

Toxicological studies and application of *Balanite aegyptiaca* seed cake in dietary formulation of albino rats

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Abstract: This study was conducted to evaluate the effect of total replacement of corn bran with *B. aegyptiaca* cake in dietary formulation of albino rats using growth performance, nutrient utilization, histopathological and blood parameters as indices. The seed cake showed a high protein value of 49.32 ± 0.13 % but low moisture, fat and ash contents of 8.09 ± 0.21 %, 10.42 ± 1.12 %, 2.13 ± 0.13 % and 1.8 ± 0.02 % respectively with a carbohydrate content of 23.91 ± 0.15 %. The mineral analysis showed that the cake contained considerable amount of potassium (4940 mg/kg), calcium (3390 mg/kg) and magnesium (1930 mg/kg). The result of the phytochemical analysis revealed the presence of tannins, flavonoids and alkaloids in the cake.

An eight-week feeding trial was conducted to determine the toxicological effect of *B. aegyptiaca* seed cake in the diet of albino rats. All diets were formulated to meet the entire nutrient requirement for young albino rats. The weekly records showed a gradual increase in body weight and a good physical appearance. Result of the pathology of the rat organs (kidney and heart) compared closely in the test and control groups but there was infiltration by mono-cellular cell in the liver of the test group rat. The haematological and blood biochemistry results revealed no adverse effect for both groups and showed no significant difference at ($p < 0.05$). *Balanite aegyptiaca* cake might be safe and suitable as good additive in feed supplements.

Keywords: *B. aegyptiaca* cake, proximate composition, corn bran, histopathology

I. Introduction

The nutritive and calorific values of plant seeds make them necessary in diets. Because of the inadequate supplies of food proteins, there has been a constant search for unconventional protein sources, for use as both functional food ingredients and nutritional supplements. The ultimate success of utilizing plant proteins as ingredients largely depends upon the beneficial qualities they impart to foods, which in turn depend largely on their nutritional and functional properties, Mcwalters et al. (1976). *B. aegyptiaca* is a perennial tropical plant of the family Balaniteceae, commonly known as desert date, is a small evergreen tree reaching a height of 6-12 m. It belongs to the Zygophyllaceae family and produces fruits, which are edible. Fresh new shoots, which are always growing during the dry season, are commonly used as animal forage but in periods of food shortage, people cut the newly growing succulent shoots with the leaves, cook and eat them. The plant is commonly found wild in Borno and Adamawa States of Nigeria, (Kubmarawa et al., 2008). It is known in Nigeria as Adowa (Yoruba), adua or Aduwa (Hausa), tanni (Fulfulde), cunjo (Kanuri) and heglig (Arabic) and widely used in food preparation and herbal medicine, especially in Africa and some developing countries (Wilson et al., 2009). It contains good quality oil and high protein content (Mohamed et al., 2002; Abu Al- Futuh, 1983).

Available reports on the nutritional and anti-nutritional profile of *B. aegyptiaca* seed powder shows that the seed powder contains a relatively high amount of protein and lipid (Samuel et al., 1997). However, in addition to the nutrients, the seed contains high level of anti-nutritional factors; tannins, oxalate and phytic acid as reported by (Chothani & Vaghasiya, 2011 and Samuel et al., 1997). Tannins are secondary plant metabolites that are rich in phenolic hydroxyl groups and have been implicated in the inhibition of non-heme iron absorption, by complex formation with iron in the gastro intestinal lumen (Brune et al., 1989). Despite of such wide spread uses, and the toxicological evaluation, toxicity of *B. aegyptiaca* seed oil in rats (Wilson et al., 2009 and Ajayi et al., 2013) there is limited literature on the defatted seeds, hence the need for this research. The aim of this work, therefore, is to determine phytochemical screening, the preliminary toxicological studies and effect of total replacement of corn bran with *B. aegyptiaca* cake on albino rats and to evaluate its suitability as an additive in feed supplement. This is in continuation of previous work on seed cake, their nutritional and possible applications in rats /animal feeding (Ajayi et al., 2004; 2008; 2012; 2014).

II. Material and methods

2.1 Raw Material, source and preparation

Mature *B. aegyptiaca* seeds used for this study were bought from Tundunwada market in Zaria, Kaduna State of Nigeria. The seeds were identified in the botanical garden of the University of Ibadan. The seeds were soaked in a large bowl of water overnight to remove the pulp from seed coats, sun dried and crushed

by a metal hammer to obtain the kernel. The kernels were grounded into paste to increase the extent of extraction. Oil was extracted from the seed flour by soxhlet extraction method using n-hexane as the solvent. *B. aegyptiaca* seed cake (BASC) obtained after the oil extraction was air dried, pulverized and passed through a 200 mesh size to obtain the defatted powder which was used as experimental material. Also reagents used for the chemical analysis were of analytical grade.

2.2 Chemical compositions

2.2.1 Proximate analysis

The ash content of *B. aegyptiaca* seed cake and the compounded feed was determined by heating at 550 °C a representative 2 g sample in a vector Muffle furnace for five hours (Ajayi, 2009). Moisture, crude fat and crude fibre contents were determined following the methods of Association of Official Analytical Chemists (AOAC, 2006). Nitrogen content of the seed cake was estimated using the micro-kjeldahl method as described by AOAC (1990) and crude protein was calculated using (N x 6.25). Carbohydrate contents were determined by difference [100 - (moisture + protein + crude fat + ash + crude fiber)]. The calorific energy value was obtained according to the methods of Olaofe et al. (2009) by multiplying the values of carbohydrate, protein and crude fat with Atwater factors of 17, 17 and 37 respectively. Determinations were in triplicate.

2.2.2 Phytochemical analysis

The phytochemical screening for the presence of saponins, tannins, alkaloids, flavonoid, steroid and glycoside were carried out according to the methods describe by Trease and Evans (1983), Hassan et al., (2004) and Ajayi et al. (2011).

2.2.3 Analysis of Mineral Elements

Exactly 1.00 g of defatted *B. aegyptiaca* was digested with 10 ml mixture of concentrated HNO₃ and perchloric acid (2:1 v/v) until the solution became clear. Thereafter, it was transferred to a 100 ml volumetric flask, diluted and made up to the mark with deionized water. The solution obtained was stored in a clean polyethylene bottle. The mineral element content was determined using an atomic absorption spectrophotometer (Perkin–Elmer model 703, USA) and flame photometer as described by Onyeike and Acheru (2002).

2.3 Animal, diets and feeding

2.3.1 Feed compounding

The feed that was used for the experiment was formulated to meet the entire nutrient requirement for young rats. The feed was prepared according to the formula and procedure used by Toyomizu et al. (2003) with little modification. The basic ingredients used were: maize (40 %), soybeans (18.21 %), bone (3.3 %), salt (0.79 %), groundnut cake (14.20 %), palm kernel cake (7.08 %), wheat (7.08 %), corn bran (7.08%) and oyster shell (2.26 %). The above were used to compound feed for the control diet (A). Corn bran was totally replaced with *B. aegyptiaca* seed cake (7.08 %) in compounding the experimental diet (B). Ingredients of the diets were mixed thoroughly to obtain a homogenous mixture which was pelletized, dried, weighed and packed into two different transparent sterile plastic containers for the analysis.

2.3.2 Experimental animal

A total number of fourteen albino rats, specific pathogen free, aged 4 weeks and weighing 60 - 80 g were obtained from the animal house of Veterinary Department, University of Ibadan, Nigeria. The animals were divided into two groups (A and B) of seven rats per group and were fed for a period of eight weeks before sacrifice. They were allowed to acclimatize for one week before the commencement of the experiment. All animals were housed in well-constructed plastic cages (7 rats/cage) placed in well ventilated rooms and kept on a 12 h light/12 h dark cycle. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from cages daily. During the 8 weeks of the experiment, the rats were fed with the compounded diets and tap water ad libitum.

2.3.3 Blood and tissue collection

At the end of the experiment, the animals were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture before incision of the abdomen; 3 ml of blood samples were collected in plain tubes, serum was collected and frozen at -30°C until the time for analysis. Liver, heart, kidney, lungs, and brain tissues were cut in small pieces and immersed in neutral buffered formalin 10 % for histopathology following the method described in Althnaian et al. (2013).

2.4 Haematological and blood biochemistry analyses

Commercial test kits obtained from Randox laboratories Co Atrium, UK were used for all biochemical parameters. 3 ml of rat blood were collected into EDTA bottles through ocular puncture for Haematological analyses. The packed cell volume, haemoglobin concentration, red blood cell, white blood cell differential counts, mean corpuscular volume and mean corpuscular haemoglobin concentration were determined and calculated respectively using the standard technique as described by Jain (1986). Blood biochemistry parameters such as aspartate amino transferase, alanine amino transferase and alkaline phosphatase were determined by the method of described in Chawla (1999).

2.5 Tissue pathology

The liver, kidney, heart and brain and of the rats after eight weeks of experiment were harvested and fixed in 10% neutral buffered formalin in labeled bottles. The tissues were processed routinely and dehydrated in graded concentration of xylene. They were infiltrated and embedded in molten paraffin 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, Shittu et al. (2006). The stained slide was observed under a light microscope. Sections were examined for histological changes following the method outlined by Jain (1986).

2.6 Statistical analysis

Results were expressed as mean \pm standard error of mean. Organ weights, biochemical and hematological determinations were analyzed by student's t-test. A probability level of $p < 0.05$ was considered significant.

III. Results and Discussion

3.1 Proximate analysis of the defatted seed and compounded feed

The proximate composition of defatted *B. aegyptiaca* as presented in Table 2 showed a very high protein content (49.32 ± 0.13 %). It is lower than the protein content of ground nut cake (50.90 ± 1.27 %) as reported by Fekria et al. (2012) and 78.70 ± 0.40 for defatted *Cucumeropsis mannii* as reported by Eunice et al. (2012), but higher than soy bean meal (44.03%) and wheat bran (14.98 %) as reported by Ahmed et al. (2009). It could be also seen as rich in crude fibre (12.86 %) and carbohydrate (23.91 ± 0.15 %). This is an indication that *B. aegyptiaca* cake is a good source of roughage in animal feeds. The ash and moisture content are quite low.

There was an increase in the crude protein and crude fibre (25.97 ± 0.13 % and 13.95 ± 0.06 %) and a lower carbohydrate values (25.50 ± 0.01 %) in the experimental diet when compared to those obtained in the control diet (13.63 ± 0.04 %, 7.95 ± 0.07 % and 36.96 ± 0.47 %) respectively. Interestingly, protein content is higher while other parameters are lower in the seed cake than in experimental groups. This result is comparable to previously reported work on *B. aegyptiaca* seed cake (Ajayi et al., 2014).

3.2 Phytochemical analysis of defatted Balaniteaegyptiaca

The phytochemical properties of *B. aegyptiaca* cake were screened and the result showed that it contains alkaloids, saponins and flavonoids in a high concentration (Table 3). These phytochemicals exhibit diverse pharmacological and biochemical actions and would have contributed to the earlier report of the medicinal properties of the plant. The results obtained are similar to those reported by (Chothani & Vaghasiya, 2011; Samuel et al., 1997). This result obtained is comparable to the reported phytochemical components which indicate the presence of alkaloids and flavonoids in *Telfairia occidentalis* (Ekpenyong et al., 2012).

3.3 Mineral composition of B. aegyptiaca seed cake

The result of the mineral elements indicates that *B. aegyptiaca* seed cake contained significant amount of important minerals (Table 3). The K concentration (4940.00 mg/kg) was the highest, Fe (30.00 mg/kg), Zn (28.00 mg/kg), Mn (17.60 mg/kg) and Cu (12.00 mg/kg). These minerals in the diet are generally required for normal growth, activities of muscles and skeletal development, cellular activity and oxygen transport, chemical reaction in the body and intestinal absorption, fluid balance and nerve transmission, as well as the regulation of acid-base balance (Ogbe and Affiku, 2011). The values obtained followed the same trend as in Ajayi et al. (2014).

3.4 Feed intake and body weight changes

Feed consumption and the change in body weight of test and control rats are shown in fig 1. There was a gradual increase in the quantity of feed consumed by rats in the two groups throughout the feeding trial that lasted for eight weeks. It was observed that there was no significant difference in the quantity of the experimental feed as well as the control feed consumed by the rats with the exception of the fifth week alone. There was steady increment in the body weight of the rats during the experimental period and no significant

differences were noted within and across the groups. From this, it could be seen clearly that the experimental diet was consumed more than the control diet.

3.5 Organ weights

There was no significant difference in the weight of the liver, kidney, heart and spleen of the control and experimental rats (Fig 2). An average Liver weight of 4.74 ± 0.63 g was noted for control rats while 4.51 ± 0.60 g was noted for the test rats. This result obtained is similar to the report given by Vishnu et al. (2010) where the control group was 4.1 ± 0.26 g and the test group ranged from 3.94 ± 0.94 g to 4.01 ± 0.31 g. On the whole, there was no significant difference between the weight of the organs of the test rats and those of the control ($p \leq 0.05$).

3.6 Haematological and biochemical parameters

Table 4 shows the result of haematological analysis of defatted *B. aegyptiaca* seed on rats. The parameters obtained for rats in group A and group B were comparable. WBC concentration in group A (6685.71 ± 1715.81) is significantly different to that of Group B (7077.42 ± 1210.66). A high value ranging from 8050 ± 330.9 to 8633 ± 513.3 was obtained by Ajayi et al. (2007). Since the haematological parameters obtained from rats fed with partially supplemented *B. aegyptiaca* seed cake compared favorably with the values obtained with the rats fed with normal feed (control group), it indicates that *B. aegyptiaca* seed cake has no adverse effect on the blood of the test rats. All serum biochemical parameters did not differ significantly from each other. The values of total protein content of 6.6 ± 0.3 and 6.77 ± 0.24 obtained respectively for the two groups (the control and BASC) are lower than those ranging from 8.81 ± 0.70 to 9.12 ± 0.30 as reported by Wilson et al. (2009) on rat fed with crude *B. aegyptiaca* seed oil and Ajayi et al. (2014) on the rat fed with wheat totally replaced with *B. aegyptiaca*. This result indicates that rats in the two groups were not anaemic, had no infection and similar to those reported for healthy rats and related murine species (Ogunsanmi et al., 2002).

3.7 Physical Appearance

Generally, the rats maintained fine and smooth hairs all through. Both the control and experimental groups have the normal rat smell. It was important to note that no mortality was recorded throughout the period of this work.

3.8 Effect of total replacement of corn bran with *B. aegyptiaca* cake on rats' body weight

The effect of total replacement of corn bran with *B. aegyptiaca* cake on rats' body weight is shown in Fig 1. It was noted that the average final weight per rat is 121.42 ± 3.72 g and 117.86 ± 3.92 g respectively for the control and experimental groups. It was also found that the test rat had 63.46 % weight gain while the control rat has 58.82 % weight gain.

3.9 Histopathology

Histopathology result revealed no major complications and no significant differences on the kidney, heart and brain organs of all the rats in both groups while there is a periportal cellular infiltration by mononuclear cell in the liver of experimental rats (table 5). The histopathological results obtained from this study for liver and heart is similar to what was reported on rats fed with wheat totally replaced with *B. aegyptiaca* seed cake (Ajayi et al., 2014).

IV. Conclusion

Based on the results obtained from this study, it is possible that *B. aegyptiaca* cake could successfully be used to totally replace corn bran in rat diet with other supplements. The high value of protein content suggests that *B. aegyptiaca* seed cake can be utilized as roughage in feed for livestock as well as in food supplements. The phytochemical analyses showed the presence of tannins, flavonoids and alkaloids which ascertain that the seed cake contains some major compounds that have remarkable biological activities, and might be helpful in preventing various diseases. The seed cake contains mineral elements that are all useful in making the body strong.

B. aegyptiaca seed cake did not produce any significant change on haematological and biochemical parameters as well as in the heart and liver of the rats in both groups. The seed cake might be a good additive in feed supplement.

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Table 1: Composition of compounded feed used for the experiment

Ingredients	Percentage (%)	Amount (g)	Control	Corn bran/BASC
Maize	40	2800	2800	2800
Soy beans	18.25	1277.5	1277.5	1277.5
Ground nut cake (DBSC)	14.6	991.2	991.2	991.2
Corn bran	7.08	495.6	495.6	495.6
Palm kernel cake	7.08	495.6	495.6	495.6
Wheat	7.08	495.6	495.6	495.6
Bone	3.30	231	231	231
Oyster shell	2.25	157.5	157.5	157.5
Salt	0.80	56	56	56
Total	100%	7000g	7000g	7000g

Table 2: Proximate Composition of *B. aegyptiaca* seed cake and compounded diets

	<i>B. aegyptiaca</i> seed cake	Control	Corn bran/BASC
Moisture content	8.45±0.03	10.12±0.16	10.51±0.03
Protein content	49.32±0.13	13.63±0.04	25.97±0.13
Crude fat	13.92±0.04	18.24±0.96	14.16±0.14
Crude fibre	12.89±0.02	7.95±0.07	13.95±0.06
Ash content	4.40±0.02	13.09±0.17	9.85±0.08
Carbohydrate	23.91±0.15	36.97±0.47	39.50±0.01
Energy(KJ/100g)	1760.00±1.28	1537.35±15.83	1637.09±0.86

Values expressed as means±SD for seven rats per group

Corn bran/BASC: Corn bran totally replaced with *B. aegyptiaca* seed cake

Table 3: Phytochemical profile and mineral composition of *B. aegyptiaca* seed cake

Phytochemical Screening	BASC
PARAMETERS	DESIGNATION
Saponins	Present
Tannins	Present
Flavonoids	Present
Steroids	Present
Cardiac Glycoside	Absent
Alkaloids	Present
Reducing Sugar	Absent
Phenol	Absent
Glycosides	Absent
Resins	Absent
Mineral composition	Concentration (mg/kg)
Na	230.00
K	4940.00
Ca	3390.00
Cu	12.00
Fe	30.00
Zn	28.70
Mg	1930.00
Mn	17.60

Table 4: Haematological and biochemical studies of rats fed with control feed and *B. aegyptiaca* seed cake

Haematological analysis*	Control group	Corn bran/BASC
PVC (%)	40.29±1.37 ^t	43.14±0.69 ^t
MCHC (%)	13.51±0.83 ^t	14.36±0.19 ^t
MCH (%)	6.48±0.50 ^t	7.41±0.11 ^t
MCV (%)	6685.71±1715.81 ^t	7077.42±1200.66 ^t
Hb (mg/dl)	62.17±2.52 ^t	58.21±4.63 ^t
RBC (10 ⁶ /ml)	20.85±0.79 ^t	19.38±0.35 ^t
WBC (10 ³ /ml)	33.53±1.11 ^t	33.28±0.26 ^t
Lymphocyte (%)	70.14±6.49 ^t	71.71±5.68 ^t
Neutrophil (%)	28.00±6.53 ^t	24.29±5.87 ^t
Monocyte (%)	1.71±0.56 ^t	1.86±0.95 ^t
Eosinophil (%)	2.14±0.50 ^t	2.14±1.07 ^t
Platelets(cells/cu.mm)x10 ⁶	125285.71±5831.51	107142.86±31845.60
Biochemical analysis*		
TP (g/dl)	6.6±0.30 ^t	6.77± 0.24 ^t
ALB (g/dl)	3.63±0.26 ^t	3.9± 0.1 ^t
GLB (g/dl)	2.86±0.15 ^t	2.81±0.14 ^t
AL/GLB	1.17±0.06 ^t	1.33±0.47 ^t
AST (g/l)	39.00±1.00 ^t	41.33±0.58
ALT (g/l)	26.67±0.58 ^t	29.66±0.58

ALP (g/l)	81.33±3.51 ^t	81.33±6.02 ^t
Urea	16.00±1.00 ^t	16.00±0.00 ^t
Creatinine	0.67±0.01 ^t	0.60±0.00 ^t

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

Hb = Haemoglobin, concentration (g %); PCV = Packed cellvolume (%); RBC = Red Blood

Cell Counts; WBC = White Blood cellcount (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

Table 5: Pathology examination of rat tissues

Tissue	Control	Corn bran/BASC
Kidney	No visible Lesion	No visible lesion
Heart	No visible Lesion	No visible Lesion
Liver	No visible lesion	There is infiltration by mono-cellular cell

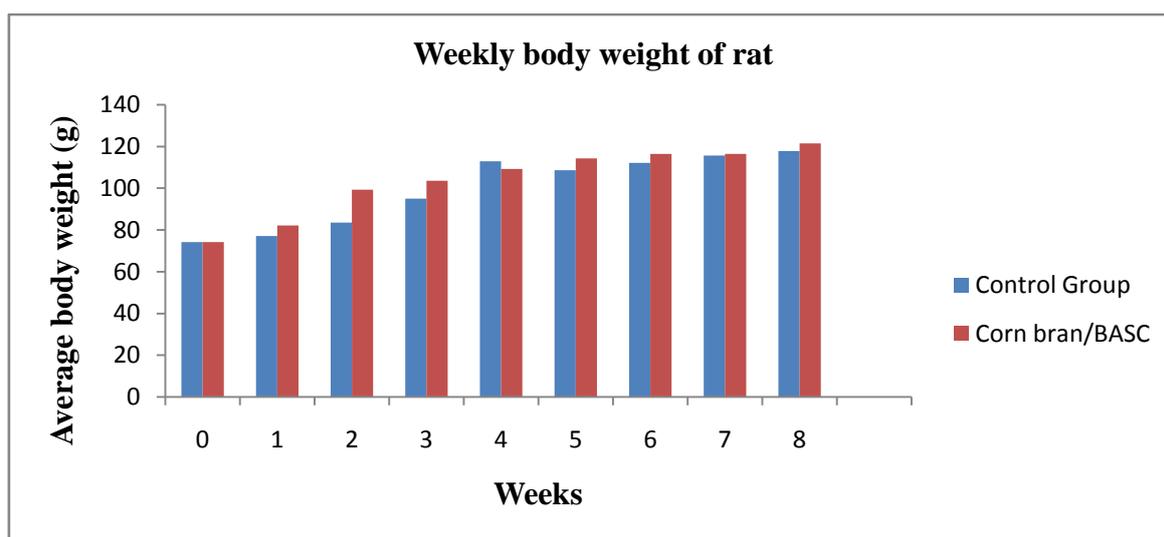


Fig 1: A chart showing the weekly body weight of the rats fed with corn bran totally replaced with *B. aegyptiaca* seed cake and the control.

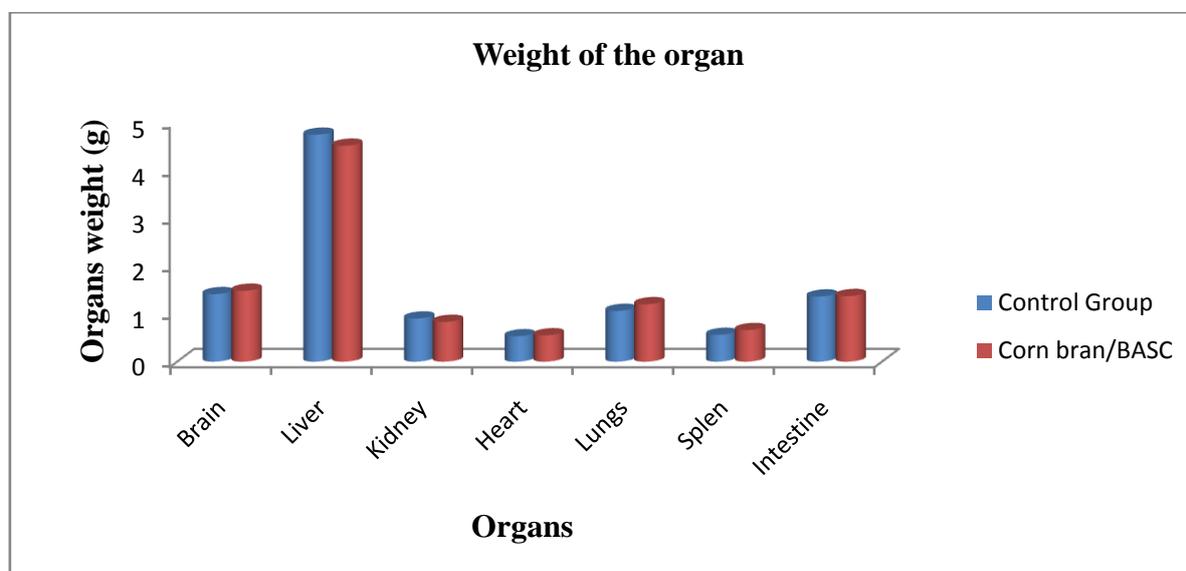


Fig 2: A chart showing the weight of the organs/tissues of the rats fed with corn bran totally replaced with *B. aegyptiaca* seed cake and the control.

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