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Effect of Preservation Methods of Oil Palm (*Elaeis guineensis*) Sap on Health Status of Male Wistar Rats

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Abstract Male Wistar rats (30) which were split into five groups of six rats each were administered four different forms of oil palm tree (*Elaeis guineensis*) sap samples by gavage based on 1.5% of their weekly body weights. The group 1 which served as control received only water, group 2 received pasteurized palm sap (PPS), group 3 received market palm wine (MPW), and group 4 received frozen palm sap (FPS), whereas group 5 received fresh palm sap (FrPS). Chemical compositions of the sap samples were determined. Normal feed and water were fed *ad libitum*. The mean body weight and feed intake of each rat group was measured every three (3) days. Blood samples were collected weekly for determination of random and fasten blood glucose. After 2 months of treatment, the bloods of each male rat group samples were collected for determination of the liver and kidney functions, while the palm sap products were subjected for microbial analyses. The sap samples content were analysed [1]. The palm sap was evaluated for its effect on the mean body weight and vital organ weight of albino rats. Results showed that palm sap had effect in reducing the mean body weight of the exposed rat groups compared to the control which could be attributed to the low alcohol content and probiotic effect of the fresh palm sap on the treated rat group. The spleen and kidney of the control rat group showed significant decrease compared to the treated rat groups, while the liver of the rat groups could be compared favourably. The percentage increases observed in spleen and kidney of the exposed rat groups was attributed to the presence of alcohol in the palm sap. However, since the liver of the palm sap treated rat groups were comparable to the control; it showed that the presence of alcohol in the palm sap administered to the Wistar rats were within normal limits. There were no significant differences on the random and fasting blood glucose of the rats which is an indication that palm sap drinking may not elevate the blood glucose levels in the blood stream. Results obtained also showed that palm sap contained mainly *Saccharomyces cerevisiea.* However, the presence of *Citrobacter freundi* in the MW could be associated with adulteration or exposure to unhygienic environment.

Keywords: *organ weight, body weight, palm sap, Liver, Kidney, Citrobacter freundi*

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1. Introduction

The unfermented pale-yellow exudate from tapped unopened spathe of oil palm tree (*Elaeis guineensis*) is referred to as palm sap [2]. It is widely consumed as a refreshing and nourishing beverage, especially in West African countries. The fermented palm sap, called palm wine or toddy is the commonest form of the beverage. In some localities, the fresh sap may be bought undiluted and given to an elderly person as a mark of respect, or processed into different products such as caramel, sugar, spirit and recently, mixed fruit juice [3,4]. Palm sap is also usually pasteurized and bottled at industrial level in order to prolong its shelf life [4], although considerations for the total elimination of yeasts in fresh palm sap, believed to aid eyesight and reproductive system, had limited its industrial demand. Both males and females are predisposed to palm sap/wine drinking, although female folks drink less of the beverage than the male counterparts [5]. Consumption of the beverage (palm sap/wine) is a common feature in virtually all ceremonies such as traditional festivals, weddings and funerals where it is served as an indication of hospitality [5,6]. Traditionally, in south-eastern part of Nigeria, the dreg of palm wine is usually reserved for newly wedded men in order to boost their sexual performance and fertility. There is a dearth of scientific evidence to prove these claims. More so, the effects of different methods of preservation of this product on vital body organs and mean body weight need to be examined. This work was conceived to examine the effect of palm sap on vital body organs and mean body weight and also how different preservation methods some of the health indices of these rats.

2. Materials and Methods

2.1. Sample Source and Preparation

The sample was prepared using the method as in [1]. Fifteen (15) litres of freshly tapped oil palm sap (FrS) were collected from a palm wine tapper at Ajuona-Nsukka, Enugu State, in a cooler packed with ice blocks, and divided into three lots. Each lot (5 L) was subjected to either heat treatment (70°C for 40 min to obtain pasteurized palm sap [PS]), freezing (- 4°C till frozen [FS]), or left untreated to serve as fresh palm sap (FrS) along with five (5 L) litre of market palm wine (MW), and tap water that served as control. The commercial rat feed (Vital Pelletized Growers Feed) that was used, was manufactured by Grand Cereals and Oil Mills Ltd. (Jos, Plateau State, Nigeria) and contained cereals/grains, animal protein, vegetable protein, minerals, salts, essential amino acids, antibiotics, antioxidants, and vitamin premix. The nutrient composition as stated in the label included: crude protein, 14.50%; fat, 7.00%; crude fibre, 7.20%; calcium, 0.80%, and metabolizable energy, 2000 kcal/kg.

2.2. Experimental Animals

Thirty (30) male, healthy Wistar rats of 4 weeks old, weighing between 47.1–172.6 g, were obtained from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The rats were divided into five groups (1, 2, 3, 4, 5) of six rats each [1]. Each study group was split into two subgroups of three rats each, and housed in a stainless steel cage with plastic bottom grid and a wire screen top, in the animal house of the Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka. The room temperature and relative humidity for the rats were 23–24.5^{o}C and 91–96%, respectively, with natural 12-h light–12-h dark cycle. The rats were served 80–100 g of the standard rat chow and at least 5 mL of water per day, ad libitum. The animals were acclimatized for 2 weeks before treatment. The group 1 rats were given normal feed and water only; group 2 rats were fed on PS in addition to the normal feed and water; group 3 rats were given normal feed, water, and MW; whereas groups 4 and five rats were given frozen palm (FP) sap and fresh (FrP), respectively, in addition to normal feed and water. The palm sap/wine was administered by gavage based on 1.5% of their weekly body weights. Each cage was supplied with 100 g of the specific diets. After three days, the remaining diets were weighed. The difference obtained between the two values was the amount of diet consumed by the rats for three days. Fresh supplies of diets were then added to the cages to maintain the total amount of 80 – 100 g. These processes were followed throughout the treatment period and the average taken after three days to determine the consumption rate per day. The male Wistar rats were weighed weekly with an electronic balance (Derive Instrument Company), and recorded. The male rats were humanely sacrificed after two months. The entire animal study was conducted in accordance with the Ethics and Regulations guiding the use of research animals as approved by the University of Nigeria, Nsukka.

2.3. Blood Glucose Test

The blood glucose of the rats was determined using Accu-check Active glucometer or blood monitoring system (Roche Diagnostics GmbH, Germany) [7]. Briefly, Scissors was used to cut a small portion of the rat's tail on weekly basis and blood droplets from the tail was dropped on the strip of the glucometer which displays the result within 5 seconds in mg/dl. The fasten blood glucose was determined using the same instrument but the male Wistar rats were fasted overnight before each determination.

2.4. Liver Function Tests

The parked cell volume (PCV) was evaluated by Microhaematocrit method as described [8]. The concentration of haemoglobin was determined by Cyanomethemoglobin method of Drabkin's haemoglobin reagent [9]. The red blood cell count, total white blood cell count and differential white blood cell count were determined using haemocytometer method [7]. The serum alanine amino transferase (ALT) and serum aspartate amino transferase (AST) were determined by colorimetric method for in-vitro determination of plasma ALT and AST in serum or plasma using QCA SGPT/ALT and QCA SGOT/AST test kits, respectively [10]. *Alkaline phosphate*, (ALP), was assayed using kits provided by Randox Laboratories Co; according to the method [11,12].

2.5. Kidney Function Tests

Serum creatinine was determined by the modified Jaffe method for the in-vitro determination of creatinine in serum, plasma or urine using the QCA creatinine test kit [13]. The bilirubin was determined by the in-vitro determination of total bilirubin in serum or plasma (QCA bilirubin test kit) [14].

2.6. Microbiological Analysis

The presence of microorganisms in the samples (both fresh and pasteurized) was determined by serial dilution of the samples into ten folds with sterile 0.1% media by the pour plate techniques [15] in triplicates. Bacterial counts were determined using nutrient agar (NA) and incubated for 24 h at 35^{o}C while the yeasts and moulds counts were determined using Sabouraud Dextrose Agar (SDA) and 14% tartaric acid. The plates were incubated at 30^{o}C for 3 days and then counted for microbial load.

2.7. Experimental Design and Statistical Analysis

The experiment was conducted in a completely randomized design [1]. Data generated were analysed using one way analysis of variance and mean separation was done by Duncan's New Multiple Range Test at 95% confidence interval using the SPSS version 17.0.

3. Results and Discussion

3.1. Results

The results obtained after going through the extensive chemical and biochemical analyses showed that the feed intake of rat group that had no treatment increased

significantly ($p<0.05$) compared to the palm sap/wine treatment rat groups.

Table 1. Feed intake of Wistar rats treated with pasteurized and unpasteurized palm sap/wine

Treatment/ Feed intake	Group 1 (Water)	Group 2 (Pasteurized palm sap)	Group 3 (Palm wine)	Group 4 (Frozen palm sap)	Group 5 (Fresh palm sap)
Feed Intake (g)	78.00±6.71^{a}	44.11±2.94^{c}	43.78±2.40^{c}	43.61±2.16^{c}	53.44±3.27^{b}
Body weight (g)	229.37±27.43^{a}	154.12±28.55^{b}	173.80±31.61^{b}	215.87±6.31^{a}	226.19±19.08^{a}

Values are means± S.D (n = 6). Values with different superscripts within a row were significantly different ($p<0.05$) (Duncan separation). Mean value for two (2) months treatments.

The rat group treated with FrP sap showed high feed intake when compared with rat groups treated with PS, MW and FS. No significant ($p>0.05$) difference was observed in the rat groups treated with PS, MW and FrS. Though, the rat group treated with FrS showed high feed intake; this was not significant compared to rat groups administered with MW, PS and FS.

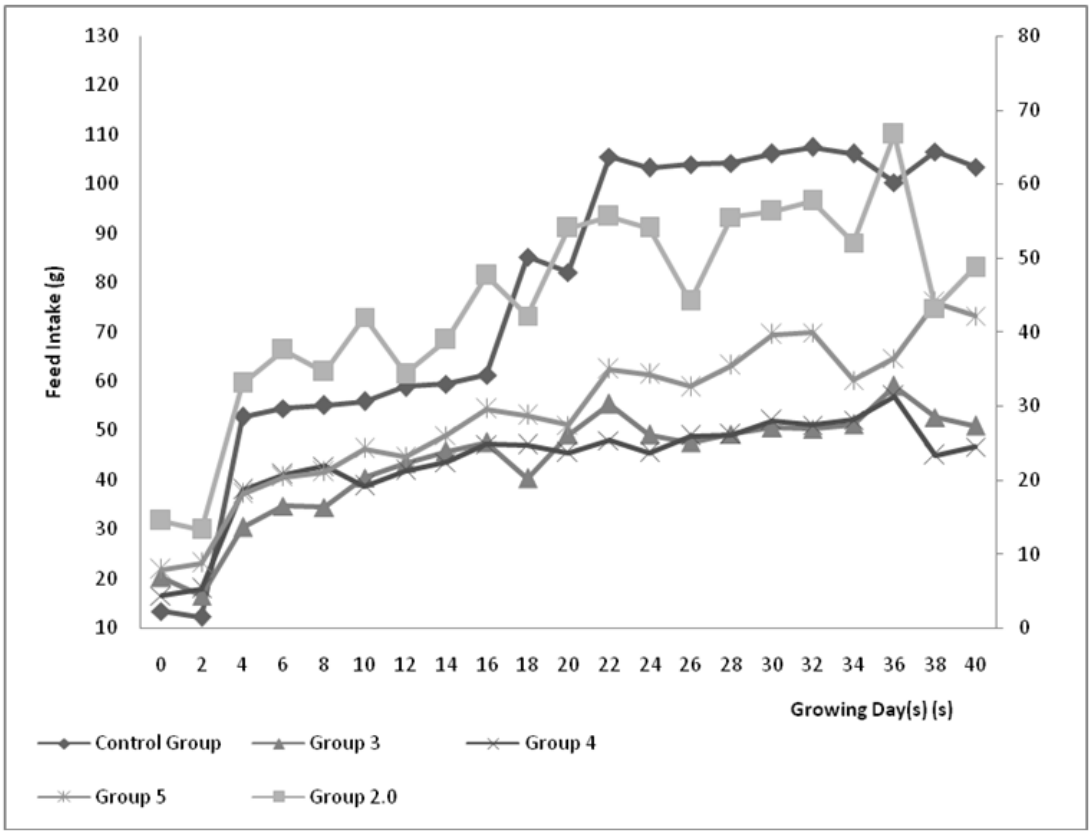

Figure 1. Weekly mean body weight of rats administered with treated palm sap/wine samples

Group 1 – Rats treated with normal feed + water
Group 2 – Rats treated with normal feed + water + pasteurized palm sap
Group 3 – Rats treated with normal feed + water + palm wine
Group 4 – Rats treated with normal feed + water + frozen palm sap
Group 5 – Rats treated with normal feed + water + freshly tapped palm sap.

The results in Table 1 showed the weekly mean body weight of the different groups of rats. The rat group that had no treatment (control) was found to have the highest body weight, which was 32.7% higher body weight than the least body weight (rats treated with PS). However, the decrease in weight was not significant ($p>0.05$) for group of rats treated with FS and FrP, but significant ($p<0.05$) for group of rats treated with MW and PS. The rat group administered with FrS showed uniform weekly mean body weight increase throughout the period of the study.

Table 2. Organ and body weight of rats treated with different and palm sap/wine samples

	Treatment Groups				
Some vital body organs and Weight	1 (Water)	**2** (Pasteurized palm sap)	**3** (Palm wine)	**4** (Frozen palm sap)	**5** (Fresh palm sap)
Spleen (%)	0.38±0.08^{b}	0.50±0.11^{a}	0.44±0.08^{ab}	0.36±0.01^{b}	0.44±0.08^{ab}
Liver (%)	3.21±0.27^{a}	3.39±0.34^{a}	3.52±0.18^{a}	3.13±0.20^{a}	3.35±0.65^{a}
Kidney (%)	0.66±0.05^{b}	0.71±0.08^{ab}	0.76±0.08^{a}	0.69±0.0^{ab}	0.70±0.06^{ab}

Values are means± S.D (n = 6). Values with different superscripts within a row were significantly different ($p<0.05$).
Key:
Group 1 – Rats treated with normal feed + water
Group 2 – Rats treated with normal feed + water + pasteurized palm sap
Group 3 – Rats treated with normal feed + water + palm wine
Group 4 – Rats treated with normal feed + water + frozen palm sap
Group 5 – Rats treated with normal feed + water + freshly tapped palm sap.

The increase or decrease in organ weights of the male Wistar rats was studied. Results obtained showed that spleen and kidney of treated rat groups increased in weight while the liver decreased in weight compared to the rat group that had no treatment (Group 1). Increases were also observed in the rats administered with PS in their spleen and kidney. The increase in the organ weight in group 2 treated rats was highly significant ($p<0.05$) when compared with the group 1, and significant ($p<0.05$) when compared with the MW or FrS. The spleen of group 4 rats administered to FS was comparable to group 1 (control).

In the study of the random and fasting blood glucose, it was found that there were no significant differences ($p>0.05$) in the random and fasting blood glucose levels of the rats among the groups. Random blood glucose is indicative of a recent food intake and therefore should have higher reference values than the fasting glucose test. Fasting refers to refraining from eating or drinking any liquids other than water for eight to twelve hours and is used as a test for diabetes. During this period, glucagon (a hormone) is stimulated which increases the plasma glucose levels in the body. The stimulation of glucagon is counterbalanced by the production of insulin which helps to maintain the glucose levels, if the host is not diabetic. Therefore, people with diabetes either could not produce enough insulin to rebalance their blood sugar (type 1 diabetes) or that their body could not be able to use the produced insulin effectively enough (type 2 diabetes). The fasting blood sugar test is usually used to test the effectiveness of different medication or dietary changes on people already diagnosed as diabetic.

Table 3. Effect of palm sap/wine administration on the random and fasting blood glucose of Wistar rats

	Treatment Groups				
Blood glucose	**1** (Water)	**2** (Pasteurized palm sap)	**3** (Palm wine)	**4** (Frozen palm sap)	**5** (Fresh palm sap)
Random blood glucose (iu/l)	109.80±5.22^{a}	113.60±7.70^{a}	113.20±10.76^{a}	116.80±5.12^{a}	115.6±17.01^{a}
Fasting blood glucose (iu/l)	93.80±17.85^{a}	92.20±22.79^{a}	85.00±13.66^{a}	95.80±13.88^{a}	84.80±11.61^{a}

Values are means± S.D (n = 6). Values with different superscripts in the same row were significantly different ($p<0.05$).
Key:
Group 1 – Rats treated with normal feed + water
Group 2 – Rats treated with normal feed + water + pasteurized palm sap
Group 3 – Rats treated with normal feed + water + palm wine
Group 4 – Rats treated with normal feed + water + frozen palm sap
Group 5 – Rats treated with normal feed + water + freshly tapped palm sap

The study of the haematological parameters in Table 4 showed that the rats treated with MW and FrS showed signs of adverse health effect when compared with the rat group that had no treatment. There were significant ($p<0.05$) differences in terms of parked cell volume (PCV), neutrophil, red blood cell and lymphocytes counts. There were also significant ($P<0.05$) differences in rat groups treated with FS and PS when compared to the control rat group in terms of their haemoglobin levels. Results obtained also showed that there were no significant ($p>0.05$) differences in the white blood cell and lymphocyte counts between group 4, group 5 rat groups and group 2 together with group 3 treated rat groups.

Table 4. Effect of palm sap/wine treatments on haematological parameters of rats

		Group of rats/feed				
Parameters		**1** (Water)	**2** (Pasteurized palm sap)	**3** (Palm wine)	**4** (Frozen palm sap)	**5** (Fresh palm sap)
PCV (%)		36.33±1.86^{a}	31.50±1.38^{b}	32.50±2.74^{b}	30.33±1.37^{b}	32.83±2.14^{b}
HB (g/dl)		12.72±0.30^{a}	9.48±1.33^{c}	10.82±1.45^{b}	8.57±0.32^{c}	11.65±1.35^{ab}
RBC (x10^{6} mm^{3})		5.79+0.51^{a}	4.99+0.23^{b}	5.16+0.54^{b}	5.05+0.05^{b}	5.64+0.60^{a}
WBC (x 10^{3} mm^{3})		9.15±2.27^{a}	10.43±1.18^{a}	9.23±0.19^{a}	10.28±4.57^{a}	8.90±1.58^{a}
Differential leucocyte counts (%)	**N**	19.83±2.79^{c}	33.75±12.15^{a}	21.50±4.76^{c}	29.50±2.43^{b}	21.67±1.51^{c}
	E	0.67±0.51^{a}	0.33±0.82^{b}	0.17±0.41^{b}	-	-
	M	-	-	-	-	-
	B	-	-	-	-	-
	L	79.83±1.72^{a}	78.25±1.25^{a}	79.25±2.81^{a}	76.33±5.24^{a}	79.20±1.93^{a}

- Values are means± S.D (n = 6). Values with different superscripts within a row were significantly different ($p<0.05$). - Not detected.

Key:
Group 1 – Rats treated with normal feed + water
Group 2 – Rats treated with normal feed + water + pasteurized palm sap
Group 3 – Rats treated with normal feed + water + palm wine
Group 4 – Rats treated with normal feed + water + frozen palm sap
Group 5 – Rats treated with normal feed + water + freshly tapped palm sap
PCV – parked Cell Volume
RBC – Red Blood Cell count
HB – Haemoglobin
WBC – White Blood Cell count
E – Eosinophil
N – Neutrophil
M – Monocytes
B – Basophile
L – Lymphocytes

The clinical chemistry/biochemistry of the male rats was equally studied. It was therefore evident from the results that rat groups treated with PS, FrS and FS exhibited significant differences ($p<0.05$) in the levels of

aspartate amino-transferase (AST), alanine amino-transferase (ALT), and alkaline phosphatase (ALP) activity, compared to the rat group that had no treatment (Table 4). The increases in serum aspartate amino-transferase (AST) and alanine amino-transferase (ALT) in the rat group treated with MW was significant ($p<0.05$) when compared to the rat group that had no treatment, but Alkaline phosphatase (ALP) activity was not significant ($p>0.05$) compared to the non-treatment rat group.

It has been reported that ethanol induces changes in the serum liver and kidneys [16]. In this study there were significant ($p<0.05$) differences in most of the liver marker and enzymes, such as aspartate amino-transferase (AST), alkaline amino-transferase (ALT), and alkaline phosphatase (ALP) activity, which are indicators of liver diseases. A rise in liver marker enzymes such as alkaline phosphatase (ALP), aspartate amino-transferase (AST) and alkaline amino-transferase (ALT) indicates lower levels of serum albumin showing reduced liver function [17]. There were no significant ($p>0.05$) differences in the levels of creatinine, except in the group treated with FrS, but significant differences existed in the levels of bilirubin between group 2 and other groups. The level of bilirubin was least in the rat group treated with PS, which could be as a result of breakdown of haemoglobin in the liver as shown in Table 5.

Table 5. Effect of palm sap/wine treatments on selected biochemical parameters of rats

	Groups				
Clinical chemistry	**Group 1** (Water)	**Group 2** (Pasteurized palm sap)	**Group 3** (Palm wine)	**Group 4** (Frozen palm sap)	**Group 5** (Fresh palm sap)
AST (iu/l)	74.50 ± 2.45^{ab}	60.50 ± 4.12^{b}	49.00 ± 0.96^{c}	55.67 ± 3.91^{bc}	82.17 ± 3.87^{a}
ALT (iu/l)	18.17 ± 3.87^{b}	18.50 ± 2.74^{ab}	16.33 ± 3.14^{b}	19.50 ± 4.28^{a}	23.00 ± 3.79^{a}
ALP (iu/l)	73.83 ± 1.91^{a}	61.67 ± 11.41^{b}	70.33 ± 5.20^{a}	64.67 ± 6.71^{b}	74.00 ± 2.79^{a}
Creatinine (mg/dl)	0.88 ± 0.12^{a}	0.82 ± 0.16^{a}	0.83 ± 0.16^{a}	0.83 ± 0.14^{a}	0.63 ± 0.18^{b}
Bilirubin (g/dl)	0.46 ± 0.09^{bc}	0.37 ± 0.06^{c}	0.54 ± 0.12^{ab}	0.63 ± 0.09^{a}	0.62 ± 0.17^{a}

Values are means± S.D (n = 3). Values with different superscripts within a row were significantly different ($p<0.05$).
Key:
Group 1 – Rats treated with normal feed + water
Group 2 – Rats treated with normal feed + water + pasteurized palm sap
Group 3 – Rats treated with normal feed + water + palm wine
Group 4 – Rats treated with normal feed + water + frozen palm sap
Group 5 – Rats treated with normal feed + water + freshly tapped palm sap
AST – Aspartate Amino Transferase
ALT – Alanine Amino Transferase
ALP – Alkaline Phosphatase.

Table 6. Microbial Isolation and counts in the palm sap/wine samples

Treatments	**Pasteurized palm sap**	**Market palm wine**	**Frozen palm sap**	**Fresh palm sap**
Yeast/Mould count (Cfu/ml)	ND	$3.78\pm0.23\times10^{8a}$	$1.27\pm0.02\times10^{8b}$	$1.76\pm0.07\times10^{8b}$
Organism present	ND	*Saccharomyces cerevisiae* and *Citrobacter fruendii*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*

Values are means± S.D (n = 3). Values with different superscripts within a row were significantly different ($p<0.05$). ND – Not detected.

Traditionally, palm sap/wine is known to contain yeast. The high demand of the product results from the presence of yeast believed to improve the eyesight of those that indulges in it. It was observed during laboratory investigation that there were microbial growth in the MW, FS and FrS, while no microbial growth was found on the PS. Microorganisms found in the MW, with higher microbial counts, (3.78×10^{8}cfu/ml) were *Saccharomyces cerevisiae* and *Citrobacter freundii* while only *Saccharomyces cerevisie* was isolated in the culture of FS and FrS.

3.2. Discussion

The investigation of the food intake of the male Wistar rats subjected to different palm products showed that the groups of rats responded differently to feed intake. Palm sap/wine is basically sugar product with high presence of glucose and fructose. The part of the brain that is responsible for food and water intake is the hypothalamus. The high glucose (glucose is regulated by phosphogluctokinase) intake can led to elevated insulin in the central nervous system which inhibits food intake in animals, including non-human primate and functions as a negative feedback signal of recent energy intake and body adiposity [18]. The body does this when it absorbs high fructose (fructose can continually enter the glycolytic pathway) intake leading to reduced insulin and leptin production (leptin is poorly secreted in the adipose tissues due to insulin production in the liver). Decreases in circulating leptin concentrations correlate with increased sensations of hunger during prolonged energy restriction in women [19], and leptin administration can reduce appetite in humans [20]. Insulin production is therefore attributed to high carbohydrate and glucose intake in the body system.

Research on the weight gain of male Wistar rats was conducted. The result obtained was consistent with Screeranjitkumar [21] that administration of toddy decreased the weight gain of dams. Increases in the weekly mean body weights were uniform throughout the period of study for the non-treated rat group, but declined as the rat advanced in age. This could be as a result of the yeast present in the palm sap and the low content of ethanol in the sample. Swathy et al. [22] reported that body weight and testis weight of rat decreased when administered with ethanol. It is, therefore, not surprising the weight gain of rats administered with different palm products were reduced since the carbohydrate intake were subsequently metabolised to alcohol in the animals' body by the *Saccharomyces cereviseae* present in the palm sap/wine.

It is important to state that the Significant ($p<0.05$) differences in the organ weight of the male Wistar rats

could be owing to the presence of high carbohydrate (glucose and fructose) intake in the treated rat groups. Lalitha et al. [23] asserted that an increase in organ weight was as a result of elevation of cholesterol content of the rats, which was in agreement with the findings of other researchers [21]. Although researches on animal study attributed the abnormal increase in organ weight to the presence of alcohol, it is necessary to state that palm sap/wine contains alcohol, and that palm sap metabolism by inherent microbial organisms results in the production of different categories of alcohol depending on the length of storage. According to Nayanatara et al. [24], alcohol fed rats showed significant increase in the weight of liver. The result of this study was in agreement with Klesges et al. [25] who showed that alcohol increases the metabolic rate of rats, thus causing more calories to be burned rather than stored in the body as fat, while Colditz et al. [26] reported that the consumption of sugar decreased as the consumption of alcohol increased, thereby leading to a loss in weight. There were no significant ($p>0.05$) differences in the organ weights of rats treated with frozen sap and freshly tapped palm sap when compared to the control group. The high content of *Saccharomyces cerevisiae* in the two groups could have contributed to the complete metabolism of food substrates in the organs. However, the spleen of rats administered with fresh palm sap were significantly ($p<0.05$) elevated from 0.38% to 0.44%, while that of the pasteurized palm sap treated rats increased from 0.38% to 0.50%.

The results of the fasting and random blood sugar showed that they were within the normal range (70 – 126 mg/dl). This implied that consumption of these products could not at 1.5% of the body weight of Wistar rats lead to high blood sugar. This finding was consistent with the report of Matawalli et al. [27] that alcohol fed rats showed no adverse health effect when administered at normal dosage. It was evident from the study that palm sap/wine treatment can not cause hypoglycaemia. The high activity of probiotic bacteria (*Zymomonas mobilis*) and yeasts have been associated with breaking down of reactive oxygen species that may be responsible for elevating blood sugar levels.

The haematology of the male Wistar rats was studied. The research study showed inconsistency with the report of Matawalli et al. [27] that alcohol fed rat administered with normal dosage shows no adverse health effect. The significant ($p<0.05$) difference in the haematological parameters of rats administered palm sap/wine when compared to the rat group that had no treatment may be as a result of chronic administration of palm sap/wine to the rat groups. The decrease in the level of haemoglobin could be a sign of anaemia in the treated male rats. WHO [28] reported that haemoglobin cut-off values that indicate anaemia vary with physiological status (e.g. age and sex), and is caused by iron deficiency. So far, no study examining the effect of palm sap/wine in vivo has been made on rats' serum parameters and haematology as the treatment model. Because of this reason, there is no scientific evidence to compare the results. The results of the haematological parameters were in agreement with the result of the blood glucose (Table 3) of the rats fed with FS and PS, though the increases observed in them were not significantly ($p>0.05$) different when compared to the control rat groups. The leucocytes counts, however, could not give specific information which necessitated the differential leucocytes counts. Neutrophil is majorly responsible for phagocytosis of pathogenic microorganisms during the first few hours after their entry into tissues [29]. There was no significant ($p>0.05$) difference in the neutrophil counts between group 5, 3 and the group 1 treated rats, but group 2 treated rats was significantly higher ($p<0.05$) compared with the group 1.

The group 4 and 5 rats treated with FS and FrS had the highest values of bilirubin. Bilirubin is formed by the breakdown of haemoglobin in the liver, bone marrow and spleen [17]. The bilirubin concentrations were within the normal limits of 0.4 – 1.7 d/dl [30,31]. The slight variations in bilirubin concentrations in the treated groups, except PS group were indicative of non–adverse effects on haemoglobin metabolism. An increase in plasma bilirubin results in jaundice. It has been shown that raised bilirubin indicates failure of the liver excretory function and increased breakdown of red blood cells [31]. This observation could not be confirmed from the results in Table 5, which showed that the treated rat groups manifested some signs of ill-health due to chronic administration of palm sap/wine based on 1.5% of the body weight per ml.

The research also examined the clinical chemistry of the male Wistar rats in order to find the effect of the treatment on the enzyme liver and kidney makers. A decreased serum alkaline phosphatase (ALP) may be due to zinc deficiency, hypothyroidism, vitamin C deficiency, folic acid deficiency, excess vitamin D intake, low phosphorus levels (hypophosphatasia), celiac disease, malnutrition with low protein assimilation, insufficient parathyroid gland function, and pernicious anaemia and vitamin B insufficiency [32]. There were no significant ($p>0.05$) differences between the serum creatinine levels in rat groups 1, 2, 3 and 4 but significant ($p<0.05$) differences exists between them and group 5 treated rats. High levels of serum creatinine are an indication of liver disease, since it shows low filtration rate of the kidney. Creatinine levels differ among individuals and groups according to age and sex. Normal levels of creatinine in the blood are approximately 0.6 to 1.2 mg/dl in adult males and 0.5 to 1.1 mg/dl in adult females [33]. The significant ($p<0.05$) low level of creatinine in the group 5 rats administered with fresh palm sap was an indication of reduced muscular mass in the treated rat group, since the level (0.63 mg/dl) falls within the normal range.

The significant increase in the neutrophil count in group 2 treated rats may be an indication of increased pathogenic organisms that necessitated increases in phagocytosis so as to eliminate the effect of pathogens in the body. Basophile and monocyte counts were not detected in the treated rat groups. Monocyte is responsible for the defence of tissue against microbial agents while basophil counts increase upon sensitization to an antigens (or allergen) [34] [16]. White blood cell is responsible for fighting germs and harmful microorganisms. Lymphocytes are responsible for humoral antibody formation and cellular immunity. The results therefore suggested that the entire rat group showed signs of immunostimulatory effect.

Probiotics can be described as live microorganisms that, when administered in adequate amounts confer a health benefit on the host. The classification of palm wine as a probiotic beverage was as a result of the presence of

Lactic acid bacterials, *Bifidobacteria* and *Saccharomyces boulardii*. However, *Saccharomyces cerevisiea* has not been characterized as a probiotic microorganism. The presence of alcohol in the palm products were elaborated by the presence of inherent *saccharomyces cerevisiae* while the presence of *Citrobacter freundii* in the MW confirms environmental contamination of the product and contributed in higher percentage weight of liver in the male animals compared to the other treated rat groups. The MW also contained the highest level of microbial count which is significant ($p<0.05$) compared to either the FS or the FrS and may be attributed to contamination by *Citrobacter freundii* while alcohol where not detected in the pasteurized palm sap. The rat treated palm sap products showed significant decrease ($p>0.05$) in the weight gain. The gain in weight for the FrS treated rat group was gradual and progressive while the control rat group progressed rapidly and fall. The progressive increase in the weight gain of rats by the FrS treated group may not be unconnected with the low alcohol content and the probiotic effect of the *Saccharomyces cerevisiea.* The weight of PS treated rat group was the lowest. The increase in the body weight of rats treated with FrS and FS could be attributed to the activities of probiotic yeasts in them compared to the body weight of rats treated with either MW or the PS. Research has shown that probiotic palm yeasts in MW by their activities increase the amino acids content of the feeding especially lysine, methionine and cysteine [35]. The significant increase in the number of microbial organisms in the MW was as a result of growth of *Citrobacter freundii*. *Citrobacter freundii* is an environmental microorganism which has been associated with the contamination of marine fish. The presence of this bacterium is suggestive of sewage contamination [36]. *Citrobacter freundii* is also associated with nitrogen fixation and is also associated with phage mediated interactions [37,38]. *Citrobacter freundi*i has not been isolated in palm wine by other researchers. Therefore, its presence in palm wine bought from the market is indicative of microbial contamination from dirty environment.

4. Conclusion

It was evident from the results obtained that palm sap drinkers may not be diabetic as a result of drinking palm sap samples. Efforts should be made to protect palm sap from contamination either by environmental microorganism or adulteration in order to exclude the presence of *Citrobacter freudi* or any other microorganism that may be injurious to health from contaminating palm sap products. The research was able to correlate the decrease in body weight to the effect of drinking palm sap products. It could therefore be asserted that the decrease in body weight resulting from drinking palm sap products could be attributed to the fact that drinkers takes less food after drinking. Since palm sap is made up of basically glucose and lactose, it is not surprising that lovers of such products rarely eats food since palm sap products supplies energy to their system and thus, they fills fulfilled. However, the drinkers of FrS sample proved otherwise, since it was comparable to the control. It may be concluded that prolonged drinking of palm sap could lead to exposure of the body to pathogenic organisms. The increase in the neutrophil count in Wistar rats treated with palm saps could be as a result of increased attack of pathogenic microorganism leading to increased response to the defence of the body against such attacks. Also, chronic administration of the palm sap samples manifested in the significant differences observed with the haematological parameters observed when compared with the control rat groups. Therefore, chronic administrations of palm sap products have effect in exposing the body to health challenges by decreasing the liver and kidney functions, and reduction in mean spleen, kidney and body weights.

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Conflict of Interest Statement

The authors have no conflict of interest to declare.

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