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Histoarchitecture of albino rat's liver

Comparative Effects of Processing on Nutritional Compositions of Chickpea (*Cicer arietinum*) Flour, and Resultant Implications on Histology of Albino Rats

Rasheed Tunde Lawal[1,*], Gboyega Oluwaseun Oyeleke[2], Wakili Tope Aborisade[3], Joshua Oluremi Odedeji[4], Abdul Dimeji Ishola[5] and Abdulwasiu Temitope Olayiwola[6]

[1]Department of Biological Sciences, Faculty of Sciences, University of Ilesa, P.M.B. 5089, Ilesa Osun State, Nigeria.
[2]Department of Science Laboratory Technology, Faculty of Science, P.M.B.301, Iree, Osun State Polytechnic, Iree, Osun State, Nigeria.
[3]Department of Microbiology, Faculty of Pure and Applied Science, Kwara State University, Malete, P.M.B. 1530, Ilorin, Kwara State, Nigeria.
[4]Department of Food Science and Technology, Faculty of Science, University of Medical Sciences Ondo City, P.M.B. 536, Ondo State, Nigeria.
[5]Department of Industrial Chemistry, Faculty of Sciences, University of Ilesa, P.M.B. 5089, Ilesa Osun State, Nigeria.
[6]Department of Biological Sciences, Ramon Adedoyin College of Natural and Applied Sciences, Oduduwa University Ipetumodu, P.M.B. 5533, Ile-Ife, Osun State, Nigeria.

*Corresponding author:
Rasheed Tunde Lawal
Department of Biological Sciences,
Faculty of Sciences,
University of Ilesa,
P.M.B. 5089,
Ilesa Osun State,
Nigeria.
Email: lawalrtunde@gmail.com

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ABSTRACT

This research examined the effects of autoclaved, cooked, and uncooked chickpea flour on animals with uncooked chickpea flour, commercial feed, and basal diets serving as comparisons and histological examination. The seeds of *Cicer arietinum* were sorted, washed, and sun-dried. The 30 kg of chickpeas were milled into powder using a blender. Three (3) 700 g portions of the powder were separated and placed in a sterile container. The fraction of chickpea flour was autoclaved for 15 minutes and cooked for 1 hour. The mineral and vitamin analysis of autoclaved, cooked, and uncooked chickpea flour was carried out using standard analytical methods. Standard procedures were used to prepare the animal tissue. The results showed that odium ranges from 15.90 to 19.19 mg/kg, potassium from 24.40 to 26.10 mg/kg, magnesium from 2.72 to 4.63 mg/kg, calcium from 3.97 to 5.33 mg/kg, and iron from 0.19 to 0.23 mg/kg in terms of mineral composition, while vitamin analysis ranges from vitamin A, 465.71 $\pm$9.27 to 1146.75 $\pm$5.63 Unit/g, vitamin B, 0.02 $\pm$ 0.01, to 0.06 $\pm$ 0.00 mg/g, and vitamin C, 0.15 $\pm$ 0.00 to 0.25 $\pm$ 0.00 mg/g. Raw chickpea flour's mineral and vitamin content was lower than that of cooked and autoclaved chickpea flour. Histological examination reveals that the experimental animals given cooked chickpea flour did not experience any changes in liver histoarchitecture. From this study, it could be concluded that animal consumption of cooked chickpeas could be safe, as it had no negative effects on their liver histology.

INTRODUCTION

A major grain legume grown for its edible seeds throughout the Mediterranean Basin, Asia, and Australia is the chickpea (*Cicer arietinum* L.). The plant becomes branched quickly and grows to a height of 20 to 60 cm, sometimes even 1 m. Its many lateral secondary roots probe the upper layers (15–30 cm) of the soil, and it has a deep taproot that reaches down to 2 m [1]. The simple, branching, straight, or curved stems are hairy. Five-centimeters-long leaves with ten to twenty sessile, oblong to elliptical leaflets. Usually, papilionaceous and solitary chickpea blossoms come in white, pink, purple, or blue. Two or three seeds are found in the rectangular, pubescent pod. The seeds range in size (5–10 mm) from spherical to angular and from creamy-white to black [1]. Regarding fat, chickpeas exhibited a high apparent digestibility coefficient. Fish pellets with chickpeas added have been shown to be harder without changing the water activity inside the pellets [2]. With so many vital vitamins and minerals, garbanzo beans

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can be very beneficial to bone health. Many of these minerals may help to increase bone mineral density and may even shield against age-related diseases like osteoporosis [1]. One of the most important organs in the human body, the liver regulates a number of biochemical processes [3]. A cool-season grain legume, chickpeas can bear high temperatures when they are maturing and fruiting. Though the earliest vestiges date back to about 7000 BC, they are believed to have originated in South-East Anatolia and nearby Syria and Iran [4].

Hepatic architecture of the extract-treated animals was nearly normal with fewer pathological alterations, according to histopathological analysis of liver tissues. The present data points to a very low acute toxicity and a great safety profile for the chickpea extract [5]. This work aims to ascertain the vitamin, mineral, and histopathological compositions of Wistar rats.

MATERIALS AND METHODS

Sample collections

Cicer arietinum seeds utilized for this research were acquired in August 2022 from Ojaja Market, Ife Central Local Government, Osun State, Nigeria.

Preparation of a *Cicer arietinum* seed

Cicer arietinum seeds were separated, rinsed, and dried in the sun. They were milled using a blender. The crushed *Cicer arietinum* seed flour was split into three parts of 700 g each and stored in a plastic container until it was utilized. 700 g each of chickpea flour was placed in six beakers and covered with aluminum foil. While the other three were cooked in a pot for an hour, the other three were placed in an autoclave and heated to 121°C for fifteen minutes. While in the autoclave, cooked and uncooked chickpea flour was used to feed experimental animals [6, 7].

Mineral analysis

The mineral analysis was measured using UV-Visible spectrophotometer after forming ammonium vanadate molybdate complex at 436 nm using known methods of [8], the nutritionally critical elements Na, Ca, Mg, and K were determined using atomic absorption spectrophotometer (A.A.S.).

Determination of vitamin content of autoclave, cooked, and raw chickpea flour

The determination of vitamin content carried out on the chickpea flour included the following: The vitamins in the fresh and dried samples were determined using the methods of association of vitamin chemists [9]. Vitamins A and B were determined using the spectrophotometer method described by [8]. Vitamin B (Niacin, thiamin, and Riboflavin) was determined using a flame photometer, while vitamin C was estimated using the 24 dinitrophenol hydrazine methods described in [9].

Growth performance of Wistar albino rats and feeding regime

The College of Health Science Animal Breeding Centre at Obafemi Awolowo University, Ile-Ife, Nigeria, supplied fifty (50) weaning albino rats. After being weighed, albino rats were arranged at random into metabolic cells. Age and weight vary, respectively, from 4 weeks and 44.23 g to 45.78 g. In their stationary digestive compartments, albino rats were fed freely from a small plastic bottle and a feeding plate. After that, the animals were weighed again and divided into groups of eight or six rats. One to ten (1–5) weights of the ten (10) experimental animals per group were not appreciably different at the start of the study. For twenty-eight days, groups one through five were fed experimental diets. They received water through a plastic bottle fastened to the cage, and the measured amount of each experimental meal that was fed to them was ad libitum in the feeding dish. After the rats were dissected, the life weight of each animal and several organs (liver, muscle, and kidney) were measured [10].

Histopathological analysis

We processed tissue using the techniques of [11, 12]. The liver sections spent ten days fixed in a 10% saline solution. Following fixation, the tissues underwent a 30-minute rising grade of alcohol—70%, 80%, 90%, and 100% alcohol—to dry them. To allow wax impregnation, the tissues were immersed in xylene overnight. Wax was stored in a thermometrically controlled, electrically heated oven set between 56 and 57 degrees Celsius. The tissues were submerged in molten wax for half an hour. Following impregnation, the tissues were covered with hardened wax.

The mold's foundation was a metal plate set on which was a Paris of L-shaped pieces of rust-proof metal. Freshly melted wax (combined with pure xylene) was nearly filling the mold. Carefully selected using warm forceps, the tissues were positioned to press the desired surface up against the partially solidified wax at the mold's base. The blocks were taken out and set firmly on wood blocks that were held in place by molten metal, which accelerated the solidification process. To the microtome were clamped the woodblocks. Five microns was the setting on the rotary microtome at which sectioning was completed. The resulting ribbons were carefully removed with Pyrex, floated in a water bath, then picked up with albumenized slides and let to drain for half an hour.

The slides were drained, and then three minutes were spent on hot plates. To remove the wax, areas on the slides were run through two cycles of absolute xylene, each lasting five minutes. Cutting alcohol grades from 90% to 70% for five minutes each will give you five minutes to drink water and stay hydrated. 20 minutes of hematoxylin, twice dipping it in 1% acid alcohol, and 15 minutes of water (blueing in water). Following mounting with Canada balsam as the mounting, coverslips were put on the slides and allowed to cure for around three days. The ready-made slides were then examined under a Livingstone Medical, England-made Leitz microscope model X52-107BN, and photomicrographs of them were shot using a Fuji camera and reviewed.

Ethical consideration of the study

The University of Ilesa Research Council and Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, and those institutions' Joint Ethical Review Committee approved this work.

Statistical analysis of data obtained in the study

Three replications and a fully randomized design were used to perform the trials. All data were first subjected to variance analysis (ANOVA), which was followed by checks for normality. L.S.D. and Turkish tests were then carried out to identify any significant changes between the treatments ($p<0.05$). The statistical analyses were performed using IBM SPSS 24.0 software (SPSS Science, Chicago, IL, U.S.A.).

RESULTS

Mineral composition of chickpea flour

The sodium content of autoclaving chickpea flour was 15.90 $\pm$ 0.01 mg/kg while cooking, and the raw chickpea flour value ranged from 17.40 $\pm$ 0.00 to 19.19 $\pm$ 0.01 mg/kg. The potassium content of autoclaving chickpea flour was 24.40 ± 0.000 mg/kg, while raw and cooked chickpea flour ranged from 20.50 ± 0.01 to 26± 10±0.01 mg/kg. The magnesium content of autoclaving, cooking, and raw chickpea flour was as follows: 2.72 ± 0.00 mg/kg, 4.63 ± 0.00 mg/kg, and 3.09 ± 0.01. The calcium content of autoclaving chickpea flour was 5.33 ± 0.00 mg/kg while cooking, and raw chickpea flour ranges from 3.97 ± 0.09 to 5.03 ± 0.00 mg/kg. The iron content had a lower value compared to the other mineral content, while the values are as follows: 0.23 ± 0.01, 0.23 ± 0.03, and 0.19 ± 0.01 mg/kg (**Table 1**).

Table 1. Mineral composition of autoclaving, cooking and raw chickpea flour (mg/kg).

Parameter	ACP	CCP	RCP
Na	15.90 ± 0.01^a	19.19 ± 0.01^a	17.40 ± 0.00^c
K	24.40 ± 0.00^a	26.10 ± 0.01^b	20.50 ± 0.01^b
Mg	2.72 ± 0.00^a	4.63 ± 0.00^a	3.09 ± 0.01^c
Ca	5.33 ± 0.00^a	3.97 ± 0.09^b	5.03 ± 0.00^a
Fe	0.23 ± 0.01^a	0.23 ± 0.03^b	0.19 ± 0.01^a

Values are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different (p < 0.05). Key: A.C.P. – Autoclaving chickpea flour, C.C.P. – Cooking chickpea flour and R.C.P. – Raw chickpea flour.

Vitamin analysis of chickpea flour

The vitamin A and B content in autoclaving chickpea flour had the lowest values of 465.71± 9.27 unit/g and 0.02 ±0.01 mg/g, while vitamin C in autoclaving chickpea flour had the highest values of 0.25 ±0.00 mg/g. The raw chickpea flour had the highest values of vitamins A and B, with a value of 1146.75 ±5.63 unit/g and 0.06 ±0.00 mg/g, while the cooking chickpea flour added a value of 667.53 ±4.26 unit/g and 0.04 ±0.01 mg/g, while vitamin C had a value of 0.19 ±0.01 mg/g (**Table 2**).

Table 2. Vitamins analysis of autoclaving, cooking and raw chickpea flour.

Parameter	ACP	CCP	RCP
Vitamin A (unit/g)	465.71 ± 9.27^a	667.53 ± 4.26^b	1146.75 ± 5.63^c
Vitamin B (mg/g)	0.02 ± 0.01^a	0.04 ± 0.01^a	0.06 ± 0.00^c
Vitamin C(mg/g)	0.25 ± 0.00^a	0.19 ± 0.01^b	0.15 ± 0.00^a

Values are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different (p < 0.05). Key: A.C.P. – Autoclaving chickpea flour, C.C.P. – Cooking chickpea flour and R.C.P. – Raw chickpea flour.

Photomicrographic sections of the liver of albino rats fed with autoclave chickpea flour

Photomicrographic sections of the liver of Albino rats fed with autoclave chickpea flour and subjected to hematoxylin and eosin staining are shown in **Fig. 1**. In animals fed with autoclave chickpea flour, the histoarchitecture had a mild disruption. The cordlike linear arrangements of the hepatocytes are altered, and the sinusoids appear clogged, which may affect free flow within the sinusoids, thus impacting the functionality of the liver.

Photomicrographic sections of the liver of albino rats fed with cooked chickpea Flour

A photomicrographic section of the liver of Albino rats fed with cooked chickpea flour subjected to hematoxylin and eosin stain is shown on **Fig. 2**. There was an observation that the portal and central veins appear normal, there is no sign of disruption, the cordlike arrangements of the hepatocytes are intact, and the sinusoids are clear and free.

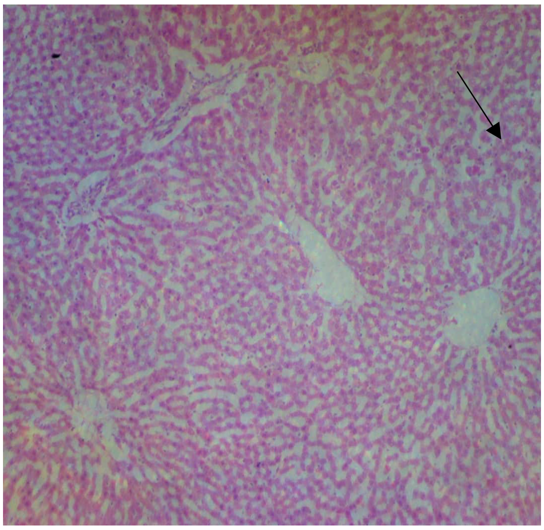

Fig. 1. Photomicrographic sections of the liver of Albino rats fed with Autoclave chickpea flour subjected to hematoxylin and eosin stain Observe: [A.C.P.] A mild disruption in the histoarchitecture is observed. The cordlike linear arrangements of the hepatocytes are altered, and the sinusoids appear clogged, which may affect free flow within the sinusoids, thus impacting on the functionality of the liver. Magnification: ×400. Key: A.C.P. - Autoclaves Chickpea.

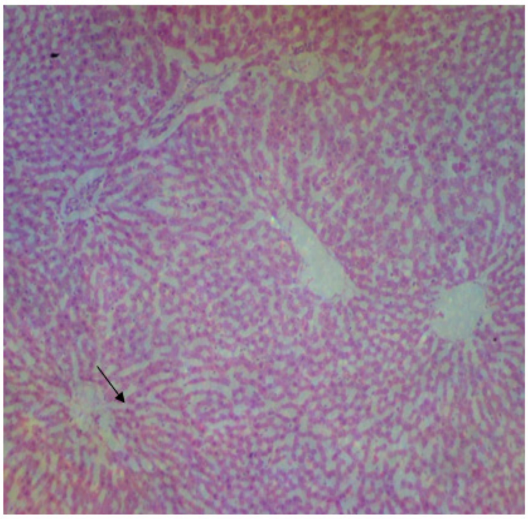

Fig. 2. Photomicrographic sections of the liver of albino rats fed with cooked chickpea flour subjected to hematoxylin and eosin stain. Observe: [C.C.P.] The portal triads and central veins appear normal; no sign of disruption is observed. The cordlike arrangements of the hepatocytes are intact, and the sinusoids are clear and free. Magnification: ×400. **Key**: C.C.P. - Cooked Chickpea

Photomicrographic sections of the liver of albino rats fed with raw chickpea flour Photomicrographic sections of the liver of albino rats fed with raw chickpea flour and subjected to heamatoxylin and eosin staining are shown on **Fig. 3.** There was an observation that histoarchitecture reveals a mild level of disruption and tissue hemorrhage; sinusoids also appear clogged.

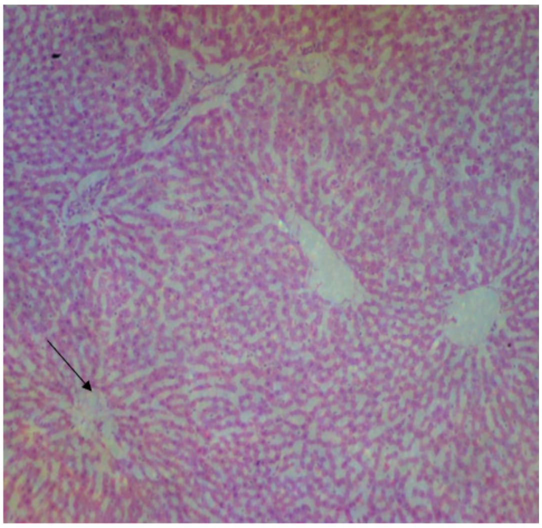

Fig. 3. Photomicrographic sections of the liver of albino rats fed with uncooked chickpea flour subjected to hematoxylin and eosin stain Observe: [R.C.P.] Histoarchitecture reveals a mild level of disruption and tissue hemorrhage. Sinusoids also appear clogged. Magnification: ×400. **Key:** R.C.P. - Raw chickpea

Photomicrographic sections of the liver of albino rats fed with commercial feed

Photomicrographic sections of the liver of albino rats fed with commercial feed were subjected to hematoxylin and eosin stain histoarchitecture, healthy hepatocyte portal triads and central veins, clear sinusoids, and very mild vascular congestion. It is shown on **Fig. 4**.

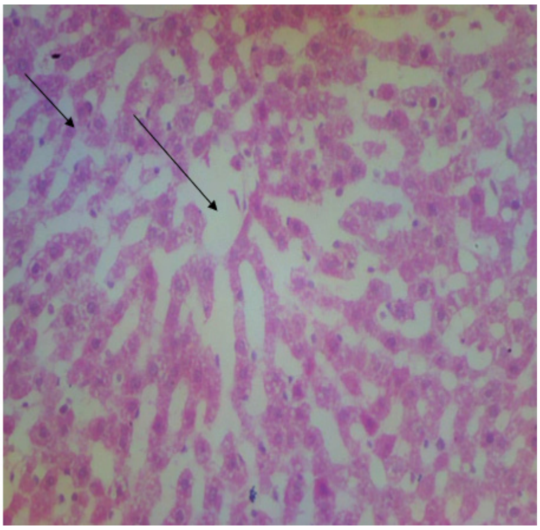

Fig. 4. Photomicrographic sections of the liver of albino rats fed with commercial feed subjected to hematoxylin and eosin stain. Observe: [C.F.] Histology reveals good histoarchitecture, healthy hepatocytes, portal triads and central veins, clear sinusoids, and very mild vascular congestion. Magnification: ×400. **Key**: C.F. – Commercial feed

Photomicrographic sections of the liver of albino rats red with a basal diet

Photomicrographic sections of the liver of Albino rats fed a basal diet subjected to hematoxylin and eosin staining are shown on **Fig. 5**. Observation was the same as in commercial feed of animals above in C.F.

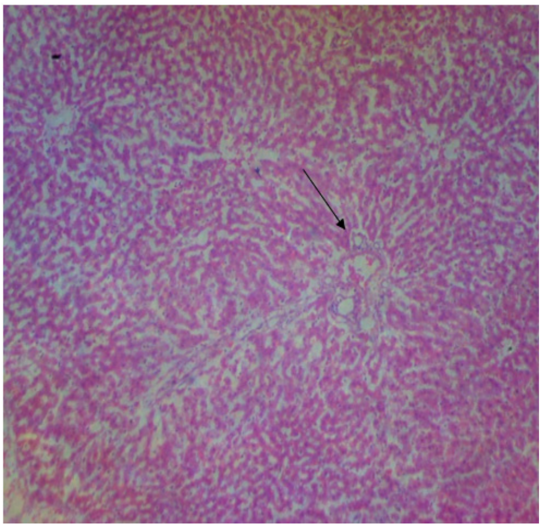

Fig. 5. Photomicrographic sections of the liver of albino rats fed with basal diet subjected to hematoxylin and eosin stain. Observe: [B.D.]: Same as in **Fig. 2** above. Magnification: ×400. **Key**: B.D. – Basal diet

DISCUSSION

The potassium content of autoclaved chickpea flour was higher than that of raw chickpea flour. The values obtained in this study were higher than the values reported by [13] for cooking chickpea flour. Potassium is essential for blood circulation and muscle contraction. The magnesium content of autoclaving chickpeas was lower than that of cooking and raw chickpea flour [14,20], which reported a loss of 22% magnesium from mature chickpeas when cooked by autoclaving. The reduction in calcium content that occurs in the cooking chickpea flour process may be due to a decrease in mineral content during cooking. The processing methods showed similar effects on calcium content in mung bean flour and with ripe and unripe plantain [15,18]. There was a lower value for autoclaving chickpea flour in vitamins A and B; the autoclaving value of chickpea flour was lower due to the combination of leaching and chemical destruction [16].

Photomicrographs of the liver of the animals fed with autoclave chickpea flour and uncooked chickpea flour showed mild disruption in the histoarchitecture, and the cordlike linear arrangements of the hepatocytes were altered, which was in agreement with the report of [17]. The animals fed with cooked chickpea flour had portal traits and central veins that appeared normal; there was no sign of disruption, and the cordlike arrangements of the hepatocytes were intact. The sinusoids were clear and free, in accordance with [12,19]. The animals fed with commercial chickpea flour revealed good histoarchitecture, healthy hepatocytes, portal traits, and clear central vein sinusoids, which were in contravention of the study of [6], who worked on a hematological and histological study of animals fed with different fermentation methods of pulverized mung bean flour (*Vigna radiata*). In conclusion, all treatments used were improved the nutrients of chickpea flour and the internal organs of experimental animals.

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