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Full Length Research Paper

Effect of fermentation methods on the mineral, amino and fatty acids composition of *Cyperus esculentus*

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Tiger nut (*Cyperus esculentus*) was subjected to different fermentation methods such as traditional, back slope and control. The raw and fermented samples were analyzed for mineral, amino and fatty acids. The results of mineral analysis revealed potassium and sodium as the most abundant mineral element with their value ranging from 546 to 91.6 mg/100 g and 64.00 to 3383.33 mg/100 g, respectively while copper was found in trace amount with value ranging from 0.03 mg/100 g to 0.05 mg/100 g. All the fermented samples shows significant increase in calcium ranging from 8.50 to 9.83 mg/100 g compared to raw samples (7.66 mg/100 g). Amino acid result showed arginine (23.02 g/100 g) as the most abundant amino acid present in back slope fermented tiger nut while tyrosine was the least amino acid (0.05 g/100 g). The oil in tiger nut showed a greater percentage of oleic acid (73.08%) which was recorded in back slope fermented milled sample. The overall result of the investigation revealed that back slope fermentation was the best method that may enhance mineral, amino and fatty acids content of tiger nut.

Key words: Tiger nut, mineral, amino acid, fatty acid, fermentation.

INTRODUCTION

Tiger nut (*Cyperus esculentus* var. *sativa*) is an under-utilized crop which belongs to the division magnoliophyta and was found to be a cosmopolitan perennial crop of the same genus as the papyrus plant (Odoemelam, 2003; Belewu and Belewu, 2007). Despite its name, tiger nut is a tuber. However, its chemical composition shares characteristics with tubers and nuts (Umerie et al., 1997). The tubers are spherical in shape and edible.

There are varieties of tiger nuts readily available in the market, which are brown and yellow varieties. The yellow

variety is preferred to all other variety because of its inherent properties such as larger size, attractive colour and fleshy body. The yellow variety is also reported to yield more soluble extracts, contains lower fat more protein and possesses less anti-nutritional factors (Okafor et al., 2003). Its tubers can be eaten raw, roasted with sugar, soaked in water or processed into starch and flour (Oladele and Aina, 2007; Cortes et al., 2005). It can be processed into a milky beverage called "Horchata de Chufa" in Spain or "Atadwe" milk in Ghana (Rita, 2009).

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In Nigeria, tiger nut is well grown and available in semi-dried form in Nigerian markets where it is sold locally and consumed uncooked (Omode et al., 1995).

Tiger nut have long been recognized to contain almost twice the quantity of starch as potato or sweet potato tubers. This tuber is a good source of energy (carbohydrate, fibre and protein), minerals (mainly phosphorus and potassium), and vitamins E and C (Arafat et al., 2009).

Processing techniques such as boiling, roasting, fermentation and germination are means of improving the nutritional value of foods (Nergiz and Gokgoz, 2007). Although little study have been carried out on the effect of fermentation on the nutritional composition of tiger nut. It is therefore important to investigate the effect of different fermentation methods on the mineral, amino and fatty acids content of tiger nut. Therefore, this research was conducted to determine the effect of fermentation methods on mineral, amino and fatty acid contents of *Cyperus esculentus*.

MATERIALS AND METHODS

Source of tiger nut

Raw tiger nut were purchased from Adedeji market in Akure, Ondo State, Nigeria. The nuts were stored in the laboratory till the second day when they were sorted, weighed and washed.

Processing of tiger nut

The sorted and washed nut were divided into six portions designated A to F. Each of the portion contained 500 g of cleaned tiger nut. Part A was analyzed raw and this serves as control. Part B was fermented whole that is, submerged in 1500 ml of portable water in a cleaned container that was covered for four days at 25°C and allowed to ferment with indigenous micro flora (spontaneous). C was milled and subjected to spontaneous fermentation. Part D and E were fermented by addition of the steep water from the previously fermented culture used as starter culture (back slope) but part E was milled before fermentation while F was allowed to undergo control fermentation, in which pure culture of *Lactobacillus plantarum* isolated in part B was used to inoculate the sixth part F. The fermented nuts were dried in oven at 50°C for 24 h and dry milled to powder using attrition mill. The milled samples were packaged in polythene prior to analysis.

Chemical analysis

Mineral analysis

The mineral composition (potassium, sodium, calcium, magnesium, zinc, iron and copper) of each sample was determined by wet ashing method followed by reading of the level of mineral. Triplicate samples of 1 g each were weighed into porcelain crucibles and placed in a muffle furnace. The temperature was raised gradually to 450°C. The sample was ashed at 450°C for 5-6 h. After cooling to room temperature, the ash was dissolved in 1 ml of 0.5% HNO₃. The sample volume was brought to 100 ml, and the levels of mineral present were analyzed by Atomic absorption spectrophotometer Buck 201 VGP. The mineral content was calculated using the formula below.

$$\text{Mineral (mg/100 g)} = \frac{R \times V \times D}{Wt}$$

Where, R = Solution concentration obtained from graph, V = Volume of sample digest, D = Dilution factor and Wt = Weight of sample. Sodium (Na) and K were analyzed using flame photometer (Perkin-Elmer, 1982).

Amino acid determination

Amino acid composition was determined by the method of Spackman et al. (2006) 2.0 g of each sample was weighed into the extraction thimble and the fat extracted with chloroform methanol mixture using a Soxhlet extraction apparatus. The extraction lasted for 5-6 h. The defatted samples (30 to 35 mg) were weighed into glass ampoules. Seven milliliters of 6 M HCl were added and oxygen was expelled by passing nitrogen gas into the ampoule (to avoid possible oxidation of some amino acid during hydrolysis). Each glass ampoule was then sealed with a Bunsen flame and put into an oven at 105 ± 5°C for 22 h. The ampoule was allowed to cool before breaking open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 ml of acetate buffer and stored in a plastic specimen bottle and kept in the deep freezer. The amount loaded was between 5 to 10 µl. This was dispensed into the cartridge of the analyzer. The TSM (technicon sequential multisample amino acid analyzer) analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 min. The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid}}$$

Fatty acid determination

Fifty milligram (50 mg) of fat extracted from raw and fermented tiger nut was esterified for 5 min at 95°C with 3.4 ml of the 0.5 M KOH in dry methanol. The mixture was neutralized using 0.7 M HCL. About 3 ml of boron trifluoride (14%) in methanol was added. The mixture was heated for 5 min at the temperature of 90°C to achieve complete methylation process. The Fatty Acid Methyl Esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for gas chromatography analysis and 1 µL was injected into injection port of gas chromatography (Alejandro, 2013).

Statistical analysis

The experiment was carried out in triplicates. Data obtained were analyzed by one-way analysis of variance and mean were compared by Duncan's multiple range tests (SPSS 17.0 version). Differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

Mineral composition (mg/100 g) of raw and fermented

Table 1. Mineral composition of raw and fermented Tiger nut (mg/100 g).

Mineral	Raw	TFM	TFW	BFM	BFW	CF
Ca	7.66 ^c ±0.28	9.33 ^{ab} ±0.28	9.50 ^{ab} ±0.50	8.50 ^{bc} ±1.00	9.00 ^{ab} ±0.50	9.83 ^a ±0.28
Cu	0.04 ^a ±0.00	0.00 ^b ±0.00	0.00 ^b ±0.00	0.05 ^a ±0.06	0.03 ^a ±0.00	0.04 ^a ±0.00
Fe	0.09 ^b ±0.00	0.23 ^{ab} ±0.30	0.14 ^b ±0.00	0.13 ^b ±0.00	0.14 ^b ±0.00	0.41 ^a ±0.00
K	606.33 ^b ±0.57	533.66 ^f ±1.15	562.00 ^d ±1.00	577.00 ^c ±1.00	546.00 ^e ±1.00	91.6 ^a ±1.00
Na	3383.33 ^a ±28.8	3250.00 ^b ±0.00	3366.67 ^a ±76.37	3166.66 ^b ±76.37	2950.00 ^b ±50.00	64.00 ^c ±1.00
Pb	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00
Zn	0.07 ^c ±0.00	0.64 ^a ±0.10	0.00 ^e ±0.00	0.51 ^b ±0.00	0.02 ^d ±0.00	0.51 ^b ±0.38

Values are (mean±SD) of replicates. Values with the same alphabet are not significantly different at ($p = 0.05$). RAW: Raw, TFM: traditional fermented milled, TFW: traditional fermented whole, BFM: back slope fermented milled, BFW: back slope fermented whole, CF: controlled fermented sample.

tiger nut is shown in Table 1. Sodium was the most abundant mineral with value 3383.3 mg/100 g which was recorded in raw sample while copper (0.03 mg/100 g) is the least mineral obtained which was found in back slope fermented whole sample. Micronutrients such as potassium, sodium and calcium were found to be appreciable in tiger nut samples analysed earlier reported by Bosch et al. (2005) and, Oladele and Aina (2007). Potassium and sodium are important in maintaining the normal water balance, conservation of osmosis and acid balance in the body. Potassium is necessary for the metabolism of carbohydrates and proteins. It also protects the internal arterial walls against any damages, prevents haemorrhages and brain/heart attack (Oladele et al., 2009). Hence, tiger nut is a good source of these elements.

The result of amino acid composition revealed that tiger nut is rich in essential amino acid such as lysine, threonine, leucine, phenylalanine and cystine. The most concentrated essential amino acid lysine (5.14 g/100 g) was recorded in back slope fermented whole sample. Tyrosine (0.50 g/100 g) was the least amino acid which was recorded in raw sample (Table 2). Some essential amino acid (threonine, leucine, phenylalanine and cystine) present in back slope fermented whole tiger nut were found to compare favourably with food and Agriculture Organization Standard (FAO, 1998). Back slope fermented whole sample showed significant increase ($p \leq 0.05$) in lysine (5.14 g/100 g), Threonine (3.15 g/100 g), leucine (4.39 g/100 g), phenylalanine (3.25 g/100 g) and cystine (2.56 g/100 g) content when compared to FAO standard (Table 3).

Oyetayo and Agbaje (2012) has earlier reported that amino acids of fermented Acha was higher than the raw sample. Also Oyetayo et al. (2007) reported that food rich in total essential amino acid will contribute to the supply of essential amino acid in diet. Amino acids distribution in controlled fermented sample is smaller to what was obtained in traditional and backslope fermented samples, this may be due to the effect of sterilization on the control sample, high temperature denature protein.

LAB fermentation has been shown to improve the nutritional value and digestibility of foods (Nout, 2009). The acidic nature of the fermentation products enhances the activity of microbial enzymes at a temperature range of 22-25°C (Mokoena et al., 2005). The enzymes, which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively. This is in line with this paper finding.

Results shown in Tables 2 and 3 show an increment in some nonessential and essential amino acid of fermented samples when compared with unfermented (raw) sample, these was in agreement with Steinkraus report (1997) that bacterial enzymatic hydrolysis may enhance the bioavailability of protein and fat and increase the production of free amino acids, short chain fatty acids and also reported that fermentation increase biological environment of food substrates with protein essential amino acids and vitamins.

The result of the fatty acid composition of oil extracted from raw and fermented tiger nut are as shown in Table 4. This study shows that all the sample contain appreciable amount of oleic acid with value recorded ranging from 64.91 to 73.08%, but low in erucic acid with values ranging from 0.02±0.00 to 0.05±0.01%. Key et al. (1986) reported that epidemiological studies also suggested that the presence of a high proportion of monounsaturated acid especially oleic acid in the diet is linked with a high reduction in the risk of coronary heart diseases. Oleic acid is also reported to be useful for building cellular membranes, attracting oxygen to tissues, to transform energy into nerve impulses, and as precursors to molecules of cellular communication such as prostaglandins (Odutuga et al., 1992).

Result also reveals that traditional fermentation had reduces the value of linolenic acid from 0.65 to 0.57%. High percentage of linolenic acid is not desirable in edible oils because of the off-flavours and potentially harmful oxidation products formed. As reported by Warner and Gupta (2003), a decrease from 2 to 0.8% linolenic acid content in oils improved flavor quality and oxidative

Table 4. Contd.

Fatty acid	Raw	TFM	TFW	BFM	BFW	CF
Lignoceri	0.11 ^b ±0.01	0.23 ^a ±0.05	0.22 ^a ±0.01	0.07±0.00	0.06 ^c ±0.02	0.00 ^a ±0.00
Linoleic	9.00 ^b ±0.01	10.12 ^a ±0.01	7.74 ^c ±0.00	10.17 ^a ±0.01	10.45 ^a ±0.01	8.86 ^c ±0.01
Linolenic	0.65 ^c ±0.01	0.71 ^c ±0.01	0.57 ^d ±0.00	3.50 ^a ±4.61	0.90 ^b ±0.00	0.66 ^c ±0.01
Margaric	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00
Myristic	1.73 ^a ±0.02	1.13 ^c ±0.01	1.15 ^c ±0.04	0.73 ^e ±0.02	1.02 ^d ±0.02	1.28 ^b ±0.01
Oleic	69.77 ^b ±0.02	70.61 ^b ±0.01	69.33 ^b ±0.03	73.08 ^a ±0.01	69.10 ^b ±0.01	64.91 ^c ±0.01
Palmitic	0.04 ^d ±0.00	0.08 ^c ±0.00	0.28 ^b ±0.01	0.03 ^d ±0.00	0.03 ^d ±0.01	0.32 ^a ±0.01
Plamitic	10.23 ^b ±0.01	9.51 ^c ±0.01	10.75 ^b ±0.18	9.16 ^c ±0.01	10.55 ^b ±0.02	12.43 ^a ±0.03
Stearic	3.38 ^c ±0.02	3.16 ^d ±0.01	5.34 ^b ±0.02	3.01 ^d ±0.01	3.40 ^c ±0.11	6.10 ^a ±0.00

Values are (mean±SD) of replicates. Values with the same alphabet are not significantly different at (p =0.05). Raw: Raw, TFM: traditional fermented milled, TFW: traditional fermented whole, BFM: back slope fermented milled, BFW: back slope fermented whole, CF: controlled fermented sample.

stability of fried foods. This therefore shows that the lower the linolenic acid content in oil, the more suitable is the oil for frying. This indicates that tiger nut oil is a good source of edible oil for cooking and frying that may be useful for the fight against cardiovascular diseases (Muhammad et al., 2011).

Conclusion

This study established the effect of different fermentation methods on the mineral, amino and fatty acids content of tiger nut (*Cyperus esculentus*). The result of mineral composition revealed that tiger nut was rich in potassium, sodium and calcium. Also tiger nut is a poor source of copper and zinc. Back slope fermented sample was found to be high in the following amino acid: Arginine, glutamic, lysine and aspartic acid. Oleic is the most abundant fatty acid present in tiger nut. In conclusion, back slope fermentation is the best processing method that enhances the mineral, amino and fatty acids content of tiger nut. This method is the best because there is an increase in essential amino acid such as lysine, threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and cystine recorded in back slope fermentation method

Conflict of interests

The authors did not declare any conflict of interest.

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Short Communication

The effect of aqueous leaf extract of fluted pumpkin on some hematological parameters and liver enzymes in 2,4-dinitrophenylhydrazine- induced anemic rats

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Anemia constitutes a serious health problem in many tropical countries including Nigeria because of the prevalence of malaria and other parasitic infections which possibly leads to decrease of hemoglobin. Fluted pumpkin has been reported to be very good in building the constituents of the blood and also replacing them. This study was designed to investigate the effects of the aqueous leaf extract of fluted pumpkin on some hematological parameters and liver enzymes; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in 2, 4-dinitrophenylhydrazine-induced anemia in experimental rat. Twelve Wister albino male rats were obtained from a nearby farm and separated into four groups of three rats each for the study. Rats in Groups 1 and 2 were injected 40 mg/kg 2, 4-dinitrophenylhydrazine for eight days to induce anemia, while rats in Groups 3 and 4 were fed with grower's mash and water *ad libitum*. All analysis was done using the standard methods. The result of this study shows that, oral administration of 50 mg/kg of aqueous leaf extract of fluted pumpkin to the rats in induced treated and normal treated groups (Groups 1 and 3), increased the hematological parameters under investigation while the rest remained significantly unchanged. Low level of ALT and AST was observed in rats in group 1 (induced treated group) suggesting a hepatoprotective property of the leaf extract which also indicate that the extract had no effect on the liver of the rats at the concentration used. The result of this research indicate that 50 mg/kg aqueous leaf extract of fluted pumpkin could elevate the packed cell volume, red blood cells and hemoglobin concentration in the rats induced with 2, 4-dinitrophenylhydrazine. Hence, oral administration of the extract could cure hemolytic anemia. The leaf extract also regulated the liver enzymes (ALT and AST) of the rats induced with 2, 4-dinitrophenylhydrazine. It can be concluded that aqueous leaf extract of fluted pumpkin is a potential blood booster and has hepatoprotective property.

Key words: Fluted pumpkin, hematological parameters, liver enzymes, 2, dinitrophenylhydrazine, anemia.

INTRODUCTION

Pumpkin as defined by the English dictionary is a large orange fruit or a round large fruit with a thick orange skinned rind, dry flesh and many seeds, cooked and eaten as a vegetable or in sweet dishes. It is known in

many different countries because of its value as most of the parts can be eaten and are rich in nutrients. It is easily grown and one plant in the garden can supply pumpkins and green leaves throughout the year. It grows

best in loose, rich soil, mainly in any old rubbish heap. There are many different varieties of pumpkin whose botanical name is 'Telfairia' which belong to the tribe, Joliffieae (Akoroda, 1990).

Fluted pumpkin is a dicotyledonous vegetable that develops long vine-like stems with trifoliolate leaves and edible large fleshy fruits which can be 5 cm high. The leaves are simple dark green if properly grown on a suitable soil. It thrives in well-drained soils and is usually cultivated in garden and family farms around homes. The dark green leaves can be 18 cm wide and 35 cm long (Iweala and Onyechi, 2009), some of its native names include 'Ugu' as known by Ibos, 'Umee' in Efik and "Umeke" in Edo (Akoroda, 1990).

It is a common tropical green leafy vegetable native to many African countries especially Eastern Nigeria (Burkett, 1968). In ethno medicine, the fresh leaves are used in treatment of malaria and convulsion (Alada, 2000; Gbile, 1986). There are various methods by which fluted pumpkin leaves can be prepared as meal and drinks. It has been observed by the users to be a vegetable that is outstanding in health nourishing. It is used in cooking different variety of food, preparing salad, stew and soup. Some local users reported that it can be used as local or native blood tonic while some suggested that it can be used as a cure for anemia precisely anemia due to blood loss (personal communication).

Anemia constitutes a serious health problem in many tropical countries including Nigeria because of the prevalence of malaria and other parasitic infections which possibly leads to decrease of hemoglobin. Over millions of people have anemia in the sense that any disease that can lead to blood shortage or loss is mostly coupled with anemia. The aim of this study, therefore, is to evaluate the effect of aqueous leaf extract of fluted pumpkin on some hematological parameters and liver enzymes in 2, 4-dinitrophenylhydrazine induced anemic rats and the effect of aqueous at unveiling the possibility of curing anemia through the use of vegetable, hence the choice of fluted pumpkin.

MATERIALS AND METHODS

Sample collection and preparation

Fresh leaves of fluted pumpkin was obtained from a nearby farm in Mubi, Adamawa State, washed and weighed on daily basis for each of the experiment.

Extraction technique

About 50 g of fluted pumpkin leaves was chopped into smaller bits and ground with mortar and pestle. 100 ml of distilled water was

poured into the ground leaves to obtain the extract which is 50 mg/ml. The aqueous extract was obtained raw using a sieve to avoid the entrance of particles. 50 mg/kg of the extract was administered to the rats according to the weight through oral gavage.

Experimental design

Twelve albino male rats weighing between 150 to 220 g were obtained from local breeders within Adamawa State University, Mubi. The rats were kept in the laboratory for one week for acclimatization before the on-set of the experiment. The rats were distributed into four groups of three rats each and were treated as follows:

Group 1 (Induced treated)

The rats in this group were labeled R₁, R₂ and R₃ and were also made anemic by daily oral administration of 40 mg/kg of 2,4-dinitro phenyl hydrazine for eight days. The rats were also fed with grower's mash and water *ad libitum*. They were treated with 50 mg/kg of the freshly prepared leaf extract for five days after being confirmed anemic.

Group 2 (Induced control)

The rats in this group; R₄, R₅ and R₆, were made anemic by daily oral administration of 2,4-dinitro phenyl hydrazine (PHZ) at 40 mg/kg for eight days during which they were fed with grower's mash and water *ad libitum* with no other treatment.

Group 3 (Control treated)

The rats in this group; R₇, R₈ and R₉ were fed normally with grower's mash and water and were given 50 mg/kg of the freshly prepared leaf extract each day for complete five days.

Group 4 (Normal control)

The rats in this group; R₁₀, R₁₁ and R₁₂ were fed with growers mash and water *ad libitum* throughout the period of experiment, with no other treatment.

Analysis of hematological parameters

Packed cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) count

The packed cell volume, white blood cell count and red blood cell count were obtained by the method described by Hoffbrand and Moss (2011).

Hemoglobin concentration determination

The hemoglobin concentration was determined using acid haematin method also known as Sahli's method (Hoffbrand and Moses, 2011).

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Table 1. Effect of the aqueous leaf extract on some hematological parameters

Parameter	Group 1 (induced treated)	Group 2 (induced control)	Group3 (normal treated)	Group 4 (normal control)
PCV ₁ (%)	45.33±1.77	48.00±0.58	46.33±0.88	44.00±0.06
PCV ₂ (%)	38.00±0.18	39.00±0.58	49.00±1.55	48.67±3.72
PCV ₃ (%)	41.67±±.88	39.67±0.88	51.67±0.88	49.33±3.39
WBC ₁ (10 ³ /mm ³)	1.92±0.05	2.08±0.05	2.00±0.03	1.90±0.06
WBC ₂ (10 ³ /mm ³)	2.72±0.05	2.48±0.12	2.12±0.02	2.02±0.08
WBC ₃ (10 ³ /mm ³)	2.27±0.12	2.35±0.03	1.62±0.05	1.88±0.05
RBC ₁ (10 ⁷ /mm ³)	2.31±0.02	2.47±0.13	2.37±0.02	2.36±0.06
RBC ₂ (10 ⁷ /mm ³)	2.18±0.02	2.27±0.06	2.72±0.02	2.69±0.09
RBC ₃ (10 ⁷ /mm ³)	3.31±0.03	3.00±0.08	3.79±0.02	3.01±0.19
Hb ₁ (g/dl)	9.67±0.18	10.00±0.12	10.10±0.16	9.87±0.37
Hb ₂ (g/dl)	8.20±0.12	8.50±0.12	10.47±0.18	10.40±0.35
Hb ₃ (g/dl)	12.37±0.13	10.07±0.07	12.97±0.04	11.47±0.37

All values are in Mean ± SEM for n=3. PCV₁, PCV₂ and PCV₃ are the packed cell volume of the rats before inducing anemia, after inducing anemia and after treatment, respectively; WBC₁, WBC₂ and WBC₃ are white blood cell count of the rats before inducing anemia, after inducing anemia and after treatment, respectively; RBC₁, RBC₂ and RBC₃ are red blood cell count of the rats before inducing anemia, after inducing anemia and after treatment, respectively; Hb₁, Hb₂ and Hb₃ are hemoglobin concentration of the rats before inducing anemia, after inducing anemia and after treatment, respectively.

Table 2. Effect of aqueous leaf extract of fluted pumpkin on the liver enzymes

Parameter	Group 1 (induced+extract)	Group 2 (induced control)	Group 3 (control treated)	Group 4 (normal control)
ALT(iu/l)	7.00±0.29	11.17±0.73	8.50±0.29	9.00±1.00
AST(iu/l)	22.17±0.44	28.33±0.88	47.33±0.34	30.00±1.48

All values are in Mean ± SEM for n=3; ALT stands for Alanine transaminase; AST stands for Aspartate transaminase.

Enzyme assays

The enzymes that were tested are Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). The test was done by the method described by Reitman and Frankel (1957) using Randox test kits.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM) for triplicate determination.

RESULTS

The results of the hematological parameters are presented in Table 1. The result shows that there was an increase in the hematological parameters of the rats as shown in Table 1. Daily intra peritoneal injection of 40 mg/kg 2, 4-dinitrophenyl hydrazine for eight days caused a decrease in the hematological parameters of the rats in group 1 and 2, while the packed cell volume dropped from 45.33 ± 1.77 and 48.00±0.58 to 38.00±0.18 and 39.00±0.58,

respectively. There was also a drop in the red blood cell count and hemoglobin concentration, whereas the white blood cell count increased.

The hematological parameters were raised after the oral administration of the aqueous leaf extract. The packed cell volume, red blood cell count and hemoglobin concentration was 38.00±0.18, 2.18±0.02 and 8.20±0.12 respectively, with those of the induced and treated rats raised to 41.67±0.88, 3.31±0.03 and 12.37±0.13 respectively, while that of the induced control group remained low.

Table 2 shows the result of the effect of the extract on the liver enzymes; ALT and AST. In the groups treated with the extract, low level of ALT was observed. The average result was 7.00±0.29 iu/l and 8.50±0.29 iu/l for groups 1 and 3, respectively. Low level of AST was observed in the rats in group 1(22.17±0.44) and high level in the rats in group 3 (47.33±0.34).

DISCUSSION

As shown in Table 1, the decrease in the hematological

parameters in Groups 1 and 2 could be as a result of breakdown of the red blood cells caused by the 2,4-dinitrophenylhydrazine. The increase in white blood cells could be due the defense mechanism against the entrance of a foreign material in the body system of the rats (2, 4-dinitrophenylhydrazine).

This study has shown that the aqueous leaf extract of fluted pumpkin caused an increase in packed cell volume, white blood cell count, red blood cell count and hemoglobin concentration in rats. The increase in hematological parameters investigated could be as a result of some constituents such as iron and some B complex vitamins which it possess as these serves as hematopoietic factors that influence directly on blood production in the bone marrow (Ganong, 2005). This study also agreed with the work of Salman et al. (2008), who reported that there was a significant increase in the hematological parameters of rats that were treated for two weeks with the aqueous leaf extract of fluted pumpkin. Some scientists have proposed the use of fluted pumpkin in treatment of anemia, following studies which reported that extracts of fluted pumpkin helps to maintain blood level in subjects given its extracts (Fiona and Latunde - Dada, 2011).

This research also shows that the leaf extract can serve as a cure for anemia as reported by some researchers. The increase in weight of the rats could be as a result of rich nutrients such as amino acids, fatty acids, mineral and vitamins (Fagbemi, 2007). Specified and suitable concentration of the aqueous extract leads to positive effects on the hematological parameters investigated.

As shown in Table 2, the lower level of ALT and AST in the rats induced with 2, 4-dinitrophenylhydrazine could be as a result of the hepatoprotective property of the plant on rats as reported (Oboh, 2005). AST of the normal rats was elevated than normal after treatment which showed that the concentration was high as regards to the health status of the rats, therefore, to some extent could be toxic to the rats. This effect attests to the observation of irregularity in the liver after a long term consumption of *Telfairia occidentalis* supplemented diet in rats (Iweala and Obidoa, 2009).

Conclusion

The result of this research indicated that 50 mg/kg aqueous leaf extract of fluted pumpkin could elevate the packed cell volume, red blood cells and hemoglobin concentration in the rats induced with 2, 4-dinitrophenylhydrazine. Hence, oral administration of the extract could cure hemolytic anemia. The leaf extract also regulated the liver enzymes (ALT and AST) of the rats induced with 2, 4-dinitrophenylhydrazine.

Conflict of interests

The authors did not declare any conflict of interest.

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