

Chemical and Preliminary Toxicological Evaluation of *Chrysophyllum albidum* Seed Flour in Dietary Formulation of Albino Rats

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Summary: The proximate, mineral analysis and phytochemical screening of Agbalumo (*Chrysophyllum albidum*) seed flour was investigated. The seed flour (CASF) contains $9.93 \pm 0.04\%$ moisture, $8.14 \pm 0.13\%$ crude protein, $12.82 \pm 0.04\%$ crude fat, $2.84 \pm 0.23\%$ crude fibre, $2.32 \pm 0.02\%$ ash and $66.79 \pm 0.12\%$ carbohydrate. The phytochemical screening revealed the presence of saponin, flavonoids and alkaloids. The seed flour was also found to contain 5100.00 mg/kg of potassium, 2100.00 mg/kg of magnesium, 1960.00 mg/kg of calcium, 210.00 mg/kg of sodium, 47.20 mg/kg of iron, 24.20 mg/kg of manganese, 12.90 mg/kg of copper and 6.70 mg/kg of zinc. Other heavy metal such as nickel, chromium and lead were not present. The low Na/K ratio (0.04) obtained showed that it would probably reduce high blood pressure.

The seed flour was also incorporated into the formulation of albino rat feed in which wheat in the control diet was totally replaced with *Chrysophyllum albidum* in the test diet. There were increments in the proximate composition of the compounded feed. The experiment which lasted for eight weeks showed that the albino rats appeared to suffer no toxicological effect and weekly monitoring showed good physical appearance. The rats in the test group recorded significant body weight gain (66.98%) when compared to (58.82%) of the control group. Blood indices (blood biochemistry and hematological) were analyzed for in the test and control rats and the results obtained revealed no adverse effect. No significant difference was observed between the histopathology of the rat tissues harvested from the two groups. *Chrysophyllum albidum* is then suggested to be a good replacement for wheat in dietary formulation of albino rats.

Key words: *Chrysophyllum albidum*, nutritional composition, toxicity, albino rats, diet.

I. Introduction

As human population continued to increase, there is a considerable interest in the utilization of underutilized plant seeds as potential sources of human and animal feeds. The nutritional value of any ingredient or feed can be evaluated by biological, chemical and physical scores (Eddy et al., 2004). Chemical score has proven to be a vital tool in food chemistry because it tends to assess the nutritional value based on the proximate composition of the food and feed which includes the protein, carbohydrate, lipid, moisture, ash and dietary fibre contents respectively. Plants are primary sources of medicine, food and shelter used by human on daily basis, their roots, leaves, fruits and seeds often provide food for humans (Amaechi et al., 2009). Due to inadequate supplies of food proteins, there has been a constant search for unconventional legumes as new source for use as functional supplements (Onweluzo et al., 1994). But many of these unconventional seeds and legumes are laying follow due to the lack of their nutritional values and toxicity.

Chrysophyllum albidum, is a tropical plant that belongs to Sapotaceae family. It is commonly found in the Central, Eastern and Western parts of Africa (Adebayo et al, 2010 and Amusa et al, 2003). It is widely distributed in Nigeria, Uganda, Benin, Niger, Cameroon and Cote d'ivoire (Adebayo et al, 2006). In Nigeria, is known by various tribal names as agbalumo (Yoruba), udara (Ibo) and ehya (Igala), (Akubor et al., 2013) and is generally regarded as a plant with diverse ethno-medicinal uses (Amusa et al, 2003). *C. albidum* is highly used and appreciated in southern Benin, where it is called azongogwe or azonbobwe in local language "Fon, Goun" and azonvivo, azonvovwe or azonbebi in their local language "Aizo" (Dah-Dovonon, 2000). It is a large berry that contains up to five seeds that are flat in shape. The leaves of the plant are alternate and nearly evergreen elliptic, slightly leathery. The fruit could be ellipsoid, round or pear shaped. It has a milky sweet pulp that houses the seeds. When the fruit is cut transversely it appears like an asterisk in the central core or like the pointed stars. This is why it is referred as "STAR APPLE" (Edem et al., 2011). In Nigeria, the fruit is gathered for household use or for sale in local markets during the months of December to April. The fruit is relished for its testy fleshy pulp. The pulp is consumed in its natural form by pressing hard and sucking the pulp. The fleshy pulp of the fruits is eaten especially as snack and relished by both young and old (Cenrad, 1999). It is reported to be an excellent source of vitamins, iron, flavours to diets and raw materials to some manufacturing industries (Adisa et al., 2000, Amusa et al., 2003). Nevertheless, while the fleshy pulp is eaten, the seeds are thrown away (Okafor and Fernandes, 1987; Umelo, 1997; Adisa, 2000).

The proximate composition, ascorbic acid content and anti-nutrients composition of *C. albidum* fruit have been reported (Edem et al 2011). While Amusa et al., (2003) also reported that the pulp starts deteriorating after 5 days and such losses in the harvest have been minimized through processing of the fruit into value added products such as juice and jam. Chukwumalume et al., (2010) reported that the seed is a good source of vegetable oil and the processing applications of the oil have been suggested by (Odowu et al., 2006). Urehigbo et al. (2010) reported on the nutrient values of *Chrysophyllum albidum* as a domestic income plantation species. Research on the proximate composition and functional properties of flour prepared from *C. albidum* has been studied and compared with those of wheat flour (Akubor et al., 2013). The extracts of the seeds and roots of *C. albidum* have good potentials as anti-inflammatory, anti-diarrheal and anti-hemorrhoidal compound and further provide a rationale for the use of the seed and root extracts of this plant in traditional medicine practice in Nigeria (Okoli, 2010). Jimoh et al. (2014) found that the replacement of maize by *Chrysophyllum albidum* seed flour in the diets of *Clarias gariepinus* fish significantly reduce the growth and nutrient utilization by *Clarias gariepinus*. No report has been cited on the effect of incorporating this nutritious seeds into rat food chain. This study, therefore, examined the toxicological evaluation and effect of total replacement of wheat flour with *C. albidum* (African star apple) seed flour in albino rat feed.

II. Material And Methods

Plant material and Preparation

Ripe and fresh *C. albidum* fruits used for this study were purchased from Ojoo market, (March 2013) in Ibadan, Oyo state of Nigeria. The fruits were washed in clean tap water and the seeds were removed from the fruits and left to air dry for three days at room temperature. The seeds were cracked manually, cleaned and separated from the hulls. The kernels were cut into thin slices of 1cm thickness and then sun-dried at 30 ± 2 °C to constant weight. The dried slices were ground through mechanical grinder and sieved through a 200 mesh sieve (British standard). The flour obtained was packed in transparent polyethylene bags prior to use.

Physical characteristics of *C. albidum* seed

The physical characterization of *C. albidum* seeds was carried out following the method of Fermia et al. (1995) and Ajayi et al.(2006). The weight of 20 seeds was taken and after which the weight, length and the width of each seed or kernel and the percentage yield of the kernel were noted. The determinations were done in triplicate and the mean values were noted. The color of the unripe, ripe seed and fruits was done by visual inspection and was noted.

Proximate analysis of *C. albidum* seed flour and compounded feed

The proximate analysis of *C. albidum* seed flour (CASF) and the feed compounded were determined according to AOAC (2010). The moisture content was determined by drying 2 g each of the samples in an oven with air circulation at 105° C until a constant weight was obtained (Ajayi, 2009). Ash was determined by weighing the incinerated residue obtained at 550 °C for 5 hours, protein content was determined using the Kjeldahl method, crude fibre and fat (solvent extraction) of the seed and the compounded feeds were determined and analyzed by the AOAC (2010) methods. Carbohydrate content was determined by the $[100 - (\text{protein} + \text{ash} + \text{crude fibre} + \text{crude fat} + \text{moisture content})]$ (Ajayi et al., 2013). The metabolizable energy was calculated using the Atwater factor 17, 37, and 17 for protein, fat and carbohydrate respectively as described in Aremu et al. (2007). All analyses were done in triplicate.

The phytochemical analysis

The phytochemical analysis of the extract was carried out to determine the presence of saponins, phenolics, alkaloids, tannin, flavonoids, glycosides, steroids, terpenes and anthraquinone using standard procedures according to Trease and Evans (1989) and Sofowora (1996).

Mineral determination in *C. albidum* seed flour

Mineral composition of *C. albidum* was determined following the method used by Ajayi et al., (2006) and Idouraine et al. (1996). One gram of the seeds was ashed in a muffle furnace at 550 °C for 5 hours (to constant weight) until a white ash was obtained. The minerals were extracted from ash by adding 5 ml of a mixture of concentrated HNO₃ and HCl at 3:1 ratio. The digest was carefully filtered into 100 ml standard bottle and made up to mark with deionized water. Sodium and potassium were determined using a flame photometer (Model, 405, Corning, UK). All other metals were determined by Atomic Absorption Spectrophotometer (Perkin - Elmer Model 703, Norwalk CT, USA). All determinations were done in triplicate.

Experimental animals and design

Fourteen male Albino rats with average body weight ranged from 70 to 75 g were used in this study. The rats were apparently clinically healthy and housed within the premises of Veterinary Department animal house, University of Ibadan, Nigeria under standard husbandry conditions (30 °C ± 2 °C, 60 - 65 % relative humidity and 12 h: 12 h day-night cycle) and fed on the rat basal diet formulated to meet the entire nutrient requirement. The diets were prepared according to the procedure described by Souza et al. (2007) and modified by (Ajayi et al., 2013). The ingredient used for the control diet were 2450 g of maize, 1260 g of soy bean, 210g of bone, 70 g of salt, 1050 g of groundnut cake, 525 g of palm kernel, 700 g of wheat, 525 g of corn bran and 210 g of oyster shell. Wheat was totally replaced with 700 g of *C. albidum* seed flour in the experimental diet. The ingredients were weighed after compounding to be 7000 g. This experiment was designed and carried out under standard husbandry conditions. The animals were divided into two groups of seven rats each and were fed for a period of 8 weeks before sacrifice. They were allowed to acclimatize for one week before the commencement of the experiment. During the 8 weeks of the experiment, the rats were fed with the compounded diets with unrestricted access to water. The feed intake and body weight gain were monitored daily and weekly. The experimental rats were fed with a compounded feed where wheat in the control group was totally replaced with *C. albidum* seed flour in the test group for 56. The physical appearance of both the control and experimental rats was monitored while the body weight of each rat was recorded weekly. Blood samples for haematology, biochemical parameters and tissue samples for histopathology were taken at the end of the eight weeks.

Collection of blood, organs and tissue samples:

After the 56 days of experiment, seven animals from each group were sacrificed under mild anesthetics with chloroform after overnight fast. The blood samples were collected by cardiac puncture into two heparinized tubes for the studies. One tube contains EDTA with calcium serving as anti-coagulant for haematological analysis while the second tube was stored at -20°C for the biochemical studies. Serum samples were collected in sample bottles without EDTA allowed to clot and centrifuged at 5000 rpm for 10 mins. The liver, intestines, heart, kidney, lungs, brain and spleen were promptly excised soon after decapitation, weighed and stored in 10 % formalin for histological examination.

Haematology and biochemistry of rat blood

Haematological analyses were carried out in about 3ml of rat blood collected into EDTA bottles through cardiac puncture. Hemoglobin and White Blood Cells were determined according to methods described by Dacie (1975), Dacie and Lewis (1991). The packed cell volume, haemoglobin (Hb) concentration, red blood cell, differential WBC counts, mean corpuscular volume and mean corpuscular haemoglobin concentration were determined and calculated respectively using standard procedures and technique as described in Ministry of Agriculture and Food manual of Veterinary investigation (MAFF, 1984). Albumin and globulin were determined by a colorimeter. The albumin/globulin ratio was obtained by dividing the calculated albumin value by the calculated globulin value. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also determined (MAFF, 1984).

Tissue pathology

For the histological analyses of the heart, liver and kidney samples, small portions of these tissues harvested and stored in formalin were fixed and put through series of dehydration in graded concentration of xylene. They were embedded in wax, sectioned at 5 µ and transferred to clean glass slides. The thin sections were stained with haematoxylin and eosin (H and E) dyes for examination under the light microscope for histological changes following the method outlined by (Ajayi et al., 2013; Jain, 1986). After H & E staining, the slides were observed and photos were taken using an optical microscope (DM750, Leica, Germany).

Statistical analysis

Results were expressed as mean standard error. Organ weights, biochemical and hematological determinations were analyzed. Differences between the groups were tested by two-way ANOVA and Duncan test. Differences between groups were considered significant at $p < 0.05$ levels.

III. Result And Discussion

Physical characteristics

The physical characterization of *C. albidum* seeds with regards to their weight, length, width, kernel percentage and the state of the oil at room temperature are listed in Table 1. The weight of twenty of seeds is 18.66 ± 1.69 g. The average length and width are 2.31 ± 0.37 cm and 1.46 ± 0.16 cm. The percentage kernel was found to be 50.00. The weight of each seed was determined to be 0.93 ± 0.07 g.

Mineral elements

Mineral element content of *C. albidum* (CASF) seed flour is presented in Table 1. CASF is rich in potassium 5100.00 mg/Kg, magnesium 2100.00 mg/Kg, calcium 1960.00 mg/Kg, sodium 210.00 mg/Kg, iron 47.20 mg/Kg, manganese 24.20 mg/Kg, copper 12.90 mg/g and zinc 6.70 mg/g. Other heavy metal such as nickel, chromium and lead were not present. The low Na/K ratio (0.04) obtained showed that it would probably reduce high blood pressure. CASF could be a good source of potassium, magnesium and calcium. These mineral in the diet are generally required for metabolic reaction, transmission of nerve impulse and rigid bone formation among others (Egwim et al., 2010). The minerals present in *C. albidum* are higher in quantity when compared to those reported for African oil bean seeds (Enujiugha and Akanbi, 2005). The value 0.04 of Na/K ratio obtained is lower than 1. This is in accordance with what was obtained by Eunice et al. (2012) for *C. mannii* seed flour. This is an indication that the seed flour would probably reduce high blood pressure. *C. albidum* seed flour showed high concentration of 2100mg / Kg for magnesium. Magnesium is found to be important mineral in its healing effect on a wide range of diseases as well as in its ability to maintain healthy bones while calcium, contributes in preventing cardiovascular diseases, regulates high blood pressure, migraines, insomnia and depression (Newsmax, 2011). The result showed that the seeds are very rich with the important mineral. The values obtained are lower than those reported on *Plukenetia conophora* seed flours (Ayoola et al., 2013).

Phytochemical analysis of *C. albidum*

C. albidum seed flour was screened for phytochemical properties and the result showed the presence of alkaloids, saponins, tannins and carbohydrate (Table 2). These phytochemicals are known to exhibit diverse pharmacological and biochemical actions and would have contributed to the earlier report of the medicinal properties of the plant. Tannin was found to have some anti-nutrient effect achieved by forming complexes with protein (Kumar and Singh 1984). The results obtained are similar to those reported by Okoli, (2010). They are also comparable to the reported phytochemical components which indicate the presence of alkaloids and flavonoids, (Assam et al., 2010).

Proximate analysis of *Chrysophyllum albidum* seed flour

The proximate composition of *Chrysophyllum albidum* seed flour is shown in Table 3. The moisture content of 9.93 ± 0.04 is high when compared to groundnut (Onyeike and Acheru, 2002) similar to the value obtained for star apple (Akubor et al., 2013). The ash content is $2.32 \pm 0.02\%$ is lower when compared to the values obtained for *Azelia Africana* (Ajayi et al., 2011) and moringa seed flour (Ogunsina et al., 2011). The high total carbohydrate value of $63.94 \pm 0.12\%$ suggests *Chrysophyllum albidum* seed flour as useful supplement in compounding animal feed. The protein content of 8.14 ± 0.13 is comparable to 8.10 ± 0.22 reported for defatted *Garcinia mangostana* (Ajayi et al., 2013), but higher than 4.5 ± 0.05 obtained for African star apple kernel (Akubor et al., 2013).

After compounding the feeds, diet sample were analyzed again to ascertain their chemical composition. It was observed that there was an increase in the protein content, crude fibre, and ash content while carbohydrate value reduced when compared with the composition of the seed flour. The calculated metabolic energy value obtained is 1699.7 KJ/100g for *C. albidum* 1524.3KJ/100g for wheat flour, 1496.5 KJ/100g for the control and 1524.3 KJ/100g for the experimental feed. This shows that the seed flour could be a source of energy. Table 4 shows the various energy values as contributed by protein, fat and carbohydrate. The proportion of total energy due to protein is 18.14 %, proportion due to fat is 27.91% and proportion due to carbohydrate is 63.95 % for *C. albidum*. This is an indication that the energy obtained from *C. albidum* seed is majorly contributed by the high carbohydrate content of the seed flours and could be useful as supplement based on carbohydrate in animal feed formulation. With the total replacement of wheat with *C. albidum* flour, there is an increase in the proportion of energy due to protein and a decrease in those of fat and carbohydrate.

Feed intake and body weight changes

Figure 1 shows the feed intake and figure 2 shows the resultant body weight changes of test and control rats. There was a gradual increase in the quantity of feed consumed by rats in the two groups from the beginning of the experiment till the end. From Fig 1, it could be seen that both the groups consumed almost the same quantity of feed within the first and fourth week while the experimental feed was greatly consumed at the last four week of experiment. At the same time, there was steady increase in the body weight of the rats at the onset of the 56 days of the study which peaked subsequently suggesting that the *C. albidum* seed flour did not exert any deteriorative effect on the weight and growth of the animals. The increase in weight of the animals suggests that they increasingly accumulated calories by consuming more of the experimental diet than the normal rat diet. The experimental groups (test rats) had a highest body weight.

Organ weight

Seven different organs (heart, liver, kidney, brain, intestine, spleen and lungs) were collected from each rat, weighed and stored. The weight of the organs collected did not differ significantly from each other in both groups as shown in Fig 3. An average kidney weight of 0.9 ± 0.05 g and 0.86 ± 0.17 were obtained for the control and test rats respectively. The liver weight obtained in control and test groups (4.74 ± 0.63 and 4.51 ± 0.45) respectively are similar to the report given by Vishnu et al., (2010) and Ajayi et al.(2013). Organ weight is an important factor of physiological and pathological status in animals. The relative organ weight is fundamental to establish whether the organ was exposed to the injury or not. The heart, liver, kidney, spleen, and lungs are the primary organs affected by metabolic reaction caused by toxicant (Jothy et al., 2001). The liver, being a key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals (Jothy et al., 2001). The absence of significant difference in the parameter obtained in this study is an indication that the seed flour did not affect the weight of the organs.

Haematological and Biochemical Parameters

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds on the blood constituents of animals. They can also be used to determine possible alterations in the levels of bio molecules, metabolic products, haematology, normal functioning and histomorphology of the organs (Jothy et al., 2001). The haematological parameters of both the control and test rats were presented on table 6. A part from WBC and Platelets values which differ from each other in both groups, all other parameter did not show any significant difference from each other in the two groups. Therefore, the diet compounded with *Chrysophyllum albidum* seed flours had no adverse effect on the blood of the rat under this study as it is comparable to the indices obtained for the diet compounded with wheat. This was in accordance to the result reported by Vishnu et al. (2010) and Ajayi et al (2012). Urea and creatinine are considered as a suitable prognostic indicator of renal dysfunction and kidney failure for any toxic compounds (Gnanamani et al., 2008). The absence of significant differences in urea and creatinine of the rat fed with CASF after 56 days means that *C. albidum* flour at this level of inclusion has no harmful effect on the kidney. All other serum biochemical parameters did not differ significantly from each other. The Mean corpuscular hemoglobin (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), which are RBC indices used in classifying types of anemia did not show any major significant changes in experimental animals when compared to the controls. The values obtained confirm that the diet compounded had no adverse effect on the rat used for this study.

Histopathological Result

The pathology result showed no major complications and no significant differences on the tissues of the rats in both groups (Table 7). No lesion was observed in the heart, liver and kidney of the rats in both the control and test groups. *Chrysophyllum albidum* seed flours might be free from any deleterious effect at this level of inclusion. This is indicating that it could probably be used to replace wheat or any other carbohydrate component in the diet of livestock and even man.

IV. Conclusion

Chrysophyllum albidum seed flour, based on the proximate analysis has a potential of being utilized successfully as a source of dietary energy and roughage in feed for livestock because of its high carbohydrate. It can as well be supplemented with other high protein residue such as groundnut cake because of its low protein and fibre values. The rats used in this experiment showed appreciable weight gain during the experimental period and the feed intake also showed that the rats consumed reasonable quantity of the diet prepared with corn bran totally replace with *Chrysophyllum albidum* seed flour. There were no significant differences in the haematological and biochemical parameters in the blood of the control and test rats. The histopathological examination of the liver, kidney and heart sections showed no visible lesion. The test rat had a higher weight gain than those of the control group. This result suggests therefore that *Chrysophyllum albidum* seed flour could be successfully used to totally replace corn bran in rat diet formulation with other supplements and might be potentially safe for human consumption.

Acknowledgements:

The author wishes to acknowledge the Department of Chemistry, Faculty of Science and Veterinary Pathology unit, Faculty of Veterinary, University of Ibadan, Ibadan, Nigeria for making their facilities available for this study.

Table 1:Physical properties and mineral composition of *C. albidum* seed and flour

Physical properties	<i>C. albidum</i>
Weight of 20 seeds(g)	18.66 ± 1.69
Weight of a seed(g)	0.93 ± 0.07
Seed length (cm)	2.31 ± 0.37
Seed width (cm)	1.46 ± 0.16
Percentage kernel	50.00 %
Colour of unripe seed	Green
Colour of ripe seed	Yellow
State of the oil at room temperature	Liquid
Mineral analysis	mg/kg (w/w)
Sodium	210.00
Potassium	5300.00
Calcium	1960.00
Magnesium	2100.00
Manganese	24.20
Iron	42.70
Copper	12.90
Zinc	6.70
Nickel	ND
Chromium	ND
Lead	ND
Na/K	0.04
Ca/Mg	0.94

Table 2:Phytochemical screening of *C. albidum* flour

Parameter	CAS
Saponin	Present
Tannins	Absent
Flavonoids	Present
Steroids	Absent
Cardiac Glycoside	Absent
Akaloids	Present
Reducing Sugar	Absent
Phenol	Absent
Anthraquinone	Absent
Glycoside	Absent
Resin	Present
Phlobatannins	Absent
Carbohydrate	Present

Table 3:Proximate composition of both *C. albidum* seed flour and the diets

	CASF	Wheat flour ^a	Control Diet	Wheat/CASF
Moisture content	9.93 ± 0.04	11.00 ± 0.34	10.12 ± 0.16	10.94 ± 0.4
Protein content	8.14 ± 0.13	11.5 ± 0.14	13.63 ± 0.04	24.00 ± 0.13
Crude fat	12.82 ± 0.04	1.50 ± 0.05	18.24 ± 0.96	14.56 ± 0.02
Crude fibre	2.84 ± 0.23	0.6 ± 0.01	7.95 ± 0.07	9.6 ± 0.03
Ash content	2.32 ± 0.02	1.00 ± 0.05	13.09 ± 0.17	8.55 ± 0.04
Carbohydrate	63.94 ± 0.12	74.90 ± 0.18	36.97 ± 0.42	32.34 ± 0.06
Dry matter	90.07	89.00	89.88	89.06
Energy (Kj/100g)	1676.76	1524.3	1535.08	1496.5

^aAkurbo et al., (2013) Mean ± SD for three replicate analyses

Values in the same row with the same superscripts are not significantly different at (P < 0.05)

CASF: *C. albidum* seed flour

Table 4: Energy values as contributed by protein, fat and carbohydrate inof *C. albidum* seed flours.

	CASF	Control	Wheat/CASF	Wheat flour
Total energy	1699.7	1535.08	1496.5	1524.3
Proportion of total energy due to Protein %	18.14	15.09	27.26	12.83
Proportion of total energy due to Fat %	27.91	43.96	35.99	3.64
Proportion of total energy due to Carbohydrate%	63.95	40.94	36.74	83.53

CASF: *C. albidum* seed flour

Wheat/CASF: Wheat totally replaced with *C. albidum* seed flour

Table 5: Haematological and biochemical studies of rats fed with control feed and *C. albidum* seed flour

Haematology		Analysis	
Parameter	Control group	Experimental(Wheat/CASF)	
PVC %	40.29±1.37 ^a	41.43±1.62 ^a	
HB	13.51±0.83 ^a	13.63±0.70 ^a	
RBC	6.48±0.50 ^a	6.46 ±0.39 ^a	
WBC	6685.71±1.715.81 ^a	6021.43±1.446.50 ^b	
MCV(fl)	62.17±2.52 ^a	56.29±2.24 ^b	
MCH (%)	20.85±0.79 ^a	18.52±1.07 ^b	
MCHC (%)	33.53±1.11 ^a	32.92±0.52 ^a	
Lymphocyte(%)	70.14±6.49 ^a	71.00 ± 0.65 ^a	
Neurophil (%)	28.00±6.53 ^a	26.57±7.06 ^b	
Monocyte(%)	28.06 ± 6.53 ^a	20.43 ±0.8.18 ^b	
Eosinophil (%)	1.14±0.50 ^a	1.43±1.15 ^a	
Platelets (cell/cu.mm)	125,285.11±35,831.51 ^a	103478.58±26318.36 ^b	
Blood		Parameter	
Total protein	6.6 ± 0.30 ^a	6.53 ± 0.3 ^a	
Albumin	3.63 ± 0.26 ^a	3.56 ± 0.44 ^a	
Globulin	2.86 ± 0.15 ^a	2.96 ± 0.43 ^a	
Alb/Glo ratio	1.17±0.06 ^a	1.33±0.55 ^b	
AST	39±1.00 ^a	37.66±2.51 ^b	
ALT	26.67±0.58 ^a	28±0.00 ^b	
ALP	81.33 ± 3.5 ^a	80±1.00 ^a	
Urea	16±1.00 ^a	15±1.00 ^a	
Creatinine	0.67±0.01 ^a	0.7±0.00 ^a	

Values are expressed as mean±SD. Values in the same row with different superscripts are significantly different at P< 0.05. Haemoglobin, concentration (g%); PCV = Packed cell volume (%), RBC = Red Blood Cell Counts, WBC = White Blood cell count (x10³/mm³), MCV = Mean Corpuscular Volume (fl), MCH = Mean Corpuscular Haemoglobin (%); MCHC = Mean Corpuscular Haemoglobin Concentration (%). AST- Aspartate aminotransferases, ALT- Alanine aminotransferases, ALP = Alkaline phosphatase; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin – Globulin ratio; TP = Total Protein. Wheat/CASF: Wheat totally replaced with *C. albidum* seed flour.

Table 6: Histopathology examination of rat tissues

Parameters	Control	Experimental (Wheat/CASF)
Heart	No visible Lesion	No visible Lesion
Liver	No visible Lesion	No visible Lesion
Kidney	No visible Lesion	No visible Lesion

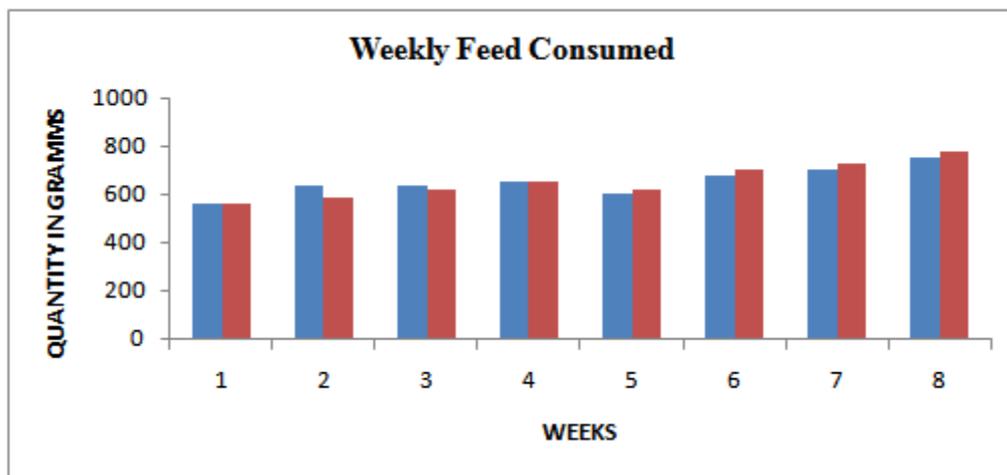


Fig 1: A chart showing the weekly feed consumption of rats fed with wheat totally replaced with *C. albidum* seed flour

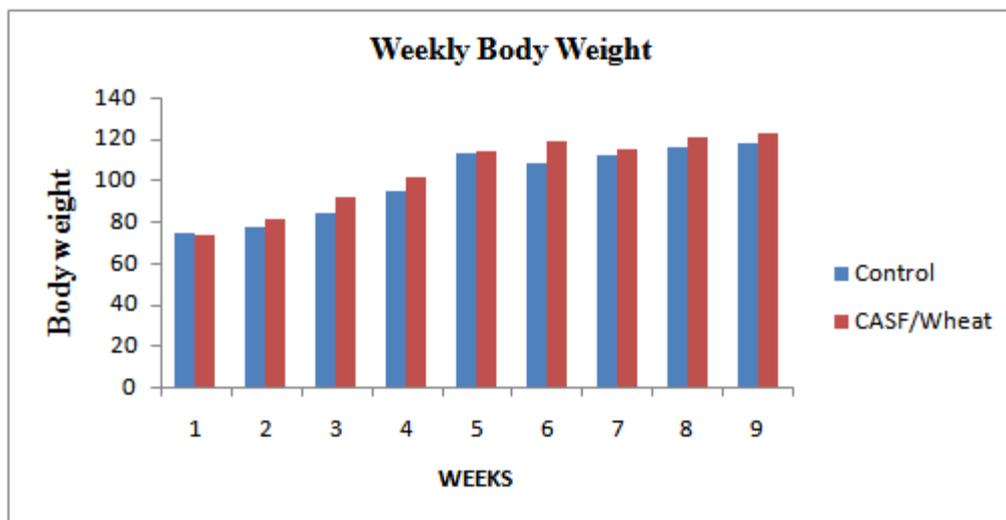


Fig 2: A chart showing the weekly body weight of the rats fed with wheat totally replaced with *Chrysophyllum albidum* seed flour

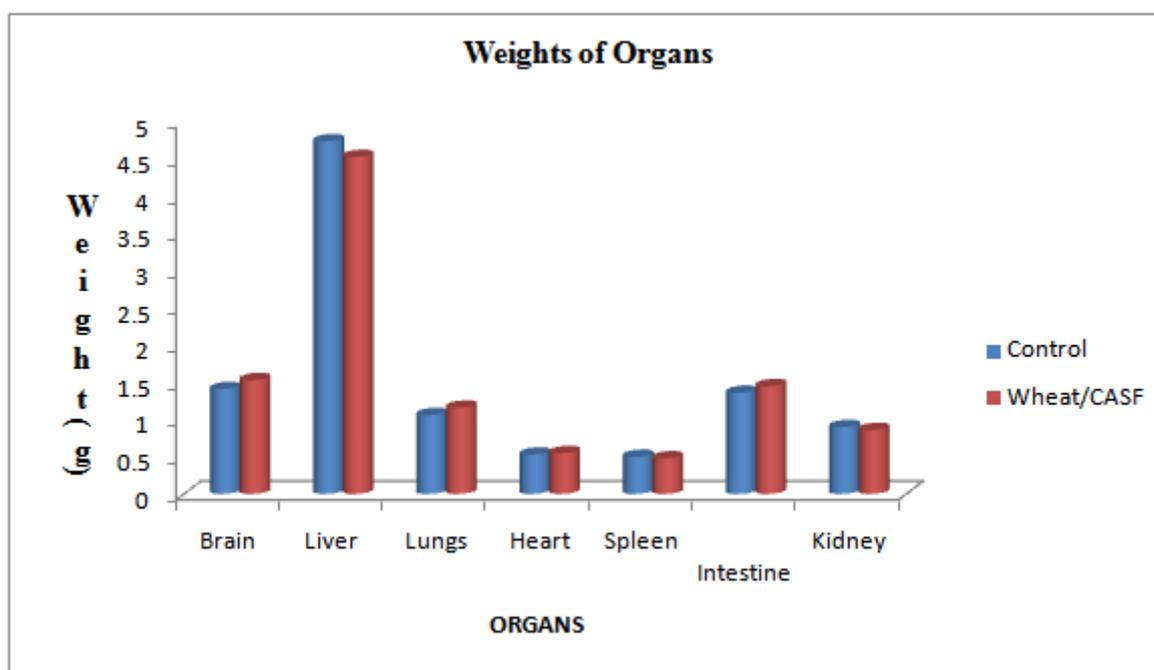


Fig 3: A chart showing the weight of the organs/tissues of the rats fed with wheat totally replaced with *C. albidum* seed cake and the control.

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