Effects of Acha-Cowpea-Carrot Based Couscous on the Hematological and Liver profile of Wistar rat

ABSTRACT

The effects of acha-cowpea-carrot based couscous on the Hematological and Liver profile of Albino Rats was investigated. Couscous was prepared from acha, cowpea and carrot flour blends at ratios of 100:0:0 (C1); 90:5:5 (F1); 80:10:10(F3); 75:15:10(F4); 70:15:15(F5); and 50:25:25 (F8), and fed to Wister rat for 42 days. The hematological and biomarker indices of wistar rat were studied. Biological assay showed high protein quality distortion of the liver. The white blood cell and lymphocytes, decreased from 2.82 – 1.75 % and 75.88 – 40.03 % respectively, with increase in added cowpea and carrot flours. There was a significant increase in ($p<0.05$) in the neutrophils, red blood cell, packed cell volume, mean cell hemoglobin (MCH), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), platelet count, and hemoglobin with increase in added cowpea and carrot flours. The glycaemic level for samples F1 F3, F4, F5 and F8 which ranged between 44.30-62.45 were lower compared with the 65.50 observed for control. However, all the tested samples are in the intermediate (medium) glycaemic level. The total protein and low density lipoprotein, decreased from 100.80 – 70.84 and 17.06 – 8.06 respectively, while the aspartate transaminase (AST), Alkaline phosphate, triglycerides (TGs), total cholesterol (TC), and HDL (high density lipoprotein) increased from 40.74 – 53.33, 105.01 – 123.53, 50.99 – 62.79, 53.78 – 69.14 and 47.57 – 56.89 respectively with increase in added cowpea and carrot flours. This study suggests that relative increase in the level of cowpea and carrot could improve the immune system and hence the health status of the consumers.

Key Words: Acha-Cowpea-Carrot, Couscous, Hematological, Liver profile,Wistar rat

INTRODUCTION

Couscous is traditional pasta like food which is commonly produced by using semolina, *acha* and sorghum in Africa and Asia, however in Turkey, traditional Turkish couscous is generally prepared by coating of bulgur granules with semolina, wheat flour; egg and water or milk. Couscous have been inscribed in 2020 on UNESCO's list of Intangible Cultural Heritage and Information on the ancestral know-how and the practices related to the production and consumption have been documented (UNESCO, 2020). Couscous is also a celebratory dish eaten at weddings, funerals, or the end of festivals (Coskun, 2013). In the literature, studies have recently been made for enrichment of couscous by either substitution of semolina with legume flours and other grain flours or adding nutritious ingredients to the composition.

Acha (*Digilaria exilis*) is a cereal grain in the family of gramineae and commonly referred to as fonio or hungry rice (Alamu, 2001). It contains 7% crude protein which is high in leucine (9.8%), methionine (5.6%) and valine (5.5%). The protein in the crop is reported to be unique in that it has greater methionine and leucine content than other cereals .which implies that acha is a very good source of protein (Jideani, 1993). Acha does not contain glutenin or gliadin proteins which are the constituents of gluten, making this cereal suitable for people with gluten intolerance *(Harlan, 1993*). Acha has been identified as a major food for diabetic patients in Nigeria (*Jideani, 1991*).

Cowpea (*Vignaun guiculata*) is an indigenous tropical legume that produces pods and grain that are highly nutritious and valuable because it contributes to the livelihood of several millions of people in West and Central Africa. Cowpea is a rich source of protein of about 30 % , this makes it a ready substitute to animal protein in addition to it being easily available to livelihoods of relatively poor people in the less developed countries (Pindar *et al.,* 2018; Boukar*et al.*, 2010). Cowpea seed is also a good source of bioactive compounds such as flavonols and hydroxyl benzoic acids, that can reduce the risk for physiological disorders such as obesity, dyslipidemia and cardiovascular complications (Sreerama *et al.,* 2012).

Carrot (*Daucus carota* L) is also one of the important nutritious root vegetables grown throughout the world. Carotenoids are potent antioxidants present in carrots which help to neutralize the effect of free radicals. Reports have showed that they have inhibitory mutagenesis activity thus, contributing to decrease risk of some cancers (Dias, 2012).

In recent years, consumption of carrot and its related products has increased steadily due to the recognition of antioxidant and anticancer activities of ß-carotene. Nutritious food products utilizing carrot had been developed and accepted by consumers (Jenkins *et al.,* 2002).

Consequently, consumption of carrot and its products would be very useful in alleviating vitamin A deficiency particularly, among children below six years and adults.

Couscous is made from durum wheat semolina and is reported to be rich in starch with low vitamins, minerals, dietary fibers, and phenolic compounds. Pasta, with legumes, herbs, and vegetables, was found as a complete, delicious, and healthy food (Kaur *et al.*, 2012). In recent years, different functional ingredients have been used to increase the nutritional as well as functional properties of pasta. Pasta has been enriched with some cereals, pseudocereals, legume flour, fruit, and vegetable powder as a source of fiber, minerals, antioxidants, and polyphenols: common bean flour (Gallegos-Infante *et al.*, 2010), carrot powder (Badwaik *et al.*, 2012), white bean, yellow pea and lentil (Wojtowicz and Moscicki, 2014).

The consumers' quest for novel and other –free food, as well as wholegrain food has prompted the couscous industry to produce and market a number of products where traditional shapes have been maintained but durum wheat has been partially or totally replaced by other cereals, pseudocereals or other flours of vegetable origin, particularly legumes (Schoenlechner, 2016).

Hunger and malnutrition remain a serious problem in third world countries like Nigeria. Non-communicable diseases (NCDs), which were formerly regarded to be diseases of affluence, have also recently emerged as a major health problem to even resource-disadvantaged members of the society, especially in developing countries.

Most of the cereal based food couscous inclusive consumed in Nigeria is made from wheat which is imported and this drains a lot of foreign exchange. The inability of Nigeria to meet the increasing demand for pasta has in sequence called for research into alternative local source of flour for producing pasta like couscous. Also, Gluten in wheat has been linked to the cause of some ailments like Control of Vitamin A deficiency (VAD), iron deficiency anaemia (IDA) and iodine deficiency disorders (IDD) poses a challenge for nutritionists and health workers.

Acha, cowpea and carrot are cultivated in Nigeria. These crops are naturally nutrient dense and can contribute immensely to good health. The crops are available and affordable all year round, but the masses are not aware of the numerous nutritional benefits to human health (Ayo et al., 2018, Ayo et al., 2016). Acha, though potentially rich in nutrients, has been classified among the lost crops with the cultivation and processing at village level technology (Ayo et al., 2016).

With the growing demand for adequate supplies of food to feed the ever increasing world population, opportunities exist for food processors to develop novel foods fortified with legume ingredients which are healthy and convenient and which take advantage of the techno-functional properties of legume flours. Several studies have focused on developing new products, such as low-fat meat balls ,extruded snacks, weaning food, bread, and macaroni, by using legume flours as ingredients. Further research is still needed to expand the availability of these legume based products and to optimize the quality of these legume-based products for specific markets. Product such as couscous can be considered a convenient vehicle for the addition of micronutrients and protein to meet these consumer health demands. Couscous is progressing towards becoming a world renowned food.

In developing countries, where protein-calorie (PCM) and micro-nutrient (MNM) malnutrition are major problems, legumes may be considered as a substitute of the expensive and sometimes scarce animal protein, to complement cereals, in lysine balance and serve as a cheap source of dietary fibre, vitamins andantioxidant compounds with beneficial health that affects the weight, intestinal function, glycaemia and LDL cholesterol levels.

Nowadays, the need for easy prepared meals increased due to the fast lifestyles and people being more aware about the importance of nutritionally valued products and its benefits to health. Growing concerns about the potential negative health impacts of consuming food with high amounts of fat and cholesterol has increased interest in using plant-derived foods such as legumes which contain low-fat and are cholesterol free in food formulation.Whole legumes can be milled into flour or fractionated into protein, starch and fiber fractions, and these components can be incorporated into commercial food products as functional or replacement ingredients, thereby facilitating their use.

Recent research studies have, furthermore, suggested that consumption of legumes may have potential health benefits including reduced risk of cardiovascular disease, cancer, diabetes, hypertension, gastro-intestinal disorder, adrenal disease and reduction of LDL cholesterol. Such studies have spurred interest in using whole legumes and their fractions in developing a variety of novel food products.

As the world population increases, increase in the commercial production of couscous, and its recognition all over the world will be supported by the studies of couscous. The broad objective of this study was to investigate the effects of acha-cowpea-carrot on the hematological and liver profile of Wister rats..

MATERIALS AND METHODS

Materials and material preparation

Acha grains (*D. exilis*); cowpea (*Phaseleous vulgaris*) and carrot *(Daucus carota)* were purchased from Bukuru market in Jos South Local Government Plateau State Nigeria. Other ingredients like vegetable oil (kings), monosodium glutamate (maggi), salt (Dangote table salt and curry spice (Tiger) were also purchased in the same market. Wistar Albino rats were purchased from Veterinary Research Institute Vom Jos.

Preparation of Acha flour: Preparation of acha flour was done by the method described by Olapade *et al.* (2012). The grains of acha were sorted manually, washed, destined (manually), dried (oven for 4 h 50 mins at 40 ^{o}C), milled (Attrition mill model no 0712098) and sieved (300 µm) to produce acha flour followed by packaging in plastic bottles prior to usage.

Preparation of cowpea flour: Preparation of cowpea flour was done by the method described by Ngomas *et al.* (2018) with slight modification. Cowpeas (2 kg) were manually sorted, moistened with portable water (500 ml) and allowed to stand for 20 mins to soften the beans). Thereafter, the soaked cowpeas were then dehulled manually and the husks removed by floatation. The clean seeds (husk freed) were then boiled(100 ^{o}C) in water for ten minutes, drained and air oven dried at 45 ^{o}C for 8 h before milling (hammer mill) sieved (~~0.3~~ 300 µm aperture size) and stored in air tight container.

Preparation of carrot flour: Preparation of carrot flour was produced by the method described by Joshua *et al.* (2021) with minor modifications. The selected carrots were manually washed (portable water), sorted, sliced into about ...cm thick using a manual slicer, blanched for 5 min and oven dried for 10 h at 40 ^{o}C. The dried carrot was then milled (Attrition mill model no 0712098), sieved (300 µm aperture) to produce carrot flour. This was packaged in Ziploc bags and stored at room temperature.

Production of couscous from acha-cowpea-carrot composite flours: Couscous samples were prepared as described by Talim (2012). Couscous samples were prepared from different blends of refined acha flour, cowpea flour and carrot powder in the respective ratios of 100:0:0; 90:5:5; 80:10:10, 75:15:10, 70:15:15, 65:20:15, 60:20:20, 55:25:20 and 50:25:25 (Table 1). The flour composite is sprinkled with clean water and rolled with hands to form small pellets and dry flour was sprinkled to keep them separate, and then sieved with a 0.4um aperture sieve. The pellets were put into colander and steamed over a pot of boiling water for

5 min. This was then spread on stainless steel trays and dried in hot air oven at 45^{o}C for 35 min, cooled and packaged in transparent polyethylene bags.

Table 1. Acha, cowpea and carrot flour blend formulation for couscous production

Treatments	Acha flour	cowpea flour	Carrot powder
C1	100	0	0
F1	90	5	5
F2	80	10	10
F3	75	15	10
F4	70	15	15
F5	65	20	15
F6	60	20	20
F7	55	25	20
F8	50	25	25

METHODS

Rat Feeding.

Animal Experiment

Preliminary investigation using sensory analysis resulted in the preference for five samples of acha-cowpea-carrot flour blends; 90:5:5 (F1), 75: 15: 10 (F3), 70:15: 15 (F4), 65: 20:15 (F5) and 50:25:25 (F8). Acha flour (100%) served as the control. The study was approved by the National Veterinary Research Institute, Federal Ministry of Agriculture and Rural Development, Vom, Nigeria, ethical committee under the ethical number AEC/02/46/18 OF 26TH July, 2018**.** were housed in clean metabolic metal cages in clean well-ventilated room which was and disinfected prior to the commencement of the feeding trials. The rats were allowed to or acclimatize for the period. Animals receive lighting schedule of 12-hr light and 12-hr darkness at room temperatures (25°C ± 2). Vita feed Palettes concentrate made by Grand Cereal and Oils Limited Bukuru Express Way, Jos was used to feed the rats for the acclimatization period of 7 days and water was given ad libitum throughout the feeding period. After the acclimatization period, the animals were reweighed and grouped into six cages, with each cage housing a total of five rats and one group of five rats was used as the control. A total of six treatments (formulations of couscous) including one control was used. Each group of six rats in each cage was given 100 g of couscous per day for 42 days. At the end of the feeding period, the animals were sacrificed; blood samples were taken for analysis.

Hematological Analysis

The blood was analyzed for packed volume cell and hemoglobin concentration, cholesterol, high- and low-density lipoprotein, very low lipoprotein using the method as described by Young *et al.* (1984). Full blood counts including packed cell volume (PCV), hemoglobin, , (Hb), red blood cell (RBC), white blood cell (WBC), respectively. Platelet count, differential WBC (lymphocytes, neutrophils and mixed), and red cell indices mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and mean cell volume (MCV) were determined using the Sysmsex® Automated Hematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan.

The animals were subjected to an overnight fast after 21 days of treatment, weighed using a weighing balance (doran-PC 500) and anesthetized using the "Drop method" (JHU, 2009). Blood samples were taken for PCV and hemoglobin estimation by the Cyanmeth hemoglobin. The sera were analysed for AST and ALT (using the Reitman and Frankel method), ALP, LDH and Amylase (using the kinetic method as described by Sood (2009). Their absorbencies were read using a spectrophotometer (APEI PD-303S). The liver was excised, blotted dry, weighed on a microbalance sensitive at 0.001mg (Precisa 125A, Switzerland) and fixed in 10% formal saline for histopathological analysis.

In-vivo determination of glycaemic index of the flour blends

The glyceamic index was determined using the method described by Wolever *et al.* (1991).. After the adaptation period, the animals were reweighed and fasted for 12 h (overnight fasting) and the blood glucose of the animals was taken at zero time from the tail vein before feeding them with the test products and glucose (a control) in a portion size that was calculated to contain 2.0 g of available carbohydrate and this consumed within 25 min. After the consumption, the serum glucose levels of the animals were measured using an automatic glucose analyzer ('Accu-chek Active' Diabetes monitoring kit; Roche Diagnostic, Indianapolis, USA) at 0, 30, 60, 90 and 120 min intervals. The glycaemic response was determined as the Incremental Area under the Blood Glucose Curve (IAUC) measured geometrically from the blood glucose concentration-time graph ignoring area beneath the fasting level.

Blood glucose curves were constructed from blood glucose values of animals at time 0, after 15, 30, 45, 60, 90 and 120 min intervals after consumption of the glucose (control) and experimental food samples of each group. The IAUC was calculated for reference food (glucose) by the trapezoidal rule in every rats in each group separately as the sum of the surface of trapezoids between the blood glucose curve and horizontal baseline going parallel to x-axis from the beginning of blood glucose curve at time 0 to the point at time 120 min to reflect the total rise in blood glucose concentration after eating the reference food (glucose).

The IAUC from the animals fed with the formulated food samples were similarly obtained. The glycaemic Index (GI) for each diet was calculated using the formular;

$$\text{GI} = \frac{Incremental\ area\ under\ 2h\ blood\ glucose\ curve\ for\ food\ samples\ (2.0g)}{Incremental\ area\ under\ 2h\ blood\ glucose\ curve\ for\ glucose\ (2.0g)} X\ 100$$

Light microscopy and photomicrography

Stained sections of the liver tissues were carefully examined under binocular compound light microscope (OLYMPUS CX41, Japan). Tissue sections from the treated groups were examined for any evidence of histopathologic changes with respect to those of the controls. After examination, photomicrograph of selected slides from both the treated and control groups were taken under a magnification of x100 and x400 objective by using (EVOS XI, China) automated built-in digital photo camera

Experimental Design and Statistical Analysis

The experimental design for the study was completely randomized design. All the data obtained from this study were subjected to analysis of variance (ANOVA) using statistical package for Social Sciences (SPSS) software version 20. Means were separated using Duncan's multiple range test at $p \leq 0.05$

RESULTS AND DISCUSSIONS

Hematological Analysis of Wistar Rats Fed with Couscous Prepared from Acha, Cowpea and Carrot Flour Blends with Acha-Iron Bean-Carrot for 42 Days *Ad libitum*

The results of hematological properties are shown in Table 2. The white blood cell and lymphocytes, decreased from 2.82 – 1.75 and 75.88 – 40.03 respectively, with increase in added cowpea and carrot flours. While the neutrophils, red blood cell, packed cell volume, MCH, MCV, and red cell indices (MCHC= mean cell hemoglobin concentration, platelet count, and hemoglobin increased from 32.00 – 58.08, 8.34 – 9.46, 45.71 – 54.28, 53.50 – 57.53, 14.55 – 15.65, 268.00 – 280.73, 658.67 – 935.50 and 126.00 – 147.12 respectively, with increase in added cowpea and carrot flours.

Table 2. Hematological Analysis of Wister Rats Fed with Acha-Cowpea-Carrot Flour

	Treatments					
Parameters	C1	F1	F3	F4	F5	F8
WBC	2.82^{a} ±0.87	2.26^{b}±1.87	2.18^{b}±1.12	2.04^{b}±0.52	2.01^{b}±0.87	1.75^{c}±0.87
Neut	32.00^{d}±0.78	38.00^{c}±0.32	40.00^{c}±0.87	46.44^{bc}±0.99	50.78^{b}±0.87	58.08^{a}±0.00
Lymph	75.88^{a}±0.45	67.54^{b}±0.87	65.50^{b}±0.33	59.44^{bc}±0.54	52.51^{c}±0.11	40.03^{d}±0.45
RBC	8.34^{b}± 0.40	8.38^{b}±0.77	8.63^{ab}±0.87	8.63^{ab}±0.14	8.76^{ab}±0.87	9.46^{a}±0.27

PVC	45.71^{b}± 1.85	46.29^{b}±0.87	46.78^{b}±0.47	46.89^{b}±0.65	49.19^{ab}±0.07	54.28^{a}±0.85
MCV	53.50^{b}± 0.71	54.12^{b}±0.11	54.50^{b}±0.18	56.02^{a}±0.77	56.03^{a}±0.08	57.53^{a}±0.87
MCH	14.55^{b}± 0.21	15.15^{a}±0.21	15.15^{a}±0.87	15.35^{a}±0.98	15.60^{a}±0.87	15.65^{a}±0.98
MCHC	268.00^{b}±4.95	271.51^{ab}±0.87	272.33^{ab}±0.87	277.01^{a}±0.87	279.14^{a}±0.55	280.73^{a}±0.57
PLT	658.67^{c}±2.79	864.55^{b}±0.87	935.11^{a}±0.43	994.09^{a}±0.44	1013.51^{a}±0.43	935.52^{a}±0.14
HGB	126.00^{b}±7.07	127.78^{b}±0.87	128.00^{b}±0.14	130.51^{ab}±0.12	131.66^{b}±0.17	147.12^{a}±0.12

Values are means ± SD of triplicate determinations. Values in the same row with different superscripts are significantly different ($p<0.05$).
Key:
C1= Couscous prepared from 100% acha flour (control sample)
F1= Couscous prepared from 90% acha flour, 5% cowpea flour and 5% carrot flour
F3= Couscous prepared from 75% acha flour, 15% cowpea flour and 10% carrot flour
F4 = Couscous prepared from 70% acha flour, 15% cowpea flour and 15% carrot flour
F5 = Couscous prepared from 65% acha flour, 20% cowpea flour and 15% carrot flour
F8= Couscous prepared from 50% acha flour, 25% cowpea flour and 25% carrot flour

Isaac *et al.* (2013) stated that hematological components, consists of red blood cells, white blood cells or leucocytes, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration are valuable in monitoring feed toxicity, especially, with feed constituents that affect the blood as well as the health status of farm animals. Aro and Akinmoyegun (2012) and Aro *et al.* (2013) reported that haematological parameters like haematocrit value, haemoglobin concentration, white blood cell count, red blood cell count among others are used in routine screening for the health and physiological status of livestock and even humans. Adejumo (2004) reported that haematological traits especially packed cell volume (PCV) and haemoglobin (Hb) were correlated with the nutritional status of the animal. Isaac *et al.* (2013) stated that PCV is involved in transport of oxygen and absorbed nutrient. Other blood parameters like blood viscosity are often neglected in routine clinical and physiological investigations.

Red blood cell indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are the most useful indicators in the diagnosis of anemia in most animals (Akpamu *et al.*, 2011, Rogers, 2011). An increased WBC count indicates that the body is fighting an infection or intoxications from chemical substance or inducing the immune response of treated animals (Ajibade *et al.*, 2012). On the other hand, significant decrease in the WBC of the blood indicates a decline in the production of leukocytes and the body is less able to fight off infections.

The evaluation of serum biochemical parameters has significant importance to evaluate toxicological changes produced by toxicants. This is because of the body's response to clinical signs and symptoms. Elevated serum levels of enzymes produced by the liver or nitrogenous wastes to be excreted by the kidney might be indications to their spillage into the blood stream as a result of necrosis of the tissues (Prakash and Manavalan, 2011). Because of the liver's strategic location between intestinal tract and the rest of the body it is the first organ to encounter ingested nutrients, vitamins, metals, drugs, and environmental toxicants as well as waste products of bacteria that enter portal blood (Curtis, 2007). As the result, liver is a primary destination for any toxic substance that entered to the body, and any abnormal change in liver will definitely affect complete metabolism of an animal (Paliwal *et al.*, 2009). The most commonly used serum liver chemistry tests include serum transaminases (alanine aminotransferase (ALT), aspartate aminotransferase (AST)), serum alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT), bilirubin and albumin. The major intracellular enzymes of the liver are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Injuries of liver cells (hepatocytes) allowing for escape of these enzymes into the bloodstream raises their levels in the blood (Thapa and Walia, 2007). The levels of ALP and GGT in the serum are important parameters for evaluation of hepatobiliary route(Weingand*et al.*, 1996).

Glycemic Index of Couscous Prepared from Acha, Cowpea and Carrot Flour Blends (%).

The results of glycemic index for 44 days (the work was for 42 days) are shown in Table 3. The control sample maintained a constant value of 65.50 % for 14 days. The glycemic index for samples F1, F3, F4, F5 and F8 decreased from 65.45 – 50.30 %, 58.03 – 49.55%, 56.37 – 44.30%, 56.55 – 50.45% and 53.42 – 50.75%, respectively, with increase in added cowpea and carrot flours. The glycemic index of couscous prepared from acha, cowpea and carrot flour fall within the range of medium glycemic index. Dona *et al.* (2010) classified glycemic index as follows: Low GI = < 55%, Medium GI = 56 - 69%, and High GI += >70% irrespective of the day of feeding.

In Nigeria, acha sellers identify diabetic patients as their major customers and it is also reported that doctors are recommending fonio to diabetic patients because it is more appropriate for them. The Glycemic Index of a food is the rate at which sugar is released from that food into the blood. The lower the value, the slower the process. Individuals with non-communicable diet related diseases such as diabetes and obese are advised to choose low GI foods like Fonio. It is also important for those with high sugar level

Table 3. Glycemic Index (%) of Couscous Prepared from Acha, Cowpea and Carrot

Study Day	Treatments					
	C1	F1	F3	F4	F5	F8
0	$65.50^{a}\pm0.41$	$62.45^{b}\pm0.71$	$58.03^{c}\pm0.11$	$56.37^{d}\pm0.14$	$56.55^{d}\pm0.07$	$53.42^{e}\pm0.14$
4	$65.50^{a}\pm0.04$	$60.10^{b}\pm0.14$	$56.20^{c}\pm0.99$	$56.81^{bc}\pm0.14$	$56.70^{bc}\pm0.14$	$53.42^{c}\pm0.00$
8	$65.50^{a}\pm0.21$	$60.35^{b}\pm0.07$	$56.15^{c}\pm0.78$	$56.85^{c}\pm0.28$	$56.55^{c}\pm0.35$	$53.13^{d}\pm0.14$
12	$65.50^{a}\pm0.08$	$60.20^{b}\pm0.28$	$57.10^{c}\pm0.84$	$56.95^{c}\pm0.21$	$56.85^{c}\pm0.07$	$53.71^{d}\pm0.42$
16	$65.50^{a}\pm0.71$	$59.05^{b}\pm0.07$	$57.50^{c}\pm1.13$	$57.55^{c}\pm0.07$	$56.35^{cd}\pm0.63$	$54.85^{d}\pm0.21$
20	$65.50^{a}\pm0.77$	$57.10^{b}\pm1.68$	$58.10^{b}\pm0.14$	$58.36^{b}\pm0.42$	$55.07^{c}\pm0.14$	$54.80^{c}\pm0.28$
24	$65.50^{a}\pm0.98$	$50.30^{d}\pm1.28$	$54.71^{c}\pm0.72$	$57.00^{b}\pm1.41$	$54.35^{c}\pm1.91$	$54.95^{c}\pm0.91$
28	$65.50^{a}\pm1.32$	$53.80^{c}\pm1.28$	$53.30^{c}\pm1.84$	$56.65^{b}\pm1.91$	$53.85^{c}\pm0.91$	$55.59^{b}\pm0.28$
32	$65.50^{a}\pm1.19$	$52.90^{c}\pm1.10$	$52.70^{c}\pm1.40$	$56.15^{b}\pm1.61$	$52.25^{c}\pm1.89$	$55.25^{b}\pm0.35$
36	$65.50^{a}\pm0.74$	$52.80^{c}\pm1.05$	$53.20^{c}\pm1.69$	$55.00^{b}\pm1.41$	$51.75^{c}\pm1.19$	$55.15^{b}\pm0.21$

40	$65.50^{a}\pm0.55$	$51.10^{c}\pm1.56$	$52.75^{c}\pm1.47$	$54.40^{b}\pm0.84$	$51.91^{c}\pm1.26$	$51.58^{c}\pm1.83$
44	$65.50^{a}\pm0.71$	$50.30^{b}\pm0.42$	$49.55^{b}\pm0.78$	$44.30^{c}\pm1.93$	$50.45^{b}\pm0.50$	$50.75^{b}\pm1.06$

Values are means ± standard deviation of triplicate determinations. Values in the same row with different superscripts are significantly different (p<0.05).
C1= Couscous prepared from 100% acha flour (control sample)
F1= Couscous prepared from 90% acha flour, 5% cowpea flour and 5% carrot flour
F3= Couscous prepared from 75% acha flour, 15% cowpea flour and 10% carrot flour
F4 = Couscous prepared from 70% acha flour, 15% cowpea flour and 15% carrot flour
F5 = Couscous prepared from 65% acha flour, 20% cowpea flour and 15% carrot flour
F8= Couscous prepared from 50% acha flour, 25% cowpea flour and 25% carrot flour

and has also been found to reduce the risk of heart disease, blood lipids and can improve the body's sensitivity to insulin (ref). Low GI means a smaller rise in blood sugar which will help control established diabetes condition, it is also shown that diets with low GI can help in weight loss

The glycemic index (GI) (a dietary measuring system relating the rate at which carbohydrate-containing foods raises blood sugar after two (2) hours or more after consumption (post-prandial glycaemia). Carbohydrate-containing foods are graded as either having a high, intermediate (medium) or low GI depending on the rate at which blood sugar level rises (Mendosa, 2000), which in turn is related to the rate of digestion and absorption of sugars and starches available in that food (FAO/UN, 1998). Thus, high GI foods (GI range: >70) will break down rapidly during digestion and cause a rapid, but short-lived rise in the blood sugar level during absorption while low-GI foods (GI-range: <55) undergoes slower but gradual release of glucose into the blood stream, while intermediate (medium) glycemic foods are those ranging between 56 and 69 on the GI scale. increased insulin secretion, caused by foods with high GI, leads to postprandial hyperinsulinemia along with an increase in both hunger and voluntary food intake (Aller *et al*., 2011). This suggests that a low-GI diet may provide some level of prevention against developing diabetes and obesity and for managing existing cardiovascular diseases (CVD). Dietary glycaemic index and glycaemic load appear to have increased in recent years because of increases in carbohydrate intake and changes in food processing (Ludwig, 2002). Dietary glycemic index is an indicator of carbohydrate quality that reflects the effect on blood glucose, and the dietary glycemic load is an indicator of both carbohydrate quality and quantity food (Wolever*et al*., 1994; Salmeron*et al*., 1997). Epidemiologic evidence suggests that a diet with a high glycemic load or glycemic index may increase the risk of coronary heart disease (Liu et al., 2000; Ford and Liu, 2001; Liu and

Manson, 2001) and type 2 diabetes (Salmeron*et al*., 1997). Glycemic index is an important tool used in treating people with diabetes and in weight loss programs. Low glycemic index foods, by virtue of the slow digestion and absorption of their carbohydrates, produce a more gradual rise in blood sugar and insulin levels and are increasingly associated with health benefits. Low glycemic index foods have thus been shown to improve the glucose tolerance in both healthy and diabetic subjects (Jenkins *et al*., 1988). Hence, the present study aimed at formulating multi-plant based functional foods with low glycemic index and antidiabetic activities. The glycemic index defined as the incremental blood glucose area under the curve following a test food, expressed as the percentage of the corresponding area following a carbohydrate equivalent load of a reference food (Bjorck *et al*., 2000). Its determination is an attempt to characterize foods according to their postprandial glycemic response rather than their chemical composition (Jenkins *et al*., 1981). Scientific studies have established that glycemic index of a food in humans is influenced by many factors like rate of digestion/gastric emptying/absorption (FAO/WHO, 1997; Liljeberg and Bjorck, 1998; Jenkins *et al*., 2002), nature of the starch/carbohydrates granules and food processing (John and Vladimir, 2004).

The high GI foods rapidly digest and increase the blood glucose level, while low-GI foods undergoes slower but gradual release of glucose into the blood stream. The fundamental health benefits of low glycaemic index and glycaemic load foods is that, these foods produce a lesser increase in the plasma glucose concentration as a result of slower rates of gastric emptying and digestion of carbohydrate in the intestinal lumen and subsequently, a slower rate of absorption of glucose into the portal and systemic circulation (Jenkins *et al*., 1981; Wolever*et al*., 1991

Liver Function Lipid Profile of Wister Rats Fed with Couscous Prepared from Acha, Cowpea and Carrot Flour Blends for 42 Days

The results of liver function properties are shown in Table 4. The total protein and low density lipoprotein, decreased from 100.80 – 70.84 g/l and 17.06 – 8.06 Mg/dl respectively, with increase in added cowpea and carrot flours. While the aspartate transaminase (AST), Alkaline phosphate, triglycerides (TGs), total cholesterol (TC), and HDL (high density lipoprotein) increased from 40.74 – 53.33 U/L, 105.01 – 123.53 U/L,, 50.99 – 62.79 Mg/dl, 53.78 – 69.14 Mg/dl and 47.57 – 56.89 Mg/dl respectively with increase in added cowpea and carrot flours.

TABLE 4: Liver Function Lipid Profile of Wister Rats Fed with Couscous Prepared from Acha, Cowpea and Carrot Flour Blends

	Parameters						
Sample	AST(U/L)	ALP(U/L)	Total Protein (g/L)	Tryglyceride Mg/dl	Cholesterol Mg/dl	High density lipoprotein Mg/dl	Low Density Lipoprotein Mg/dl
C1	40.74^{b}±0.81	105.01^{b}±1.02	100.88^{a}±0.28	50.99^{b}±0.29	53.78^{c}±0.02	47.57^{c}±0.01	17.06^{a}±0.05
F1	42.75^{b}±0.58	106.48^{b}±1.03	97.06^{a}±0.72	51.24^{b}±0.12	58.90^{b}±0.20	49.27^{bc}±0.52	16.09^{a}±0.10
F3	45.34^{b}±0.17	113.45^{ab}±0.54	84.77^{b}±0.88	51.78^{b}±0.81	63.08^{ab}±0.26	50.54^{b}±0.03	12.82^{b}±0.04
F4	48.60^{ab}±0.29	118.19^{a}±0.97	79.21^{b}±0.95	54.55^{ab}±0.92	68.84^{a}±0.71	54.01^{a}±0.18	11.73^{b}±0.04
F5	50.32^{a}±0.89	123.00^{a}±1.27	73.17^{c}±0.39	59.12^{a}±0.60	68.91^{a}±0.28	55.40^{a}±0.65	9.26^{c}±0.44
F8	55.33^{a}±0.31	123.53^{a}±0.10	70.84^{c}±0.29	62.79^{a}±3.00	69.14^{a}±0.33	56.89^{a}±0.16	8.06^{c}±0.13

Values are means ± standard deviation of triplicate determinations. Values in the same column with different superscripts are significantly different ($p<0.05$).

Key:

C1= Couscous prepared from 100% acha flour (control sample)
F1= Couscous prepared from 90% acha flour, 5% cowpea flour and 5% carrot flour
F3= Couscous prepared from 75% acha flour, 15% cowpea flour and 10% carrot flour
F4 = Couscous prepared from 70% acha flour, 15% cowpea flour and 15% carrot flour
F5 = Couscous prepared from 65% acha flour, 20% cowpea flour and 15% carrot flour
F8= Couscous prepared from 50% acha flour, 25% cowpea flour and 25% carrot flour

Light Microscopy

Plates 1-6 showed Liver of Albino rat fed with couscous made with 100% acha flour (C1) and drinking water ad libitum for forty-two days, presenting a gradual distortion of the liver as increase in the cowpea and carrot flours increases. Samples with 100% acha and 90%acha: 5% cowpea and 5% carrot have normal morphology while samples with higher levels of cowpea and carrot have inflammation eviden in tmassive cellular degeneration (necrosis).

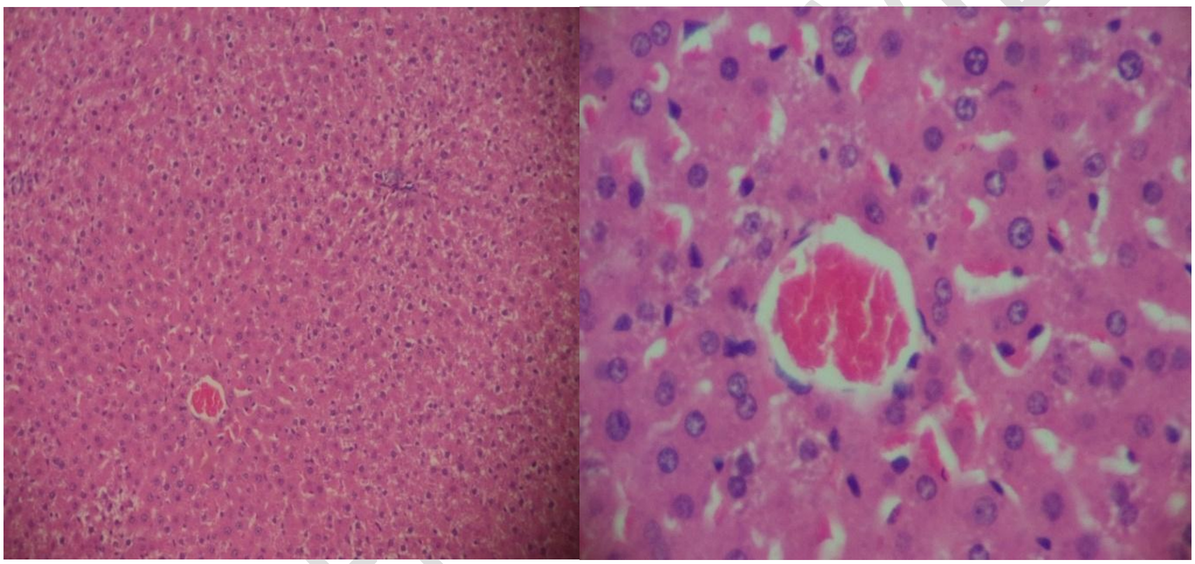

Plate 1 (sample C1): Liver of Albino rat fed with couscous made with 100% acha flour and drinking water ad libitum for forty-two days, presenting with normal morphology evident by the presence of intact nuclei (white arrows) surrounded by intact cytoplasm (Black arrows). The hepatocytes are interspersed by hepatic sinusoids (black arrowheads) which are linked to the central vein (white stars). H&E **A**: X100 **B**: X400.

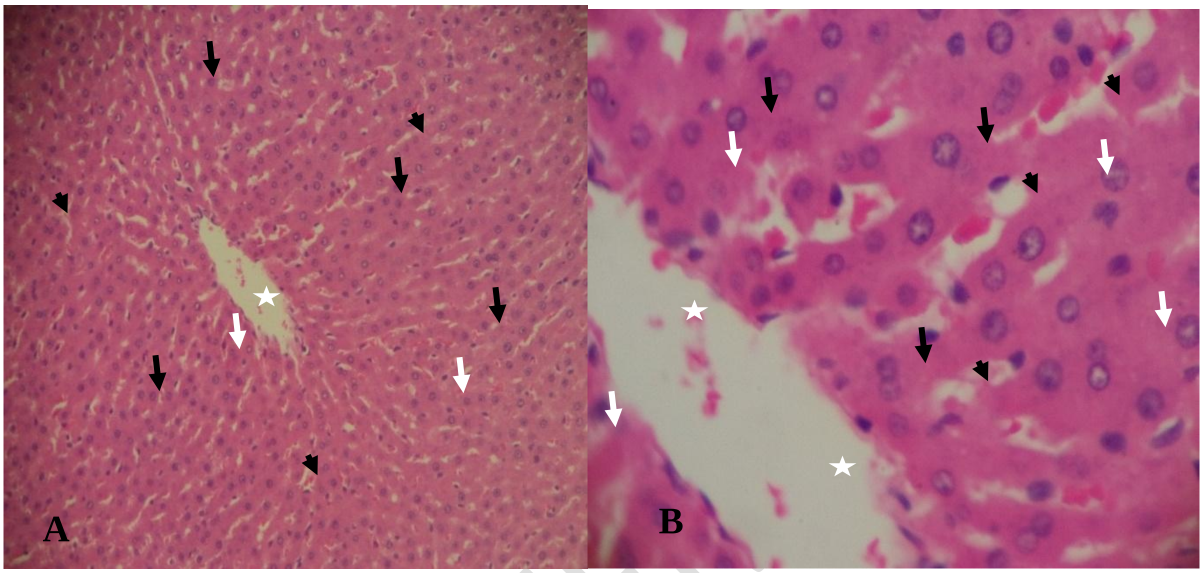

Plate 2 (sample F1): Liver of Albino rat fed with couscous made with 90% acha flour, 5% cowpea flour, 5% carrot flour and drinking water ad libitum for forty-two days, showing normal tissue architecture. White stars= central vein, black arrows= cell nuclei, white arrows= cytoplasm, black arrowheads= sinusoids. H&E **A**: X100 **B**: X400.

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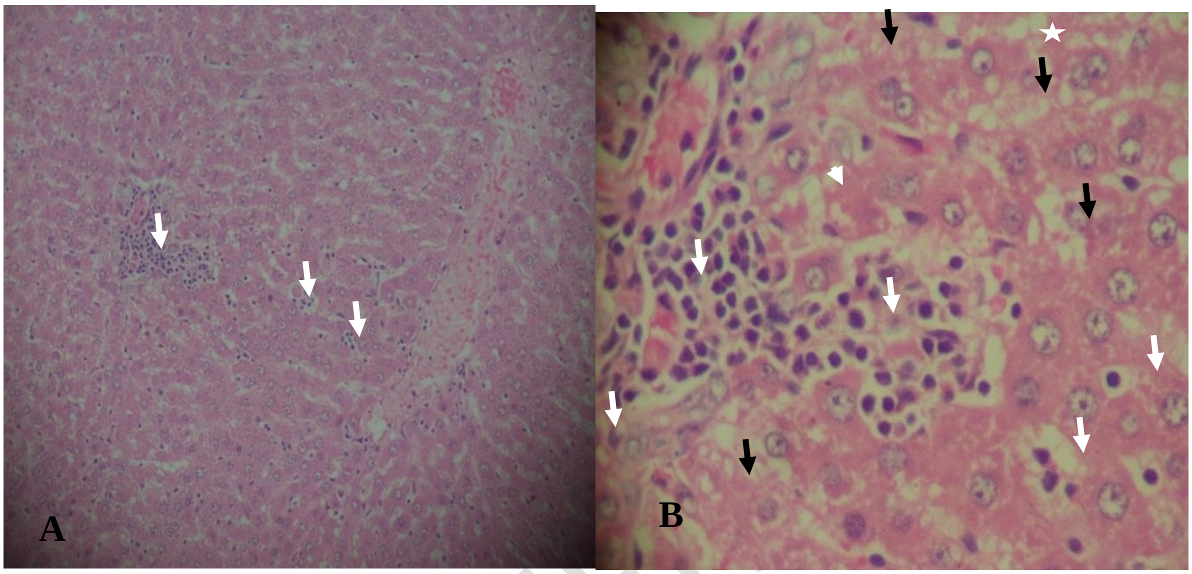

Plate 3 (Sample F3): Liver of Albino ratfed with 75% couscous made with 75% acha flour, 15% cowpea, 10% carrot flour and drinking water ad libitum for forty-two days, showing dissolution of both nuclear and cytoplasmic components (necrosis) (black arrows) and intracellular inflammation evident by the presence of inflammatory cells (white arrows) within the tissue some of which have taken over the hepatic cords. The demarcations between the hepatocytes is not clear due to tissue degeneration. H&E **A**: X100 **B**: X400

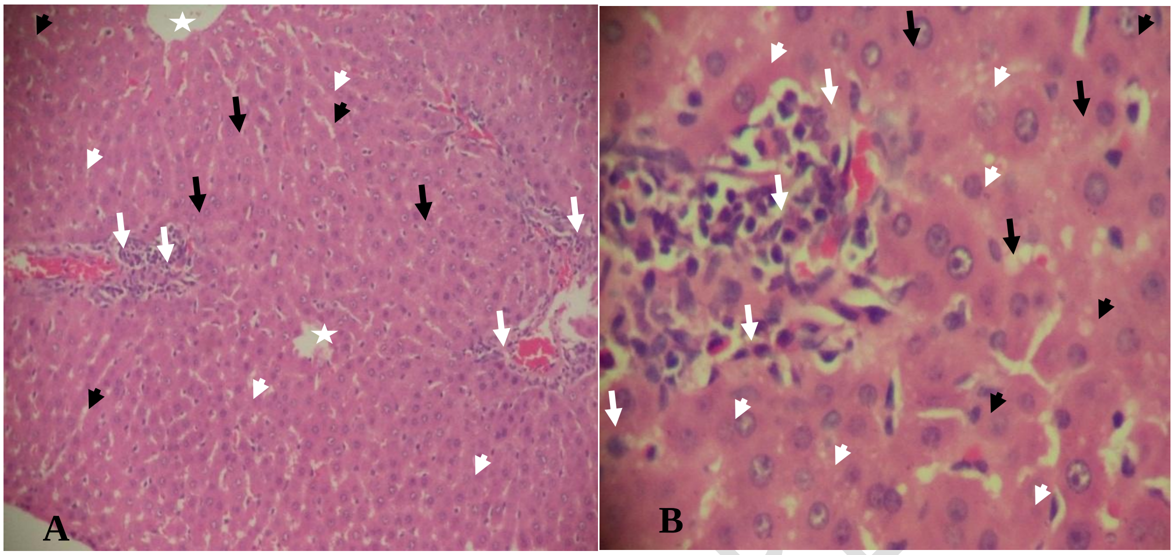

Plate 4 (Sample F4): Liver of Albino rat fed with couscous made with 70% acha flour, 15% cowpea flour, 15% carrot flour and drinking water ad libitum for forty-two days, showing perivascular inflammation as seen by the presence of inflammatory cells (white arrows) within and around the blood vessels. Most parts of the tissue appear normal with intact nuclei (black arrows) surrounded by intact cytoplasmic components (white arrowheads) and interspersed by hepatic sinusoids (Black arrowheads) which are linked to the central veins (white stars).H&E **A**: X100 **B**: X400

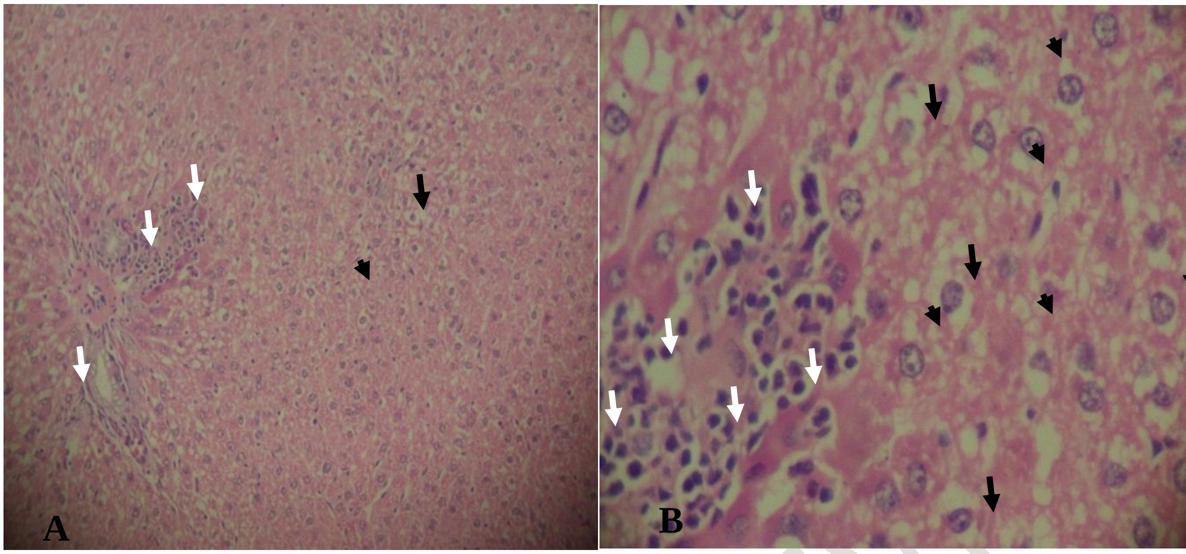

Plate 5 (Sample F5): Liver of Albino rat fed with couscous made with 65% acha flour, 20% cowpea flour, 15% carrot flour and drinking water ad libitum for forty-two days, showing massive cellular degeneration (necrosis) evident by the dissolution of both nuclear and cytoplasmic components thereby presenting with naked nuclei (black arrows) and excessive vacuolation of the cytoplasm (black arrowheads). Evidence of inflammation is shown by the presence of inflammatory cells (white arrows) some of which have completely taken over the hepatocytes. H&E **A**: X100 **B**: X400

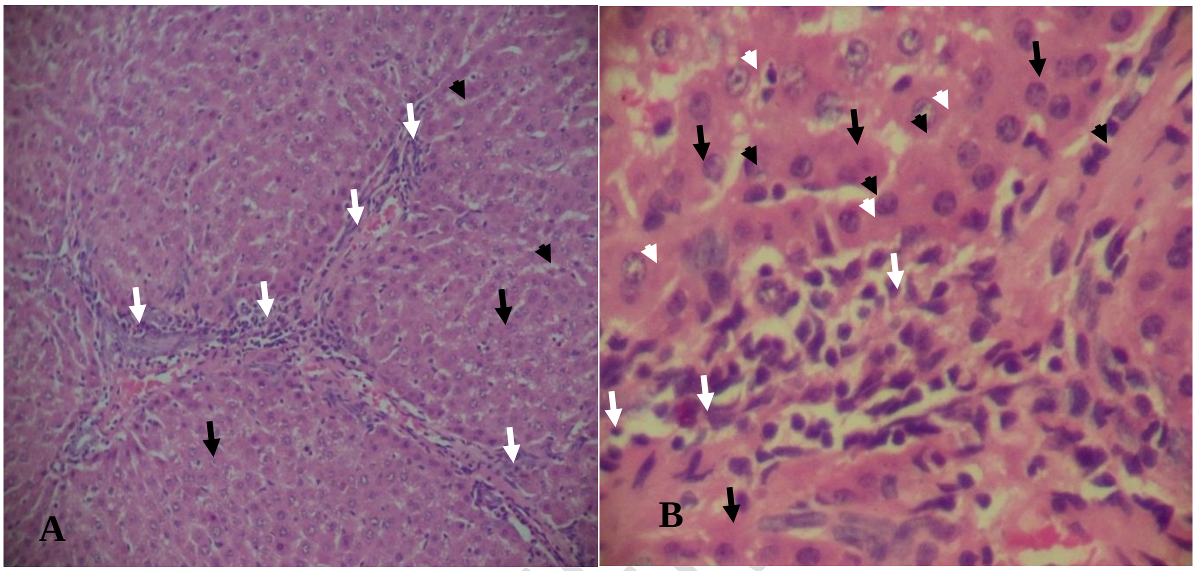

Plate 6 (Sample F8): Liver of Albino rat fed with couscous made with 55% acha flour,25% cowpea flour, 20% carrot flour, and drinking water ad libitum for forty-two days, showing perivascular inflammation evident by the presence of inflammatory cells (white arrows) within and around the blood vessels. White arrowheads show nuclei surrounded by intact cytoplasmic components (black arrows). The hepatocytes are interspersed by hepatic sinusoids (black arrowheads). H&E **A**: X100 **B**: X400

CONCLUSIONS

The study have shown that increase in the added cowpea and carrot flour increased the neutrophils, red blood cell, packed cell volume, mean cell hemoglobin (MCH), mean cell volume(MCV), and hemoglobin concentration while the glycemic indices decreased. These are positive indices in the wellbeing of the consumer. However, a gradual distortion of the liver with increase in the cowpea and carrot flours was observed which called for caution at excess consumption of the blends as it over work the liver in breaking down the protein and excretion of the byproducts. Inflammations observed as cowpea and carrot content is increased could be due to antinutritional factors in the cowpea.

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