrificed after 28 days under anaesthesia (chloroform suffocation) and blood sample collected through cardiac puncture into EDTA and Heparin bottles. The liver was collected through abdomino-thoracic dissection into plain bottles containing Bouin's fixatives which is made up of 75ml Picric acid, 25ml raw formaldehyde and 5ml acetic acid for histological study.

### Determination of serum biochemical parameters

Alanine amino transferase was determined by monitoring the concentration of pyruvate hydrazine formed with 2, 4-dinitrophenyl hydrazine. Aspartate amino transferase was

determined by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenyl hydrazine. Alkaline phosphatase was determined using colorimetric end point method. Total protein was determined using biuret method and Albumin was determined using the bromocresol green method.

### Determination of haematological parameters

Packed cell volume (PCV) is determined using the method of filling blood into capillary tubes as described [9]. Haemoglobin is determined using the haemoglobinocyanide technique, red blood cell count and white blood cell count were determined by counting chamber method.

### Statistical analysis

All data were subjected to statistical analysis. The values reported as mean  $\pm$  standard error mean (SEM) while one way anova was used to test for differences between test groups and control groups using statistical package for social sciences (SPSS) version 21. The results were considered significant at p-values of less than 0.05 that is at 95% confidence level ( $p < 0.05$ ).

### Results

The qualitative phytochemical constituents of tiger nut and Date showed the presence of carbohydrate, reducing sugars, flavonoids, tannins and phenols in both Tiger nuts and Dates fruit. Alkaloids was found only in tiger nuts whereas steroids was present only in Dates. On the other hand, both Tiger nuts and Dates fruit were devoid of Triterpenoids, oxalates, and phlobatanins.

Results of the proximate composition of Tiger nuts and Dates showed that the Moisture content ( $3.5 \pm 0.21\%$ ), crude protein ( $6.5 \pm 0.12\%$ ), crude lipid ( $22.6 \pm 1.02\%$ ) and energy ( $436.2 \text{Kcal}/100\text{g}$ ) value were higher in Tiger nuts as compared to Dates fruit. However Dates fruit recorded higher levels of Ash ( $10.8 \pm 0.21\%$ ) and carbohydrates ( $72.97 \pm 2.28\%$ ) as compared with Tiger nuts.

**Table 2:** Phytochemical constituents of tiger nut and Date.

Phytochemical	Tiger Nuts	Dates
Alkaloids	+	-
Carbohydrates	+	+
Reducing Sugars	+	+
Flavonoids	+	+
Tanins	+	+
Triterpenoids	-	-
Saponins	+	+
Oxalate	-	-
Glycosides	+	+
Steroids	-	+
Phlobatanins	-	-
Phenols	+	+

Absent (-); Present (+)

**Table 3:** Proximate composition of tiger nut and Date

Parameters	Tiger nut	Date
Moisture (%)	$3.5 \pm 0.21$	$3.03 \pm 0.24$
Ash (%)	$9.90 \pm 0.47$	$10.8 \pm 0.21$
Crude protein (%)	$6.5 \pm 0.12$	$2.7 \pm 0.23$
Crude lipid (%)	$22.6 \pm 1.02$	$1.5 \pm 0.15$
Fibre (%)	$8.33 \pm 0.78$	$8.1 \pm 0.31$
Carbohydrate (%)	$51.7 \pm 0.66$	$72.97 \pm 2.28$
Energy (Kcal/100g)	436.2	316.18

The result of the Liver biomarkers of albino rats given normal feed with tiger nut and Date for 28 days showed that level of ALT increased significantly ( $p \leq 0.05$ ) in group III which was administered feed and Dates only ( $60.80 \pm 4.61$  IU/L) when compared with the control group ( $41.20 \pm 3.20$  IU/L). The level of AST as seen in the study shows that there was a significant ( $p \leq 0.05$ ) increase in groups IV, V, and VI ( $212.00 \pm 2.00$  IU/L,  $229.80 \pm 7.91$  IU/L and  $232.00 \pm 14.67$  IU/L) respectively with group VI recording the highest level when compared with the control. On the other hand, ALP level results showed a significant ( $p \leq 0.05$ ) decrease in ALP level in group III and VI

( $52.20 \pm 2.78$  IU/L and  $67.40 \pm 1.98$  IU/L respectively) when compared with the control ( $90.40 \pm 3.54$  IU/L). Total protein level increased in all the groups with significant ( $p \leq 0.05$ ) increase in group II which was administered Feed and Tiger nuts only and group VI which was administered feed, Tiger nuts and Dates in ratio 2:1 only when compared with the control. Albumin levels was slightly increased in group III and significantly ( $p \leq 0.05$ ) increased in group VI ( $40.60 \pm 0.67$  g/l) when compared with the control group ( $34.00 \pm 1.22$  g/l).

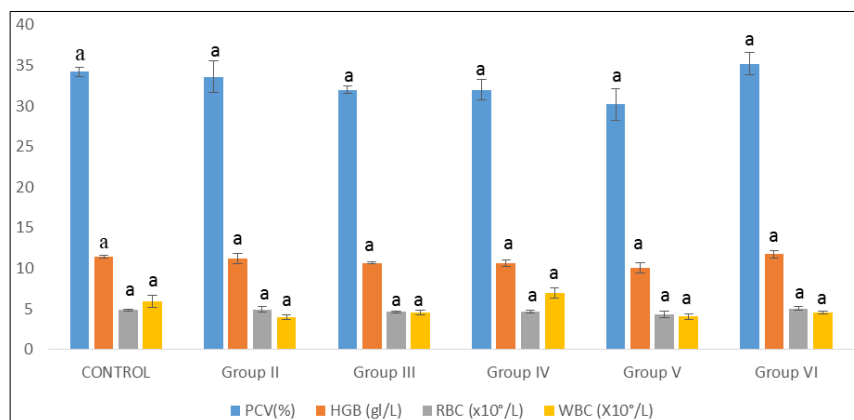
**Table 4:** Liver biomarkers of albino Wistar rats fed normal feed, tiger nut and Date flour for 28 days.

Test Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/l)	ALB (g/l)
Control	$41.20 \pm 3.20^a$	$145.20 \pm 2.93^a$	$90.40 \pm 3.54^a$	$65.20 \pm 2.70^a$	$34.00 \pm 1.22^a$
Group II	$56.40 \pm 3.61^a$	$176.20 \pm 11.49^a$	$92.40 \pm 3.20^a$	$74.20 \pm 1.77^b$	$34.00 \pm 1.41^a$
Group III	$60.80 \pm 4.61^b$	$159.60 \pm 16.52^a$	$52.20 \pm 2.78^b$	$73.60 \pm 1.32^a$	$35.80 \pm 1.01^a$
Group IV	$40.40 \pm 0.67^a$	$212.00 \pm 2.00^b$	$81.40 \pm 2.20^a$	$67.40 \pm 1.16^a$	$34.60 \pm 1.36^a$
Group V	$50.80 \pm 3.96^a$	$229.80 \pm 7.91^b$	$81.00 \pm 3.91^a$	$67.40 \pm 1.32^a$	$34.20 \pm 1.06^a$
Group VI	$46.00 \pm 6.97^a$	$232.00 \pm 14.67^b$	$67.40 \pm 1.98^b$	$85.60 \pm 1.32^b$	$40.60 \pm 0.67^b$

Data are mean values  $\pm$  standard error of mean of three determinations. Values that are bearing similar superscript letters are not significant ( $p \leq 0.05$ ) compared to the control whereas values that are bearing different superscript letters are significant ( $p \leq 0.05$ ) compared to the control.

The haematological parameters of albino Wistar rats fed normal feed, tiger nut and Date for 28 days showed that there was no significant effect of the feed to the Haematological

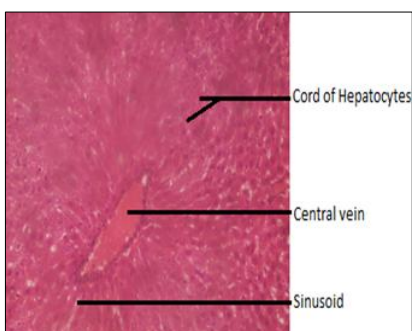
parameters such as packed cell volume (PCV), Haemoglobin (HGB), Red blood cell (RBC) and White Blood Cell (WBC), across the groups when compared with the control.



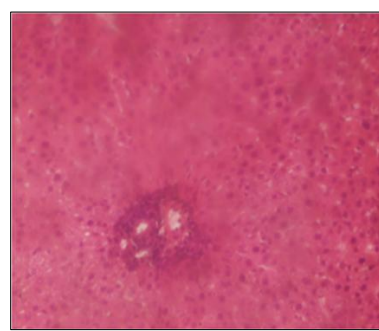
**Fig 1:** Result of Heamatological parameters of wistar albino Rats after 28 days. PCV = Packed Cell Volume, HGB= haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell

Data are mean values  $\pm$  standard error of mean of three determinations. Values in chat bearing similar superscript letters are not significant ( $p \leq 0.05$ ) compared to the control whereas values with different superscript letters are significant ( $p \leq 0.05$ ) compared to the control.

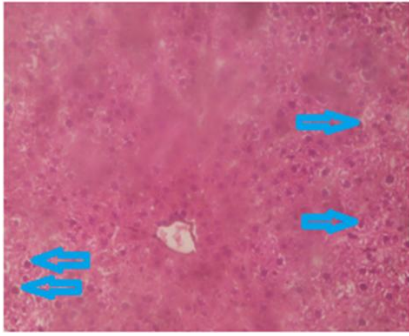
**Histological examination of the liver rats given normal feed, tiger nut and Date for 28 days.**



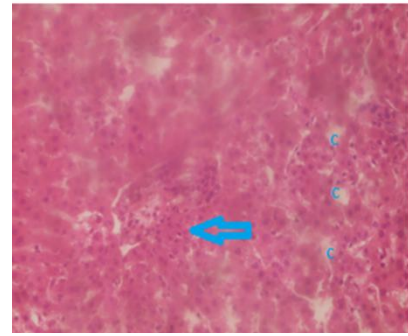
**Plate 1:** Showing photomicrograph of the liver of rats in control group (H and E staining, 400x). Central vein showing no pathology, normal hepatic cords, kuuffer cells and sinusoids.



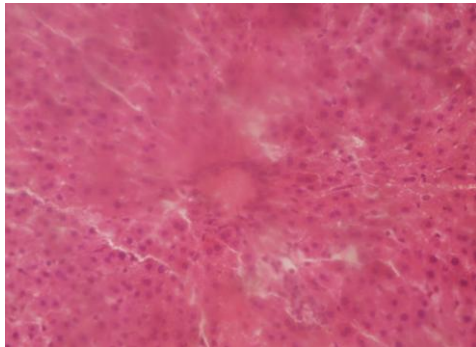
**Plate 2.** Showing photomicrograph of the liver of group 2 rat (H and E staining, 400x) stain. Showing four regenerated central veins with no pathology, normal kuffer cells, sinoids, portal triad, which indicates a normal study.



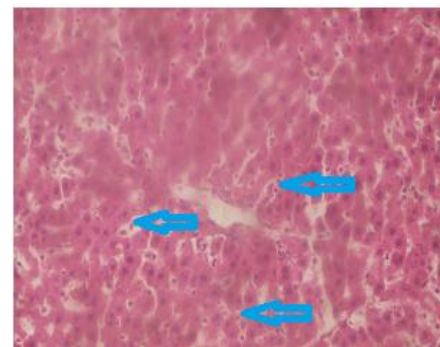
**Plate 3:** Showing the photomicrograph of the liver of group 3 rat. (H and E staining, 400x) stain. Central vein spherical with no pathology, normal mild distended kuffer cells and normal sinusoids and inflamed cells as indicated by the arrow.



**Plate 4:** Showing photomicrograph of the liver of group 4 rat (H and E staining, 400x) stain. Normal kuffer cells with no pathology, distended cords of hepatocytes, normal sinoids with mild inflammation.



**Plate 5:** Showing photomicrograph of the liver of group 5 rat. (H and E staining, 400x) stain. Showing normal hepatocytes, cords of hepatocytes, kupfer cells and sinusoid.



**Plate 6:** Showing photomicrograph of the liver of group 6 rat. (H and E staining, 400x) stain. Constricted proliferated kupfer cells, Vein, sinusoids, and distended hepatic cords. The photomicrograph of this slide shows that there are inflammatory cells as indicated by the arrows.

### Discussions

Phenolic compounds found in most plants are known to possess some biological properties such as antiaging, anticarcinogen, antiinflammation, anti-diabetic, anti apoptosis, anti atherosclerosis, cardiovascular protection and improvement of endothelial functions as well as inhibition of angiogenesis and cell proliferation activities. Flavonoids are phenolic substances known to be synthesized by plants in response to microbial infection. Studies have shown that they are good antimicrobial, anticancer and antioxidant substances. They act as these substance because of their ability to form complex with extracellular and soluble proteins, and with bacterial cell wall [10]. Alkaloids, tannins and saponins are known to have antimicrobial activity and other physiological activities [11]. Some alkaloids are toxic such as those that inhibits enzymic activities and affects glucagon, thyroid stimulating hormone and are carcinogenic [12]. The non-toxic alkaloids are those that are used as anagelsic, antispasmodic and bactericidal agents. Saponins can be used as detergents, foaming and emulsifying agents and can help reduce inflammation of upper respiratory passage [13]. Also saponin has the ability to coagulate and precipitate red blood cells [10]. The astringent property of tannins makes it useful in the healing of wounds, and can also be used as bactericidal agents [14]. According to reports steroids are very important compounds that has antibacterial properties and Glycosides help to lower blood pressure as described [10].

The result of the qualitative phytochemical constituents of Dates flour carried out in this study indicated the presence of some phytochemicals such as Phenols, Steroids, Glycosides, Saponins, Tannins and Flavonoids. The presence of phenols, glycosides and flavonoids is in this study agrees with some

report that Dates contains some type of Phenols, Flavonoids and Glycosides and that the concentration of these phytochemicals decreases with advancing stage of fruit maturity [15]. The presence of tannins in Dates is in agreement with the findings [16] and [17], who characterised the presence of tannins in Dates as the astringent taste found in it and that this taste decreases as the fruits mature. The result of the phytochemical screening of *Cyperus esculentus* (Tiger nuts) flour as shown in table 2 shows the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenols. A study [18] confirmed the presence of alkaloids, glycosides, tannins and saponins in raw tubers of tiger nut, also another study [19] confirmed the presence of alkaloids, flavonoids, tannins, saponins and glycosides in both the big yellow and small brown variety of tiger nuts.

A report [20] stated that Dates were not considered a good source of proteins. The value (2.7g/100g) of protein obtained in this study supports this claim. This is contrary to other view [21] who obtained a higher value (17.09%) of protein. Also another report [22] analysed eleven Tunisian cultivars of Date for protein and found that the highest protein content of 2.85g/100g dry matter. This suggests that some varieties of Dates might not be rich in protein while some might be very rich in protein. Dates has a high value (72.97g/100g) of carbohydrates in this study. This claim supports the findings [21] that the amount of carbohydrates obtained in Dates was very high which would be useful for getting the energy for metabolic processes.

The low level of lipid content of Dates in this study supports the study [21] on the lipid contents of Dates. This low levels of lipid in Dates and its content of sugars means that Date palm is safe for the heart and high blood pressure patients because



it contains a low level of fatty acids and cholesterol. On the contrary, a report <sup>[23]</sup> stated that drying of Dates increased their fat contents.

Ash content is an index to the nutritive value of foods <sup>[24]</sup>. The ash content value (10.8g/100g) of Dates in this work, is in the same range as the ash content in the work described <sup>[21]</sup>. The ash content value of Dates investigated are higher than the ash range mean values of legumes which are between 2.4-5.0% as described <sup>[25]</sup>.

The caloric value of *Phoenix dactylifera* (Dates) in the table shows it is a good source of energy and can provide a large portion of the daily requirement for adult if large quantities are consumed.

The protein content of tiger nuts in this study as shown in the table supports the claim <sup>[26]</sup>, whose findings stated that the protein content of tiger nuts was quite low but fell within the range of hickory nuts (3.60g), chestnuts (4.53g), coconuts (2.06g) and pine nuts (6.81g). The higher lipid content of tiger nuts in this study when compared with Dates is in agreement with <sup>[27]</sup> whose value of lipids in Dates is in the same range with the value of lipid in Dates in this study. Also as described <sup>[28]</sup> tiger nut oil was found to contain 18% saturated (palmitic and stearic acid) and 82% of unsaturated (oleic and linoleic acid). The presence of other nutrients like carbohydrates and fibre and the high energy value present in tiger nuts as shown in the table shows it is a good source of nutrient and energy and has the ability to curb the problem of malnutrition.

ALT, ALP and AST activity are used for the assessment of the normal physiological state of the liver and are significant in amino acid metabolism <sup>[29]</sup>. In this study, the significantly increased ALT observed in Group III given feed with Date only, and other notable increase though not significant in Group II (feed with tiger nut only), Group IV (feed with tiger nut and Date in 1:1), Group V (feed with tiger nut and Date in 1:2), and Group VI (feed with tiger nut and Date 2:1) when compared to the normal control values as shown in the table is not suggestive of any hepatocellular damage. Also notable increase in AST in all the groups with significant increase in Group IV (feed with tiger nuts and Dates in ratio 1:1), Group V (feed with tiger nuts and Date in ratio 1:2), and Group VI (feed with tiger nuts in ratio 2:1) as shown in the table is also not suggestive of a hepatocellular damage. The increase in ALT and AST could be from extra hepatic tissues as these enzymes are found in other tissues. Also the increase of these enzymes could be as a result of high protein diet of the mixture therapy of tiger nuts and Date flour. The report as described <sup>[30]</sup> showed that the levels of ALT and AST increased following a high protein diet consumption. Increased level of ALP reflects impaired excretion and bile flow as in the obstruction that affects the biliary system <sup>[31]</sup>. In this study the concentration of alkaline phosphatase was decreased across the groups with significant ( $p \leq 0.05$ ) decrease in Group III and Group VI. This indicates that there was no hepatobiliary obstruction caused by the feed. The increase in total protein in all the groups with significant increase in Group II and Group VI, and also increase in albumin in Group III, IV, V with significant increase in Group VI, shows the non-toxic effect of the feed when fed singly and in a mixture. The liver synthesizes protein and low levels might indicate impaired synthesis. Also the raised level of total protein and albumin might be as a result of increased rate of protein synthesis or from high protein diet consumption. Total protein and albumin are generally influenced by protein intake <sup>[32]</sup>.

The effect of the administration of the various feed mixture on haematological parameters such as Red Blood Cells (RBC), Haemoglobin (HGB), Packed Cell Volume (PCV), and White Blood Cell (WBC), presented on the table shows no significant effect on these parameters. This could be related to the nutritional composition of the mixture therapy on the health status of the animals because none of them died all were reported healthy. This shows that the feed mixture is healthy and do not have the ability to cause anaemia. This report is in line with the claim as described <sup>[33]</sup> on the influence of food components on haematological traits. Histology of the liver of the rats given the various mixed diets showed central vein, kuffer cells, and sinusoids with no pathology respectively as shown in plate 1-6. The mild inflammation shown in plate 3, 4 and 6 might be as a result of increased protein synthesis which might have over worked the liver as this is evidenced by an increase in the liver marker parameters in these groups respectively as shown in the table. As described <sup>[34]</sup> there would be increase in liver enzymes where the cells had been inflamed, and in a liver that is not diseased, the metabolic and tissue regenerative functions require some amount of inflammation <sup>[35]</sup>. The dependence of blood protein on the quality and quantity of protein source <sup>[36]</sup>. This proves that the mixture is histologically safe to the liver and kidney.

## Conclusion

The mixture of tiger nuts and Dates did not pose damage to the liver as can be seen in the histopathology results of the liver and the liver function test. It can also be said to be rich in nutrients especially protein, carbohydrates and lipids which can help curb the problem of malnutrition. Also the combination therapy of Tiger nuts and Dates is safe to consume and does not cause anaemia.

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