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***Full Length Research Paper***

# Toxicological Properties of the Seed of Chinese Fan Palm *(Livistona chinensis)* Using Animal Model

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***Abstract***

*An acute toxicity study was carried out on the seed during which albino rats were differently fed the raw and also the cooked and roasted samples at 25:75, 50:50, 75:25 and 100: 0 sample-to-commercial feed (Emii feed) ratios respectively for 14 days. Haematological studies showed a selective effect on some albino rat blood parameters (packed cell volume: 39%, hemoglobin: 13.00g/dl, platelets: 864.21 x 10g/L for albino rats fed 50:50 cooked sample-to-commercial feed mix) and also its ability to cause hyposplenism, thromboembolism and thrombocytosis with possible vascular accidents at higher ratios. The roasted (110°C, 15 min) sample of L. chinensis seed had a significant increasing effect($p<0.05$) on the white blood cells (WBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and platelets. With increase in the ratio (concentration) of the sample (L. chinensis seed) of the feed mix, there was no definite pattern (continuous rise or fall) established for the red blood cells (RBC), lymphocytes and neutrophils while there was totally no significant effect ($p<0.05$) on the mean corpuscular hemoglobin concentration (MCHC), monocytes, eosinophils and basophils.*

***Key words:*** *Toxicity, platelets, haematological, cooked, roasted*

## Introduction

*Livistona chinensis* commonly known as Chinese Fan palm or Chinese Fountain palm is of the family, Arecaceae (palm family) and belongs to the genus *Livistona* (Naoto *et al*., 2000). It Is native to southern Japan, Taiwan and several Islands in the southern China sea. It is a medium-sized, slow-growing, single-trunked palm tree that reaches about 15.2m tall in its natural habitat but often seen at much shorter heights of 3 – 8m (Forest, 2003). The leaf sheaths are fibrous, fluffy and brown-coloured somewhat like nests of birds. It has oval to round olive-like fruits that change from green to blue-black when ripe (Wagner *et al.,* 1999).

In most developing tropical countries, the food situation is worsening owing to increasing population, shortage of fertile land, high prices of available staples and restrictions on the importation of food (Nwosu, 2011). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women are most vulnerable (Potter and Hotchkiss, 1995; Nwosu, 2011). Prediction of future rates of population increase and food production emphasizes the seriousness of this problem (FAO, 1990). Okaka *et al.* (1992) and Nwosu (2011) noted that there is no single solution to the problem of food shortages and crisis. In essence, all information on new sources of food will be of value in the food security struggle. As recommended by Okaka *et al.* (1992) and noted by Nwosu (2011) that although measures are being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast number of less familiar food plant resources. Many of such plants have been identified but lack of data on their chemical composition has limited the prospects for their broad utilization (Viano *et al.,* 1995). Most reports on some lesser-known and unconventional crops indicate that they could be good sources of nutrients and many have the potentials of broadening the present narrow food base for humans (Nwosu, 2011).

*Livistona chinensis* (Chinese Fan Palm) tree resembles that of *Cocos nucifera* (coconut), the cross-sectional profile of the seed also resembles that of coconut and both belong to the same family, Arecaceae (Genini *et al.,* 2009).

It is mainly planted for ornamental reasons (Juliana *et al.,* 2003). The seeds have been noted to be astringent, contain phenolic compounds and used traditionally by the Chinese as an anti-cancer agent (Juliana *et al*., 2003; Gurpreet and Roman, 2008; Singh and Kaur, 2008; Tao *et al.,* 2009). But in areas where this plant is found in Nigeria, their seeds are left to waste after maturity. Also its sparing distribution, astringent nature, high phenolic compounds composition, lack of knowledge and documentation of its chemical composition has restricted its use to traditional medication rather than food.

Application of different processing methods to *Livistona chinensis* (Chinese Fan palm) seed, its seed oil properties' determination, proximate composition determination, and its toxicological evaluation will give some useful information, which may increase the utilization of Chinese Fan palm seeds and enhance its potential in food formulations. It is envisaged that a more suitable process for

the reduction or elimination of any detected anti-nutritional factors may be found for the production of safer Chinese Fan Palm seed products.

Despite the importance of the palm family, *Arecaceae*, and particularly *Livistona chinensis* which has been used traditionally by the Chinese as an anti-cancer agent (Singh and Kaur, 2008), little has been systematically documented about its utilization as food; the proximate composition, oil extract and effect of processing on the anti-nutrients it contains.

Furthermore, they are mainly planted for ornamental reasons (Corlett, 2005) and the fruits are not utilized as food but rather left to waste after maturity in many places they are found in Nigeria. The fruit *of Livistona chinensis* has also been noted by many researchers (Gurpreet and Raman, 2008; Juliana *et al.,* 2003; Singh and Kaur, 2008; Fabiana *et al.,* 2006) to be astringent and contain phenolic compounds which could be part of the reason why it is not utilized as food. This research will seek to find answers to some of the problems of its utilization through appropriate processing. The objective of this research therefore is to evaluate the toxicological effect of *Livistona chinensis* seed on albino rats. This study will give an insight on the effect of blanching, cooking and roasting on its toxicological effects. Through this way, some possible level of utilization of *Livistona chinensis* seeds will be achieved. This in essence will also be a step forward in the food security struggle in Nigeria through non-conventional food sources.

## Materials and methods

### Source of Raw Material

The fresh fruits of *Livistonia chinensis* were obtained from Amaigbo in Nwangele L.G.A of Imo State, Nigeria.

### Equipment and Chemicals Used

All equipments and chemicals used are available at National Root Crops Research Institute (NRCRI), Umudike and Federal University of Technology (FUTO), Owerri, Imo State.

### Sample Preparation

The pulp of the fruit was removed manually with a knife. The separated seed was dried in an oven (Gallenkamp hot box oven) at $60^0$C for 3h. The dried sample was milled and kept in airtight containers. The cooked and roasted samples were used for rat feeding. A completely randomized design and one-way analysis of variance (ANOVA) was used for the experiment.

**Plate 1:** *Livistona chinensis* (Chinese fan palm) fruit

**Plate 2***: Livistona chinensis* (Chinese fan palm) seeds

### Blanching, Cooking and Roasting

Blanching and cooking were done by the procedures described by Nwosu (2011). The fruit was manually removed of its pulp and the seed was taken in its whole form (without milling) for blanching, cooking and roasting treatments. The seed was divided into Nine (9) batches (1, 2, 3, 4, 5, 6, 7, 8 and 9) of 300g each. Batches 1, 2 and 3 were given a hot water ($100^0$C) blanching treatment for 4, 6 and 8 min respectively. Batches 4, 5 and 6 were cooked for 20, 40 and 60 min respectively. While batches 7, 8 and 9 were roasted at $110^0$C for 5, 10 and 15 minutes respectively). The samples were left to cool after the treatments. The blanched and cooked samples were dried (Gallenkamp hot box oven) at $60^0$C for 3h to a moisture content of 35%. The Nine (9) processed batches were all milled, allowed to cool and stored in airtight containers. Samples were taken from the airtight containers for rat feeding (toxicity study).

## Acute toxicity study

The method used by the Akinnawo *et al.,* (2002) was adopted for the acute toxicity study.

### Animals

Adult (8 weeks) male albino rats (Wistar strain) weighing 80 – 100mg from Emii veterinary farm at 120 Royce Road, Owerri, Imo State were used in this study. The rats were housed in metal cages placed under a well ventilated laboratory with temperature (27+2), Relative humidity (85%) and an alternate 12 hour natural light/12 hour dark cycles. The rats were allowed to acclimatize for a

minimum of 5 days in the environment where the experiment was carried out. A commercial rat feed (Emii Finishers feed) and water was provided *ad libitum.*

**Treatment**
The albino rats were completely randomized into ten (10) groups (A, B, C, D, E, F, G, H, I and J) consisting of five (5) rats in each group. Group A (control) was fed 100% of the commercial rat feed (Emii feed) and water while group B was fed 100% of the raw sample (unprocessed sample) and water for 14 days.

Groups C, D, E and F were treated like the control except that they were fed the cooked sample (60min cooked) which was incorporated into the commercial feed (Emii finishers Feed) at 25%, 50%, 75% and 100% levels respectively.
Rats in groups G, H, I and J were also treated like the control except that they were fed the roasted ($110^0$C, 15min) sample incorporated into the commercial feed (Emii finishers feed) at 25%, 50%, 75%, and 100% levels respectively.

## General observations
All animals were observed twice daily for morbidity and mortality. Any abnormal physical and behavioral changes (skin, fur, eyes, posture, and response to handling) were observed. The time of onset, intensity and duration of such symptom if any were recorded.
Individual animal body weights for treatment and control groups were recorded weekly, beginning on the day before the initiation of treatment. Final body weights were recorded a day prior to the final day of experiment (the 13th day). The amount of feed consumed by each animal was recorded daily.

**Hematological analysis**
This was done by the method described by Akinnawo *et al*. (2002). At the end of the experiment, the rats were anaesthetized with chloroform and blood was collected by cardiac puncture. Two (2) ml of blood from each rat was out into sample bottles containing disodium EDTA and used to determine hematological parameters.

The hematological parameters examined are white blood cells (WBC), Red blood cells (RBC), packed Cell Volume (PCV), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Neutrophiles, Monocytes, Lymphocytes, Eosinophils and Basophils.

**Red blood cell count (RBC)**
The red blood cell (RBC) count was done using the conventional method of Dacie and Lewis (2001). Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with an improved Neubauer counting chamber under a light microscope (Mc Arthur Microscopr) using a X40 objective in an area of 1/5 sqmm. Their characteristic pink-red colour was used for their identification. The number was then calculated thus:
Red blood cells = Cells counted x blood dilution x chamber depth
Area of chamber counted

**White blood cell count (WBC)**
The counting of total white blood cells was done using a diluting fluid (Turk's fluid) in a ratio of 1:20 and then counted with an improved Neubauer counting chamber under a light microscope (McArthur Microscope) using a X10 objective in an area of 4sqmm. The cells appeared as small black dots. The number was thus calculated:

White blood cells = Cells counted x blood dilution x chamber depth
Area of chamber counted

**Hemoglobin estimation**
The conventional method (Sahli's hemoglobinometer) was employed for the estimation of hemoglobin (Hb) content of the blood.
Using the Sahli hemoglobinometer, the colour of the test solution was matched against a colored glass standard. A graduation test tube was filled to 20ml mark with 10N hydrochloric acid. 0.02ml of blood was added and the content of the test tube was mixed with a glass rod. It was left for 5 min (for the hemoglobin to be changed into acid haematin). More acid was thereafter added and the mixture was stirred until the color of the test solution matched that of the colored glass standard. The level of the fluid in the tube was read and the hemoglobin content was expressed as a percentage.

**Packed cell volume (PCV)**
The packed cell volume (PVC) was done using the macrohaematocrit method (Dacie and Lewis, 2001). The blood sample was added to a bottle containing heparin (0.1mg/ml of blood). The haematocrit tube was filled to 100mm with a capillary pipette and it was centrifuged at 3,000 rpm for 30 minutes. The height of the red blood cells was read and the result was expressed as a percentage.

**Determination of platelets**

The platelets were determined by diluting the blood in one percent (1%) ammonium oxalate which haemolysed the red blood cells. The platelets were then counted in a definite area using the rulings of an improved Neubauer counting chamber. Their characteristic Mauve-pink colour was used in their identification.

**Differential white blood cell count**

The differential white blood cell count (Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils) was done by making a thin film of blood on a smooth edged slide. It was allowed to dry on a bench protected from dust, ants, flies, and other insects. The blood film was fixed in a covered staining jar of methyl alcohol for 3 min.

Ten (10) ml of May Grunwald Stain (mixture of 5g of May Grunwald powder and 1 litre of methanol) and 10ml of buffered water (pH 6.8) was mixed thoroughly and the smear was covered with the diluted May Grunwald stain for 3 min. The stain was tipped off and replaced with diluted Giemsa's stain (5%) for 9 min. The stin was washed off with buffered water (pH 6.8) and clean water was dropped on the slide which was allowed to stay for 30 sec. The water was tipped off and the slide was allowed to dry. It was then examined microscopically (McArthur Microscope) for the identification of Neutrophils (cytoplasm stained pink with small mauve granules), Eosinophils (cytoplasm stained pink with large red granules), Basophils (cytoplasm contained dark mauve-blue granules), Monocytes (cytoplasm stained dull grey-blue) while lymphoctes (cytoplasm stained blue).

**Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) determination.**

The mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated from the values obtained from red blood cells (RBC), packed cell volume (PCV) and Hemoglobin (Hb) content. They were calculated thus:

$$\text{Mean Corpuscular Hemoglobin (MCH)} = \frac{\text{Hemoglobin content}}{\text{Red Blood Cell count}} \times \frac{10}{1}$$

$$\text{Mean Corpuscular Hemoglobin Concentration (MCHC)} = \frac{\text{Hemoglobin content}}{\text{Packed Cell Volume}} \times \frac{100}{1}$$

$$\text{Mean Corpuscular Volume (MCV)} = \frac{\text{Packed Cell Volume}}{\text{Red Blood Cell count}} \times \frac{10}{1}$$

**Data analysis**

The results obtained from the data were subjected to Analysis of Variance (ANOVA) according to Onuh and Igwemma (2000) and SAS (1999). Significant means at $p<0.05$ were separated using Fisher's Least Significant Difference (LSD) test (Onuh and Igwemma, 2000).

## Results and Discussion

**Feed intake and weight gain**

The albino rats feed intake levels are shown in Table 1 while their weekly weight gain record is shown in Table 2.

From Table 4, groups B, E, F, I and J fed raw, 75:25 and 100:0 cooked and roasted *L. Chinensis* seed sample-to-commercial feed (Emii Finishers Feed) respectively showed a decreased feed consumption. This may be as a result of the non-palatability of the *L. chinensis* seed sample at these relatively high ratios/concentrations.

Groups C, D, G and H feed consumptions were very comparable to that of group A (control group) that was fed the normal commercial feed (Emii Finishers Feed). This may likely be as a result of the lower ratios/concentrations of the *L. chinensis* seed which possibly did not affect the palatability of the feed mix.

Rats from the test group displayed fairly similar body weight gain to those from the normal control group (group A) (table 2).

**General observations on the albino rats**

Albino rats in groups B and I died after 5 days while those in groups E, F and J died after 6, 4 and 11 days respectively (table 1). A very dull behaviour, dim, dull and partially closed eyes with a fur arrangement that revealed some very minute uncovered straight line skin areas were always observed a day prior to the death of the albino rats.

**Effect of cooked (60 min) *Livistona chinesis* seed on the hematological parameters of albino rats**

The effect of cooked (60 minutes) *L. chinensis* seed on the hematological parameters of albino rats are presented in table 6.

Assessment of hematological parameters of albino rats can be used to determine the extent of deleterious effect of a foreign compound, including plant extracts, on the blood (Odeyemi *et al.*, 2009). It can also be used to explain blood-relating functions of a plant extract or its products (Odeyemi *et al*., 2009; Ajayi *et al*., 2005; Akinnawo *et al*., 2002).

The cooked (60 minutes) sample of *L. chinensis* seed had a significant increasing effect ($p<0.05$) on the packed cell volume (PCV), hemoglobin (Hb) and platelets but did not produce any definite pattern (continuous rise or fall) in white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Lymphocytes, Neutrophils, and Eosinophils. It had no significant effect ($p<0.05$) on the mean corpuscular hemoglobin concentration (MCHC), basophils and monocytes (table 3).

The white blood cells showed a significant increase ($p<0.05$) with the increase in sample (*L. chinensis* seed) concentration but did not show a definite rise in pattern while there was no significant difference ($p<0.05$) for the monocytes, basophils and eosinophils. It showed no definite pattern of rise or fall of neutrophils and lymphocytes but there was a significant difference between them ($p<0.05$). White blood cells (WBC) are important in defending the body against infection (Aboderin and Oyetayo, 2006). The white blood cell count however cannot give specific information but a differential white blood cell count (neutrophils, basophils, monocytes, lymphocytes, eosinophils) narrows down to give specific information about toxicity, poisoning, infections, allergy or immuno-suppression (Aboderin and Oyetayo, 2006).

The primary role of lymphocytes is in humoral antibody formation and cellular immunity (Aboderin and Oyetayo, 2006; Hoffbrand *et al.*, 2004). Neutrophil is mainly responsible for phagocytosis of pathogenic micro-organisms during the first few hours after their entry into tissues (Aboderin and Oyetayo, 2006). Basophils counts increase upon sensitization to an antigen (or allergen), monocytes are responsible for defense of tissues against microbial agents; it increases with bacterial infection and decreases with stress while eosinophils are responsible for allergic reactions and disorders; it increases with allergic conditions and decreases with stress and/or infection (Lewis *et al*., 2006; Aboderin and Oyetayo, 2006; Odeyemi *et al*., 2009).

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) which are all important in the diagnosis of anaemic conditions all increased significantly($p<0.05$) when compared with the control though they did not show any definite pattern as sample (*L. chinensis* seed) ratio(Concentration) was increased. The red blood cells also did not show a definite pattern (continuous rise or fall) also implying dose independent effect (Odeyemi *et al*., 2009).

The packed cell volume (PCV), platelets and hemoglobin (Hb) all showed significant increase ($p<0.05$) as the sample (cooked *L. chinensis* seed) ratio (concentration) was increased in the sample-to-feed mix that was given to the albino rats. The significant increase in packed cell volume (PCV), platelets and hemoglobin (Hb) is an indication that the rats did not suffer anaemia but rather the sample (cooked 60min *L. chinensis* seed) enhanced the PCV, Hb and platelets at these ratios (concentration) tested. This enhancement is possibly as a result of the increase in the ash content of the processed *L. chinesis* seed (Table 2) which is an indication of the amount of minerals present including iron(Fe) and copper(Cu) which are important in hemoglobin synthesis.

The significant increase($p<0.05$) in packed cell volume(PCV), hemoglobin (Hb) and platelets with increased concentration of the sample suggests that increased concentration of the sample in the feed mix at those higher concentrations would have also likely increased the values of these parameters (packed cell volume, hemoglobin and platelets). High packed cell volume (PCV) beyond normal limits suggests venous thromboembolism/vascular accidents (heart failure/block, renal failure, ishaemi stroke, possible retinopathy). High hemoglobin levels also suggest thromboembolism while very high platelet counts suggest hyposplenism, myeloproliferative disorders or thrombocytosis (Lewis *et al*., 2006; Hoffbrand *et al*., 2004). Thus it is possible that the deaths recorded may be due to the conditions mentioned.

The deem, dull and partially closed eyes usually observed a day prior to the death of the albino rats may likely be connected to this possible retinopathy mentioned above.

**Effect of roasted ($110^0$c, 15min) *Livistona chinesis* seed on the hematological parameters of albino rats**

The effect of roasted ($110^0$C, 15min) *L. chinensis* seed on the hematological parameters of albino rats are presented in table 4.

The roasted ($110^0$C, 15min sample of *L. chinensis* seed had a significant increasing effect($p<0.05$) on the white blood cells (WBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and platelets. With increase in the ratio (concentration) of the sample (*L. chinensis* seed) of the feed mix, there was no definite pattern (continuous rise or fall) established for the red blood cells (*RBC*), lymphocytes and neutrophils while there was totally no significant effect ($p<0.05$) on the mean corpuscular hemoglobin concentration (MCHC), monocytes, eosinophils and basophils.

It was observed that the roasted ($110^0$C, 15min) sample of *L. chinensis* exerted a significant increasing effect ($p<0.05$) (definite rising pattern) on the packed cell volume (PCV), hemoglobin (Hb) and platelets just like that of the cooked (60 min) sample except that the roasted ($110^0$C, 15 min) sample also showed an additional significant effect($p<0.05$) on the white blood cells (WBC), mean

corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). The increase in the hemoglobin content would most likely be connected with the increase in the ash content of roasted ($110^0$C, 15min) sample as shown in table 2. The ash content of a sample is an indication of the amount of minerals including iron (Fe) and copper (Cu) in the sample which are both particularly important in hemoglobin synthesis.

The white blood cells showed a significant increase ($p<0.05$) with increase in the ratio (concentration) of the *L. chinensis* seed in the feed mix but with the examination of the differential white blood cell count no significant effect was observed on the monocytes, eosinophils and basophils while lymphocytes and neutrophils did not show any pattern (continuous rise or fall) though there was a significant effect of the sample on them. The lymphocyte is responsible for immunity, eosinophils are responsible for allergy, neutrophils are responsible for phagocytosis of pathogens, monocytes are responsible for defense against microbial agents while Basophils increase upon sensitization to an antigen (or allergy) (Lewis *et al*; 2006; Aboderin and Oyetaya, 2006). The non-significant effect observed for the monocytes, eosinophils and basiphils, and the indefinite pattern (continuous rise or fall) observed for the lymphocytes and neutrophils suggests that the significant increase in the white blood cells is not dependent on the increased ratio of roasted *L. chinensis* seed in the feed mix.

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) relate to individual red blood cells and are of particular importance in the diagnosis of anaemia (Odeyemi *et al*., 2009). The significant increase($p<0.05$) in the hemoglobin level, mean corpuscular volume (MCV), mean corpuscular hemoglobin and the non-significant effect on the mean corpuscular hemoglobin concentration (MCHC) implies that the sample enhanced the incorporation of hemoglobin (Hb) into the red blood cells (RBC) and by implication, the animal did not suffer anaemia (Odeyemi *et al*., 2009).

There was also a significant increase in the platelets ($p<0.05$). Platelets are the blood cells involved in coagulation (Odeyemi *et al.*, 2009). Coagulation of blood requires that the platelets should be in sufficient size, number and function. The increase in the platelets level may be explained by stimulatory effect on thrombopoietin (Odeyemi *et al*., 2009).

The significant increase ($p<0.05$) in the packed cell volume (PCV) and hemoglobin (Hb) which are linked to the total population of red blood cells (Odeyemi *et al*., 2009) and the non-definite pattern (definite continuous rise or fall) observed in the red blood cell count may imply that the rats were not anaemia and that the osmotic fragility of the red blood cells was not adversely affected (Odeyemi *et al*., 2009). The non-definite pattern (continuous rise or fall) observed for the red blood cells may be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) may not have been altered by the roasted sample (*L. chinensis* seed) at the levels which the test animals survived.

The deaths of the albino rats recorded at higher sample-to-commercial feed mix (75:25 and 100:0) may likely be as a result of possible increases in the packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and platelets to high levels beyond normal limits. Very high levels of these parameters (PCV, Hb, MCV, MCH and Platelets) have been implicated in the raising of blood to hypercoaguable state (Lewis *et al*., 2006) which probably predisposed the animals to hyposplenism, myeloproliferative disorders, and thromboembolism with possible vascular accidents like heart failure/block, renal failure, Ischaemic stroke and possible retinopathy (eye problems) (Lewis *et al*., 2006), and consequently led to their deaths.

The deem, dull and partially closed eyes usually observed a day prior to the death of the albino rats may likely be connected to this possible retinopathy mentioned above. It is worthy of note that these effects are very similar to those observed in the albino rats fed the cooked (60min) *Livistona chinensis* seed sample.

**Table 1:** Feed intake (g) of albino rats

| DAY | ALBINO RAT GROUPS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **J** |
| 1 | 14 | 8 | 12 | 13 | 7 | 7 | 12 | 13 | 5 | 6 |
| 2 | 15 | 7 | 14 | 15 | 8 | 7 | 14 | 14 | 8 | 6 |
| 3 | 13 | 7 | 14 | 15 | 8 | 8 | 14 | 14 | 7 | 5 |
| 4 | 14 | 7 | 15 | 14 | 8 | 6 | 16 | 16 | 5 | AD |
| 5 | 15 | AD | 16 | 14 | 10 | 7 | 15 | 13 | AD | - |
| 6 | 16 | - | 13 | 14 | 8 | AD | 15 | 16 | - | - |
| 7 | 14 | - | 14 | 15 | 9 | - | 15 | 14 | - | - |
| 8 | 15 | - | 15 | 16 | 8 | - | 13 | 14 | - | - |
| 9 | 13 | - | 15 | 14 | 10 | - | 14 | 16 | - | - |
| 10 | 14 | - | 15 | 13 | 9 | - | 14 | 13 | - | - |
| 11 | 14 | - | 15 | 14 | AD | - | 13 | 13 | - | - |
| 12 | 16 | - | 14 | 14 | - | - | 14 | 13 | - | - |
| 13 | 15 | - | 14 | 14 | - | - | 16 | 16 | - | - |
| 14 | 15 | - | 15 | 14 | - | - | 13 | 14 | - | - |

NOTE: The values are the means for each albino rat group

AD = ALL DIED = No remaining rats
Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)
Group B albino rats = Albino rats fed raw sample *( L. chinensis* seed*)*
Group C albino rats = Albino rats fed 25:75 cooked sample-to-commercial feed mix.
Group D albino rats = Albino rats fed 50:50 cooked sample-to-commercial feed mix.
Group E albino rats = Albino rats fed 75:25 cooked sample-to-commercial feed mix
Group F albino rats = Albino rats fed 100:0 cooked sample-to-commercial feed mix
Group G albino rats = Albino rats fed 25:75 roasted sample-to-commercial feed mix.
Group H albino rats = Albino rats fed 50:50 roasted sample-to-commercial feed mix.
Group I albino rats = Albino rats fed 75:25 roasted sample-to-commercial feed mix
Group J albino rats = Albino rats fed 100:0 roasted sample-to-commercial feed mix

**Table 2:** Weekly weight record for the rat groups that survived the 14 days duration of the experiment

| **WEEK** | **ALBINO RAT GROUPS** | | | | |
|---|---|---|---|---|---|
| | **A** | **C** | **D** | **G** | **H** |
| Initial weight (on day 1) | 92 | 83 | 89 | 97 | 100 |
| Week 1 | 101 | 93 | 97 | 106 | 105 |
| Week 2 | 109 | 98 | 104 | 11 | 109 |

NOTE: The values are the means for each group

Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)
Group C albino rats = Albino rats fed 25:75 cooked sample-to-commercial feed mix.
Group D albino rats = Albino rats fed 50:50 cooked sample-to-commercial feed mix.
Group G albino rats = Albino rats fed 25:75 roasted sample-to-commercial feed mix.
Group H albino rats = Albino rats fed 50:50 roasted sample-to-commercial feed mix.

**Table 3:** Effect of cooked (60 minutes) *L. chinensis* seed on the haematological parameters of albino rats

| **Haematological parameter** | **White blood cells (x106/mm3)** | **Red blood cells (x 106/mm3)** | **Packed cell volume (%)** | **Haemogolobin (g/dL)** | **Mean corpuscular volume (CUμ)** | **Mean corpuscular hemoglobin (pg)** | **Mean corpuscular hemoglobin concentration (%)** | **Platelets (x 109/L)** | **Monocytes (%)** | **Lymphocytes (%)** | **Eosinophils (%)** | **Neutrophils (%)** | **Basophils (%)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP A ALBINO RATS | $3.60^{a}$ | $4.90^{b}$ | $32.00^{a}$ | $10.80^{a}$ | $65.30^{a}$ | $22.04^{a}$ | $33.75^{a}$ | $780.12^{a}$ | $0.00^{a}$ | $51.00^{b}$ | $2.00^{a}$ | $47.00^{b}$ | $0.00^{a}$ |
| GROUP C ALBINO RATS | $4.80^{c}$ | $4.00^{a}$ | $38.00^{b}$ | $12.60^{b}$ | $95.00^{c}$ | $31.50^{c}$ | $33.15^{a}$ | $830.06^{b}$ | $0.00^{a}$ | $68.00^{c}$ | $0.00^{b}$ | $32.00^{a}$ | $0.00^{a}$ |
| GROUP D ALBINO RATS | $4.00^{b}$ | $5.50^{c}$ | $39.00^{c}$ | $13.00^{c}$ | $70.90^{b}$ | $23.63^{b}$ | $33.33^{a}$ | $864.21^{c}$ | $1.00^{a}$ | $49.00^{a}$ | $2.00^{a}$ | $48.00^{c}$ | $0.00^{a}$ |
| LSD | 0.06 | 0.06 | 0.65 | 0.06 | 1.47 | 0.47 | 0.53 | 5.15 | 0.00 | 0.79 | 0.00 | 0.79 | 0.00 |

NOTE: Means with different superscripts along the same column are significantly different at $P < 0.05$

LSD: Least Significant Difference
Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)
Group C albino rats = Albino rats fed 25:75 cooked sample-to-commercial feed mix.
Group D albino rats = Albino rats fed 50:50 cooked sample-to-commercial feed mix.

**Table 4:** Effect of roasted ($110^0$C, 15 min) *L. chinensis* seed on the haematological parameters of albino rats.

| Haematological parameter | White blood cells (x106/mm3) | Red blood cells (x 106/mm3) | Packed cell volume (%) | Haemogolobin (g/dL) | Mean corpuscular volume (CUμ) | Mean corpuscular hemoglobin (pg) | Mean corpuscular hemoglobin concentration (%) | Platelets (x 109/L) | Monocytes (%) | Lymphocytes (%) | Eosinophils (%) | Neutrophils (%) | Basophils (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP A ALBINO RATS | 3.60$^a$ | 4.90$^a$ | 32.00$^a$ | 10.80$^a$ | 65.30$^a$ | 22.04$^a$ | 33.75$^a$ | 780.12$^a$ | 0.00$^a$ | 51.00$^b$ | 2.00$^a$ | 47.00$^b$ | 0.00$^a$ |
| GROUP G ALBINO RATS | 3.90$^b$ | 5.30$^c$ | 35.00$^b$ | 11.80$^b$ | 66.03$^{ab}$ | 22.26$^{ab}$ | 33.71$^a$ | 798.17$^b$ | 0.00$^a$ | 49.00$^a$ | 0.00$^a$ | 51.00$^c$ | 0.00$^a$ |
| GROUP H ALBINO RATS | 4.00$^c$ | 5.00$^b$ | 38.00$^c$ | 12.80$^c$ | 76.00$^b$ | 25.60$^b$ | 33.68$^a$ | 806.09$^c$ | 1.00$^a$ | 59.00$^c$ | 0.00$^a$ | 40.00$^a$ | 0.00$^a$ |
| LSD | 0.06 | 0.06 | 0.79 | 0.01 | 2.35 | 0.47 | 0.51 | 2.17 | 0.00 | 1.56 | 0.00 | 1.50 | 0.00 |

NOTE: Means with different superscripts along the same column are significantly different at $P < 0.05$

LSD: Least Significant Difference
Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)
Group G albino rats = Albino rats fed 25:75 roasted sample-to-commercial feed mix.
Group H albino rats = Albino rats fed 50:50 roasted sample-to-commercial feed mix.

## Conclusion

From the evidences of the toxicity study which showed significant effect on packed cell volume, hemoglobin, mean cell volume, mean cell hemoglobin and platelets, it is concluded that *L. chinensis* seed is capable of causing thromboembolism, thrombocytosis, hyposplenism or myeloproliferative disorders with possible vascular accidents like heart failure, renal failure and retinopathy. Raw, 60 minutes cooked and 15 minutes roasted *L. chinensis* seed all caused death of albino rats. The death of the albino rats leads to the conclusion that *L. chinensis* seed is unsafe for human consumption at the levels tested.

## Recommendation

A chronic and histopathological toxicity study should further be carried out to ascertain the effect of *L. chinensis* seed on the different organs of albino rats and *L. chinensis* seed should not be used as food at these levels of treatment.

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