Original Research Article

Assessment of the Safety of Aqueous Extract of Corn (*Zea mays*) Husks in Wistar Albino Rats

ABSTRACT

Aims: This study was designed to investigate the possible toxic impact of aqueous extract of corn husks (HA) on Wistar albino rats.
Study design: Experimental design.
Place and Duration of Study: This study was conducted at the Federal University of Technology, Akure (FUTA) Ondo State, Nigeria.
Methodology: The extract was prepared adopting the cold extraction procedure. Acute toxicity study was carried out by administering HA at a single dose of 2000, 4000 and 8000 mg/kg body weight to the rats by oral gavage. The rats were observed for 14 days for any mortality or signs of toxicity. For sub-acute study, doses of 200, 400 and 800 mg/kg body weight were orally administered daily for 28 days. Biochemical and haematological parameters as well as histopathological studies were carried out on the rats after the in vivo experiments.
Results: Acute toxicity results indicated that the median lethal dose (LD_{50}) of HA was greater than 8000 mg/kg. There was remarkable body weight gain ($P \leq 0.05$) in both male and female rats in all the sub-acute groups and acute group treated with 8000mg/kg. In the acute and sub-acute toxicity study, slight difference was recorded between the biochemical and hematological parameters of the treated rats dosed with the extract and the control. There was a significant increase in Packed Cell Volume (PCV) of female rats and lymphocytes of male rats treated with 800 mg/mL and 400 mg/mL respectively. The total protein, glucose and urea level of male rats treated with 200 mg/mL reduced while urea level of females treated with 200 mg/mL and 400 mg/mL also reduced remarkably. However, histological data showed no significant difference.
Conclusion: In general, the extract was found to show no toxic effect on the rats and hence it is safe for potential therapeutic use.

Keywords: [Toxicity, corn husks, acute toxicity, sub-acute toxicity)

1. INTRODUCTION

Higher plants are described as good sources of drugs having contributed to the welfare and quality of life in rural and urban communities [1]. Many plants have been reported to possess certain compounds that offer therapeutic properties in the treatment of human and animal diseases even though most of these plants are yet to be explored for their curative properties [2]. It was estimated by the World Health Organization [3] that about 80% of people living in developing countries adopt traditional medicine as their major health care source. However, many plants have been reported to be detrimental to the health of humans and animals and the toxicity of many natural products from plants being used are greatly underestimated [4]. Treatments of various infectious diseases with diverse herbal preparations have been adopted by Nigerian traditional medical practitioners [5].

Corn is an annual grass plant of about 2-3m tall with a fibrous root system and an erect stalk belonging to the family Poaceae. It is scientifically known as *Zea mays* and believed to be first cultivated in North America (Mexico) but now commonly cultivated all over the world especially in Asia and Africa. The stalk is spanned from bottom to the top with long narrow leaves arranged in two vertical rows alternately on opposite sides of the stalk. The husk is the multiple leaf-like structure covering an ear of corn and part of the silk [6].*Zea mays* is widely distributed around the world and used as either foods, industrial raw material or as animal feed [7]. Itis majorly used as staple foods in Nigeria and some other developing countries. Whereas, in the developed countries more than 60 percent of it is used as animal feed [8]. Beyond its nutritive benefits, the leaves, silks, stalk, maize grain and inflorescence are also used in ethnomedicine for treatment of various illnesses [9].

Corn silks have been reported to be efficient in the treatment of urinary tract infections and other renal diseases such as chronic nephritis [10]. Sani [8] stated that corn silks have been extensively used for many decades in China as antidiabetic agent. In Nigeria, the decoctions of corn husks have been reportedly used for traditional treatment of malaria, arthritis and pains [6, 9]. In this study, the safety or otherwise of aqueous extract obtained from corn husks was investigated in Wistar albino rats.

Comment [G1]: why is the aqueous extract tested, is it empirically used in the community using this type of extract?

2. MATERIAL AND METHODS

2.1 Plant material collection and aqueous extraction

Fresh corn husks were collected from a self-cultivated farm located at Bolorunduro area of Ilesa, Osun State, Nigeria, with geographic coordinates 7.4905° N, 4.7096° E. Husks were carefully separated from the cobs and silks, washed with clean water to remove any dust and other unwanted particles. They were allowed to air dry on a clean surface and then pulverized to powder using a grinder. The cold maceration extraction method was adopted, whereby 250 g of ground husks was macerated with 2 L of distilled water for 72 hours with intermittent stirring. The mixture was sieved with a clean muslin cloth. Further filtration was carried out with No. 1Whatmann filter paper. The filtrate was then evaporated to dryness at low temperature in a hot air oven. The dried extract was weighed, kept in sterile container, labeled HA and stored in the refrigerator.

2.2 Preparation of Experimental Animals

The animals used for this study were 48 (8 weeks old) male and female Wistar albino rats with body weight of 98 – 152 g. They were obtained from animal breeding stock at Federal University of Technology Akure (FUTA) and were acclimatized to the laboratory condition at the Department of Microbiology, FUTA for one week before the commencement of the experiment. The rats were housed in plastic cages with food and water supplied ad-libitum. However, they were made to fast for 24 hours prior to the administration of the extracts.

2.3 Acute Toxicity Study

To investigate the acute toxicity of corn husks, twenty-four (24) rats comprising 12 males and 12 females were divided into four (4) groups of six (6) rats (3 males with body weight of 110 – 150 g and 3 females with 98 – 135 g body weight in different cages) for each group. A single dose of 2000, 4000 and 8000 mg/kg body weight was administered to both the male and female experimental groups while normal saline was administered in same volume to the control group using a beaded intra-gastric catheter. The animals were monitored for any changes in behaviour, clinical signs and mortality for 14 days with special attention given during the first 24 h. On the 14th day all the animals were sacrificed and were euthanized by cervical dislocation. Blood was collected and organs (kidneys and livers) were harvested for biochemical and histopathological examinations respectively [11].

2.4 Sub-acute Toxicity Study

The sub-acute toxicity study was carried out on twelve (12) male rats with body weight of 118 -152 g and 12 females with 117-133 g body weight were divided into four (4) groups of six (6) rats (3 males and 3 females in different cages) for each group. Daily doses of 200, 400 and 800 mg/kg body weight of the extract as well as normal saline were administered to the experimental and control groups respectively. The animals were monitored for any clinical changes and mortality for 28 days. All the animals were sacrificed and euthanized by cervical dislocation, blood was collected via cardiac puncture for hematological and biochemical examinations while organs were harvested for histopathological examinations [11, 12].

2.5 Haematology and Serum Biochemistry

After each experiment, all animals were sacrificed and blood samples collected were subjected to haematological and serum biochemical analysis as described by Olukunle*et al*. [13].

2.6 Histopathological Examination

The kidney and liver samples of the various groups were harvested, weighed and fixed in 10% natural buffered formalin. The organs were then dissected and stained with hematoxylin and eosin stain after which histopathological examination was performed using light microscope [14, 15].

2.7 Statistical Analysis

Results are expressed as mean ± standard deviation and subjected to Analysis of Variance (ANOVA) with level of significance documented at $P \leq 0.05$. Separation of means were performed using Duncan's new multiple range test (DNMRT) at 95 % confidence level.

3. RESULTS

3.1 Acute Toxicity study

A single dose of aqueous extract of *Zea mays* husks administered at 2000, 4000 and 8000 mg/kg body weight caused no mortality on any of the test rats throughout the 14 days study. However, the test groups administered 4000 and 8000 mg/kg passed loose stool after 10 hours of administration which resolved completely after 48 hours. There was no significant change in weight among the male and female rats treated with 2000 mg/kg and the female rats treated with 4000 mg/kg. There was a significant weight gain ($P \leq 0.05$) in male rats treated with 4000 mg/kg as well as male and female rats treated with 8000 mg/kg as shown in Table 1.

Table 1: Effects of different concentrations of aqueous extract of Corn Husks (HA)on the weight of Wistar albino rats in the acute toxicity study

Dose (mg/kg)	Gender	Pre-Treatment weight (g)	Post-treatment weight (g)	Weight gain (g)

Control	**M**	106.67 ± 4.93	112.33 ± 7.51	5.67 ± 2.57[a]
	F	111.33 ± 3.51	115.67 ± 6.11	4.33 ± 2.60[a]
2000	**M**	146.67 ± 2.08	157.67 ± 8.08	11.00 ± 6.00[a]
	F	148.67 ± 1.53	153.67 ± 6.43	5.00 ± 4.90[a]
4000	**M**	128.67 ± 1.53	144.00 ± 6.25	15.33 ± 4.72[b]
	F	128.33 ± 1.53	133.67 ± 7.23	5.33 ± 5.71[a]
8000	**M**	104.33 ± 6.03	126.00 ± 4.58	21.67 ± 1.45[c]
	F	100.67 ± 1.53	117.00 ± 11.27	16.33 ± 9.74[b]

Values are mean ± standard deviation. Values with the same superscript letter along the same column are not significantly different ($P \leq 0.05$).Key: M = Male, F = Female

3.1.1 Serum Biochemical analysis

Single oral dose of aqueous extract of corn husk administered to the test rats led to the significant decrease ($P \leq 0.05$) in the cholesterol, triglycerides, Low-density Lipoproteins and urea level of female rats treated with 8000 mg/kg. The cholesterol, calcium and albumin/globulin ratio level of male rats increased in group treated with 8000 mg/kg while globulin and urea level decreased. Female rats treated with 2000 mg/kg and male treated with 4000 mg/kg had a significant decrease in Aspartate Aminotransferase and triglycerides level respectively. The results of the serum biochemical analysis are shown in Table 2 & 3.

Comment [G2]: Why these parameters increased when treated with HA dose 8000 mg?kg?

Table 2: Effects of HA on Cholesterol Level of Albino Rats in the Acute Toxicity Study

Parameters	Gender	Control	2000 mg/kg	4000 mg/kg	8000 mg/kg
Total Cholesterol	Male	93.41 ± 1.07[a]	89.05 ± 5.75[a]	93.94 ± 4.52[a]	103.47 ± 5.58[b]
(mg/dL)	Female	85.89 ± 2.24[a]	82.76 ± 3.75[a]	91.55 ± 2.70[a]	63.96 ± 3.47[e]
HDL (mg/dL)	Male	38.97 ± 1.42[a]	39.34 ± 2.19[a]	34.87 ± 1.82[a]	43.16 ± 3.48[a]
	Female	28.23 ± 1.84[a]	28.27 ± 1.29[a]	28.58 ± 2.47[a]	24.89 ± 0.88[a]
Triglycerides	Male	40.72 ± 1.14[a]	39.34 ± 2.19[a]	34.87 ± 1.82[c]	43.16 ± 3.48[a]
(mg/dL)	Female	30.86 ± 0.56[a]	28.27 ± 1.29[a]	28.58 ± 2.47[a]	24.89 ± 0.88[c]
LDL (mg/dL)	Male	46.29 ± 2.66[a]	41.68 ± 6.60[a]	51.61 ± 2.17[a]	51.76 ± 5.07[a]
	Female	51.48 ± 3.79[a]	48.20 ± 3.22[a]	56.93 ± 2.85[a]	32.64 ± 4.10[d]

Values are mean ± standard deviation. Values with different superscripts along the same column for each parameter are significantly different ($P \leq 0.05$). HDL: High-densityLipoproteins, LDL: Low-density Lipoproteins.

Table 3: Effects of HA on other serum biochemical parameters of Albino Rats for Acute toxicity Study

Parameters	Gender	Control	2000 mg/kg	4000 mg/kg	8000 mg/kg
Sodium (mmol/L)	Male	40.28 ± 1.64[a]	39.87 ± 1.59[a]	40.50 ± 1.29[a]	40.46 ± 0.62[a]
	Female	34.56 ± 1.93[a]	34.09 ± 2.84[a]	34.47 ± 1.75[a]	34.13 ± 1.42[a]
Calcium	Male	0.56 ± 0.03[a]	0.57 ± 0.01[a]	0.55 ± 0.02[a]	0.62 ± 0.05[b]

(mmol/L)	Female	0.53 ± 0.01[a]	0.52 ± 0.01[a]	0.53 ± 0.01[a]	0.53 ± 0.01[a]
Total Protein	Male	319.01 ± 27.82[a]	337.48 ± 19.43[a]	355.16 ± 44.43[a]	261.97 ± 54.87[a]
(g/L)	Female	364.59 ± 28.19[a]	322.73 ± 29.23[a]	364.22 ± 5.57[a]	410.77 ± 16.92[a]
Albumin (g/L)	Male	125.18 ± 12.20[a]	126.33 ± 7.45[a]	124.24 ± 5.92[a]	121.83 ± 29.42[a]
	Female	103.12 ± 7.01[a]	103.57 ± 4.94[a]	100.21 ± 7.93[a]	77.58 ± 10.85[a]
Globulin (g/L)	Male	276.63 ± 40.02[a]	229.45 ± 54.17[a]	302.38 ± 62.38[a]	101.96 ± 26.42[d]
	Female	328.75 ± 28.01[a]	311.83 ± 23.44[a]	278.43 ± 21.52[a]	350.14 ± 42.80[a]
A/G ratio	Male	0.456 ± 0.04[a]	0.57 ± 0.10[a]	0.42 ± 0.09[a]	1.30 ± 0.60[c]
	Female	0.31 ± 0.03[a]	0.33 ± 0.04[a]	0.36 ± 0.04[a]	0.23 ± 0.06[a]
Glucose (mg/dL)	Male	127.69 ± 1.22[a]	117.51 ± 6.73[a]	118.83 ± 8.11[a]	118.31 ± 15.89[a]
	Female	91.10 ± 5.01[a]	86.53 ± 4.16[a]	98.95 ± 18.92[a]	66.58 ± 41.73[a]
ALT (U/L)	Male	86.27 ± 6.10[a]	81.07 ± 7.49[a]	80.83 ± 5.57[a]	70.54 ± 2.17[a]
	Female	64.79 ± 7.31[a]	64.59 ± 4.60[a]	58.96 ± 2.76[a]	46.93 ± 24.24[a]
AST (U/L)	Male	454.81 ± 51.31[a]	355.32 ± 51.86[a]	462.13 ± 41.49[a]	487.87 ± 27.63[a]
	Female	467.45 ± 39.55[a]	347.94 ± 54.70[b]	403.17 ± 80.06[a]	569.58 ± 39.98[a]
Urea (mg/dL)	Male	41.30 ± 5.66[a]	37.30 ± 6.50[a]	42.76 ± 3.53[a]	19.19 ± 4.83[d]
	Female	43.26 ± 2.59[a]	39.77 ± 1.70[a]	36.69 ± 5.81[a]	22.72 ± 7.32[d]

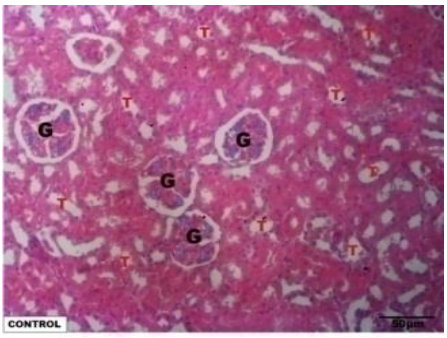
Values are mean ± standard deviation. Values with different superscripts along the same column for each parameter are significantly different ($P \leq 0.05$). A/G ratio: Albumin/Globulin ratio, ALT: Alanine Transaminase, AST: Aspartate Aminotransferase.

3.1.2 Histopathology

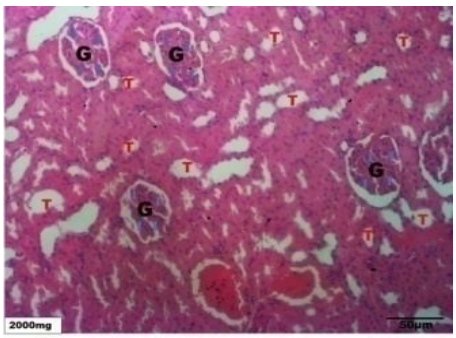
Histopathological examination of kidneys of the rats treated with 2000 mg/kg, 4000 mg/kg and 8000 mg/kg showed normal histology. The microscopic structure of the kidney section showed complete regular glomeruli, clear capillary network, the renal tubules had clear outline, the surface of the brush was clear, and the epithelial cells were arranged regularly in both the male and female treated groups and the control (Figure 1).

A

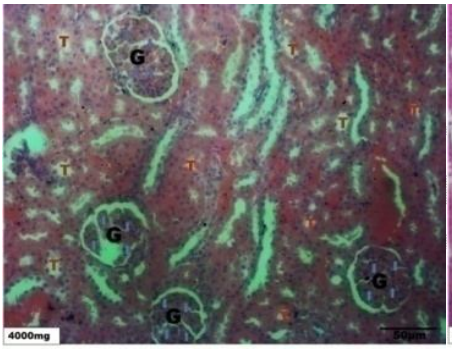
Control



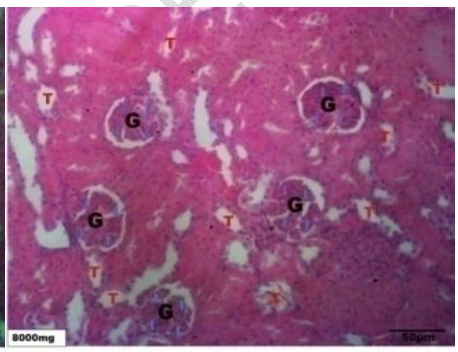
2000 mg/kg



4000 mg/kg

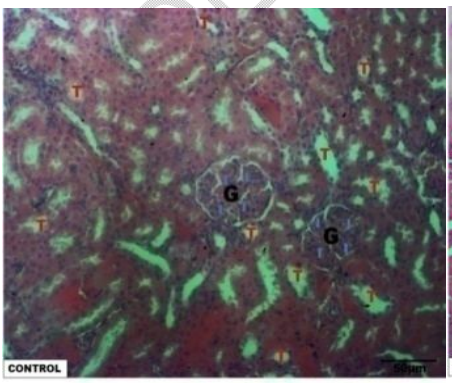


8000 mg/kg

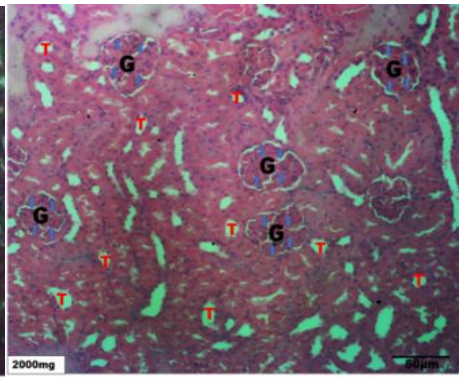


B

Control



2000 mg/kg



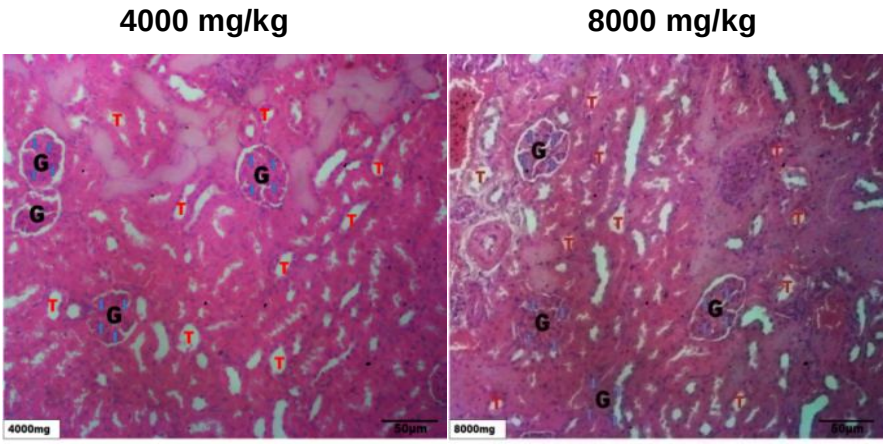
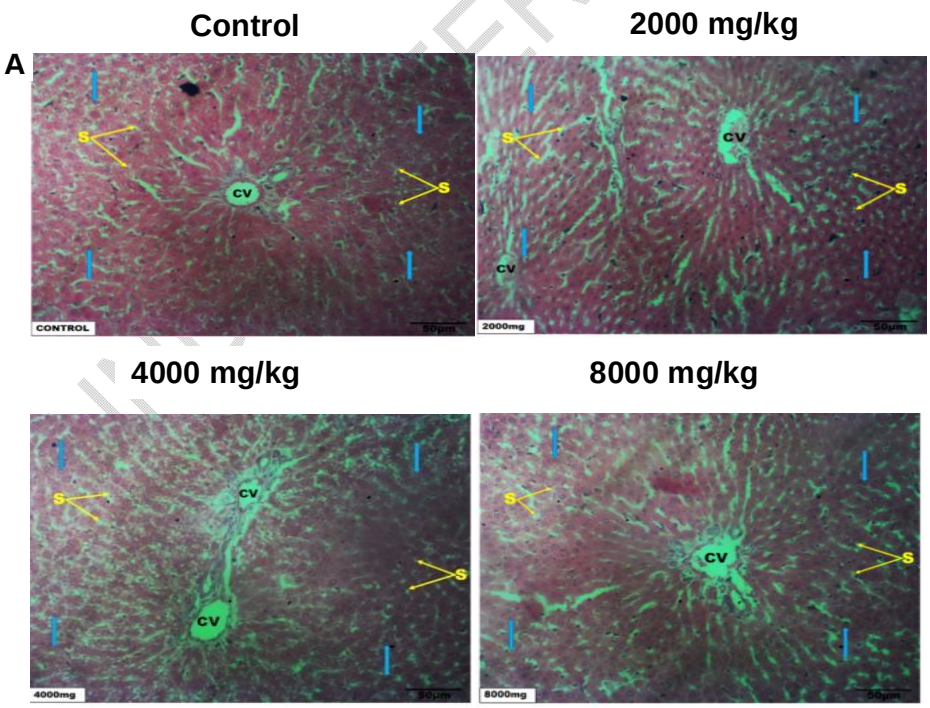


Figure 1: Photomicrographs from Kidneys of male (A) and female (B) rats in the acute groups treated with 2000, 4000 and 8000 mg/Kg of HA. G: Glomerulus; T: Renal tubules; Arrows: Renal capillaries. (Stains: Heamatoxylin& Eosin. Mg: x100)

Comment [G3]: Please correct the writing of Heamatoxylin, supposed to be: Haematoxylin

The histology of the liver sections from the treated and control groups revealed normal histo-architecture of the liver tissues characterized by normal and organized layer of hepatic tissues. Sinusoids symmetrically arranged with visible central vein surrounded by hepatocytes with polyhedral shape. (Figure 2)



B

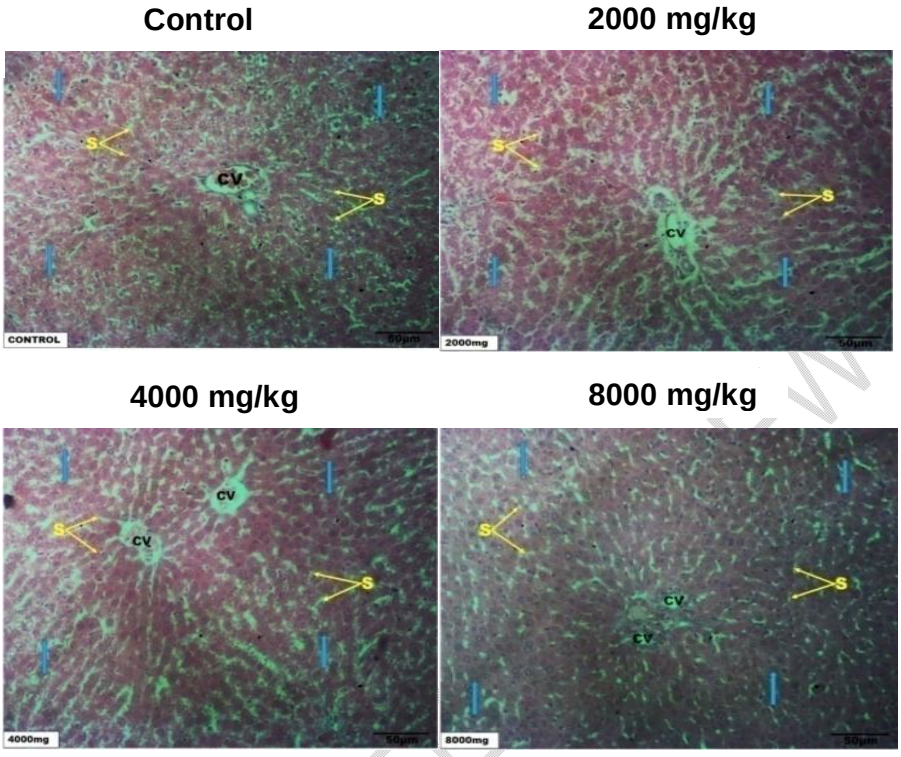


Figure 2: Photomicrographs from Livers of male (A) and female (B) rats in the acute group treated with 2000, 4000 and 8000 mg/Kg of HA. CV: Central vein; S: Sinusoids; Arrows: Hepatocytes. (Stains: Heamatoxylin& Eosin. Mg: x100)

3.2 Sub-Acute Toxicity study

The single daily dose of aqueous extract of corn husks showed remarkable weight gain on both the male and female rats treated with 200 mg/kg and 400 mg/kg body weight. The extract (800 mg/kg body weight) increased the weight of rats in this group but was only significant on the male rats (Table 4). There was no change in the behaviour and physiological characteristics of the rats throughout this study.

Table 4: Effects of different concentrations of aqueous extract of Corn Husks (HA)on the weights of Wistar Albino Rat for the Sub-Acute toxicity study

Dose (mg/kg)	Gender	Pre-Treatment weight (g)	Post treatment weight(g)	Weight gain (g)
Control	**M**	125.33 ± 4.04	153.66 ± 10.12	28.33 ± 6.07^{a}
	F	125.67 ± 6.11	135.00 ± 5.57	9.33 ± 0.54^{a}
200	**M**	150.00 ± 2.00	197.67 ± 3.06	47.67 ± 1.06^{b}
	F	124.00 ± 2.00	167.67 ± 2.52	43.67 ± 0.52^{b}
400	**M**	139.67 ± 1.53	187.00 ± 1.73	47.33 ± 0.20^{b}
	F	130.67 ± 2.52	161.67 ± 3.06	31.00 ± 0.54^{c}
800	**M**	120.00 ± 2.00	165.00 ± 4.36	45.00 ± 2.36^{b}
	F	118.67 ± 2.08	147.00 ± 11.27	28.33 ± 9.19^{a}

Values are mean ± standard deviation. Values with different superscripts along the same column for each parameter are significantly different (P ≤ 0.05). Key:M = Male, F = Female

3.2.1 Heamatological parameters

The extract showed very little impact on the heamatological parameters of the test rats compared with the control. Slight significant increase was observed in the Packed Cell Volume of the female rats treated with 800 mg/kg and the lymphocytes of the male rats treated with 400 mg/kg while other parameters were not significantly different from that of the control (Table 5).

Table 5: Effects of HA on the heamatological parameters of Wistar Albino Rats for sub-acute study

		Sub-Acute Doses (mg/kg)			
Parameters	**Gender**	**Control**	**200**	**400**	**800**
PCV (%)	M	48.37 ± 1.82[a]	48.57 ± 1.14[a]	49.80 ± 0.95[a]	49.20 ± 0.66[a]
	F	45.53 ± 1.34[a]	46.07 ± 0.57[a]	46.63 ± 0.14[a]	49.53 ± 1.13[b]
Hb (g/dL)	M	14.77 ± 1.27[a]	14.47 ± 1.19[a]	15.83 ± 0.91[a]	13.50 ± 0.66[a]
	F	13.73 ± 0.40[a]	13.87 ± 1.14[a]	13.73 ± 1.37[a]	13.17 ± 0.78[a]
RBC ($x10^6/mm^3$)	M	6.74 ± 0.67[a]	7.02 ± 0.65[a]	7.08 ± 0.97[a]	7.10 ± 0.13[a]
	F	6.90 ± 0.86[a]	6.76 ± 0.48[a]	6.94 ± 0.92[a]	7.23 ± 0.49[a]
WBC ($x10^3/mm^3$)	M	9.20 ± 0.89[a]	9.50 ± 2.43[a]	10.03 ± 1.14[a]	10.57 ± 2.55[a]
	F	8.73 ± 1.74[a]	8.47 ± 2.08[a]	8.37 ± 1.08[a]	10.50 ± 1.47[a]
Lymphocytes ($10^3/mm^3$)	M	62.77 ± 0.64[a]	66.04 ± 0.34[a]	67.67 ± 1.23[b]	65.15 ± 2.38[a]
	F	62.21 ± 0.97[a]	63.13 ± 1.89[a]	62.95 ± 1.45[a]	62.97 ± 1.26[a]
Eosinophils ($10^3/mm^3$)	M	3.47 ± 0.42[a]	3.33 ± 0.40[a]	4.40 ± 0.53[a]	3.50 ± 0.40[a]
	F	3.33 ± 0.35[a]	3.23 ± 0.15[a]	3.93 ± 0.06[a]	3.27 ± 0.15[a]
Neutrophils ($10^3/mm^3$)	M	23.17 ± 1.09[a]	24.17 ± 2.51[a]	24.97 ± 1.10[a]	27.47 ± 1.70[a]
	F	23.33 ± 1.86[a]	24.00 ± 1.93[a]	24.10 ± 2.98[a]	24.53 ± 0.81[a]
Monocytes ($10^3/mm^3$)	M	3.53 ± 0.38[a]	3.50±0.53[a]	3.37 ± 0.46[a]	3.87 ± 0.15[a]
	F	3.43 ± 0.41[a]	3.33 ± 0.40[a]	3.43 ± 0.31[a]	3.37 ± 0.25[a]

Values are mean ± standard deviation. Values with different superscripts along the same column for each parameter are significantly different (*P* ≤ 0.05). PCV = packed cell volume; Hb = hemoglobin concentration; RBC = red blood cell; WBC = white blood cell; M = Male and F = Female.

3.2.2 Serum Biochemical Analysis

There was no consistent effect on the serum biochemical parameters of the rats in relation to the doses of the extract administered. The total protein, glucose and urea level were significantly reduced in the male rats treated with 200 mg/kg. All the rats treated with 800 mg/kg had no significant difference in their serum biochemical parameter from the control. The Aspartate Aminotransferase increased while urea level decreased in females treated with 400 mg/kg and 800 mg/kg respectively. The serum biochemical results are shown in Table 6 & 7.

Table 6: Effects of different concentrations of HA on Cholesterol level of Albino Rats in Sub-Acute Study

Parameters	Gender	Control	200mg/kg	400mg/kg	800mg/kg
Total Cholesterol (mg/dL)	Male	97.95 ± 9.99[a]	99.77 ± 8.33[a]	97.30 ± 4.53[a]	101.23 ± 15.95[a]
	Female	75.80 ± 1.31[a]	90.96 ± 2.23[a]	75.58 ± 0.55[a]	75.75 ± 1.95[a]
HDL (mg/dL)	Male	39.95 ± 1.61[a]	40.19 ± 1.71[a]	39.35 ± 2.95[a]	40.50 ± 3.88[a]
	Female	29.86 ± 1.85[a]	37.87 ± 1.19[a]	29.81 ± 0.94[a]	31.23 ± 1.79[a]
Triglycerides (mg/dL)	Male	39.85 ± 2.05[a]	40.40 ± 1.55[a]	40.16 ± 4.37[a]	40.73 ± 1.02[a]
	Female	34.54 ± 2.38[a]	33.78 ± 1.10[a]	32.34 ± 0.73[a]	31.89 ± 0.48[a]
LDL (mg/dL)	Male	50.02 ± 9.16[a]	51.50 ± 6.96[a]	49.93 ± 2.66[a]	52.58 ± 12.36[a]
	Female	39.04 ± 2.10[a]	46.34 ± 0.88[a]	39.30 ± 1.35[a]	38.14 ± 3.47[a]

Values are mean ± standard deviation. Values with different superscripts along the same column for each parameter are significantly different ($P \leq 0.05$). HDL: High-density Lipoproteins, LDL: Low-density Lipoproteins.

Table 7: Effects of different concentrations of HA on other serum biochemical parameters of Albino Rats for Sub-Acute Study

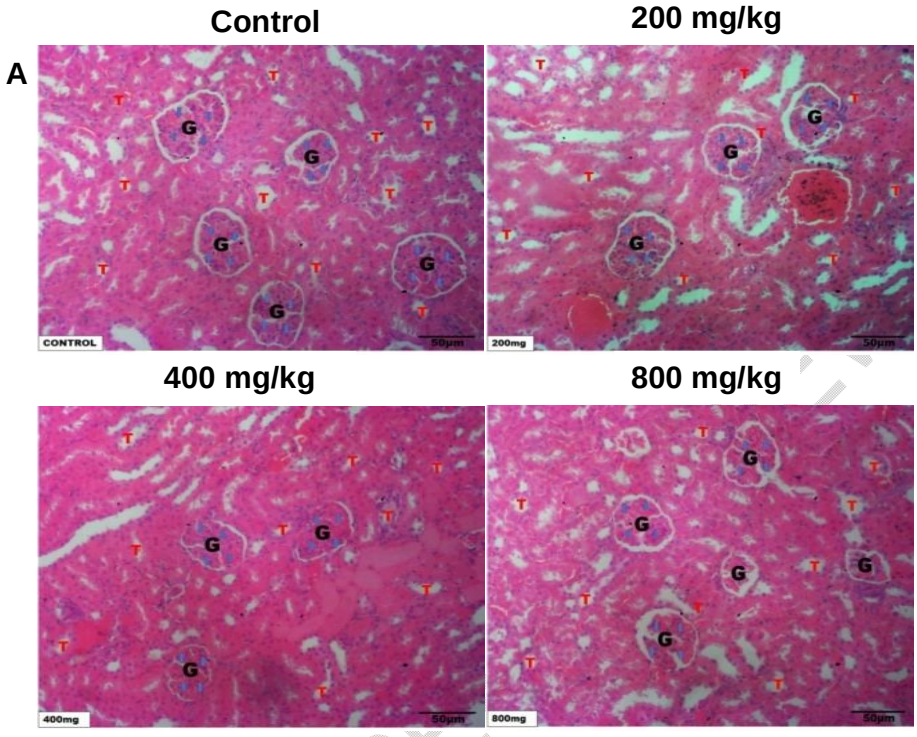
Parameters	Gender	Control	200mg/kg	400mg/kg	800mg/kg
Sodium (mmol/L)	Male	40.05 ± 0.57[a]	39.74 ± 0.67[a]	40.29 ± 1.18[a]	39.59 ± 1.56[a]
	Female	37.30 ± 0.61[a]	36.74 ± 0.60[a]	39.38 ± 1.22[a]	37.61 ± 1.99[a]
Calcium (mmol/L)	Male	0.59 ± 0.058[a]	0.60 ± 0.03[a]	0.56 ± 0.03[a]	0.56 ± 0.00[a]
	Female	0.53 ± 0.01[a]	0.52 ± 0.01[a]	0.57 ± 0.01[a]	0.56 ± 0.03[a]
Total Protein (g/L)	Male	544.32 ± 48.24[a]	292.03 ± 77.87[c]	481.61 ± 211.69[a]	440.94 ± 6.20[a]
	Female	525.17 ± 24.60[a]	412.56 ± 11.79[a]	353.50 ± 45.62[a]	380.12 ± 18.57[a]
Albumin (g/L)	Male	212.03 ± 20.68[a]	143.94 ± 5.25[a]	196.48 ± 80.66[a]	180.24 ± 42.12[a]
	Female	100.12 ± 6.50[a]	162.47 ± 12.29[a]	88.99 ± 3.31[a]	130.05 ± 22.64[a]
Globulin (g/L)	Male	316.81 ± 24.59[a]	186.35 ± 102.89[a]	288.42 ± 131.81[a]	282.65 ± 40.43[a]
	Female	415.77 ± 32.03[a]	270.71 ± 29.71[a]	250.17 ± 31.35[b]	296.10 ± 76.02[a]
A/G ratio	Male	0.67 ± 0.02[a]	1.07 ± 0.82[a]	0.70 ± 0.09[a]	0.66 ± 0.23[a]
	Female	0.24 ± 0.03[a]	0.61 ± 0.12[a]	0.36 ± 0.03[a]	0.46 ± 0.15[a]
Glucose (mg/dL)	Male	152.69 ± 13.17[a]	97.06 ± 3.92[c]	107.43 ± 30.21[b]	127.67 ± 39.99[a]
	Female	95.00 ± 2.48[a]	93.73 ± 3.05[a]	75.66 ± 6.79[a]	90.34 ± 2.99[a]
ALT (U/L)	Male	80.58 ± 6.03[a]	61.45 ± 15.78[a]	76.99 ± 22.56[a]	81.01 ± 20.37[a]
	Female	55.03 ± 4.34[a]	57.61 ± 3.37[a]	45.00 ± 0.94[a]	46.01 ± 4.96[a]
AST (U/L)	Male	276.86 ± 25.78[a]	221.46 ± 8.38[a]	204.79 ± 36.96[a]	220.00 ± 55.39[a]
	Female	171.85 ± 10.14[a]	448.48 ± 121.83[d]	467.49 ± 90.89[e]	168.13 ± 3.62[a]
Urea (mg/dL)	Male	44.55 ± 4.82[a]	17.24 ± 3.97[e]	37.27 ± 7.76[a]	34.92 ± 5.63[a]
	Female	50.05 ± 2.11[a]	24.00 ± 10.03[e]	32.22 ± 2.14[c]	47.45 ± 1.17[a]

Values are mean ± standard deviation. Values with different superscripts along the same column for each parameter are significantly different ($P \leq 0.05$). ALT: Alanine Transaminase, AST: Aspartate Aminotransferase, A/G ratio: Albumin/Globulin ratio.

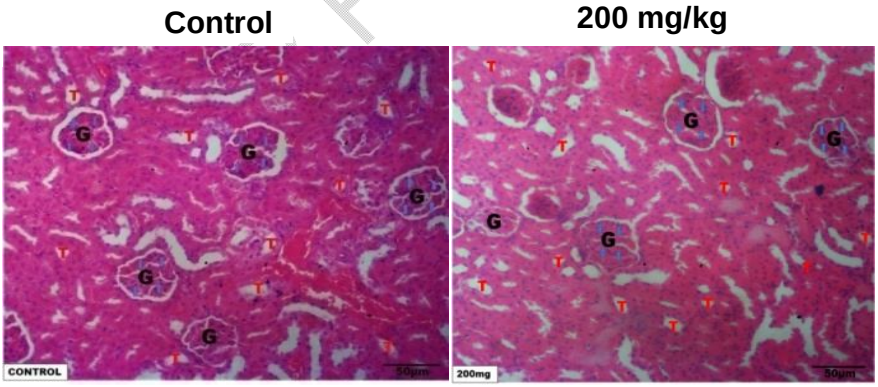
3.2.3 Histopathological Examination

Histological examination of kidneys of the rats treated with 200 mg/kg, 400 mg/kg and 800 mg/kg showed no pathological changes in the features of the treated kidneys and the control. The glomeruli, interstitium and tubules appeared normal in the test group when compared with the control group as shown in Figure 3.

A


Control
200 mg/kg
400 mg/kg
800 mg/kg
CONTROL
200mg
400mg
800mg
50µm

B


Control
200 mg/kg
CONTROL
200mg
50µm

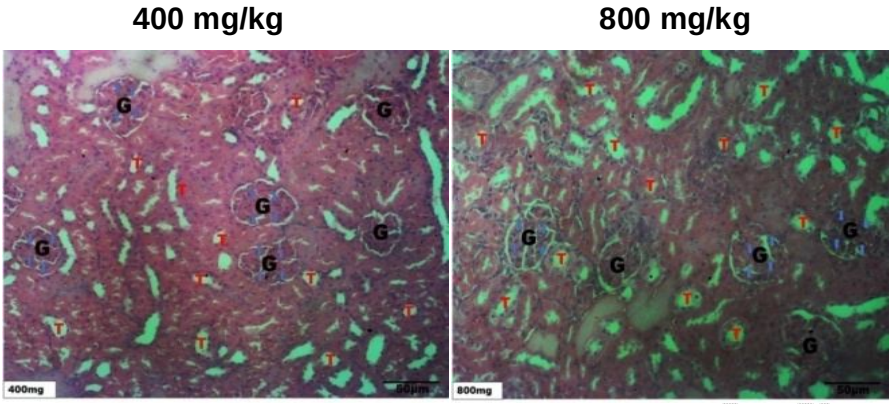
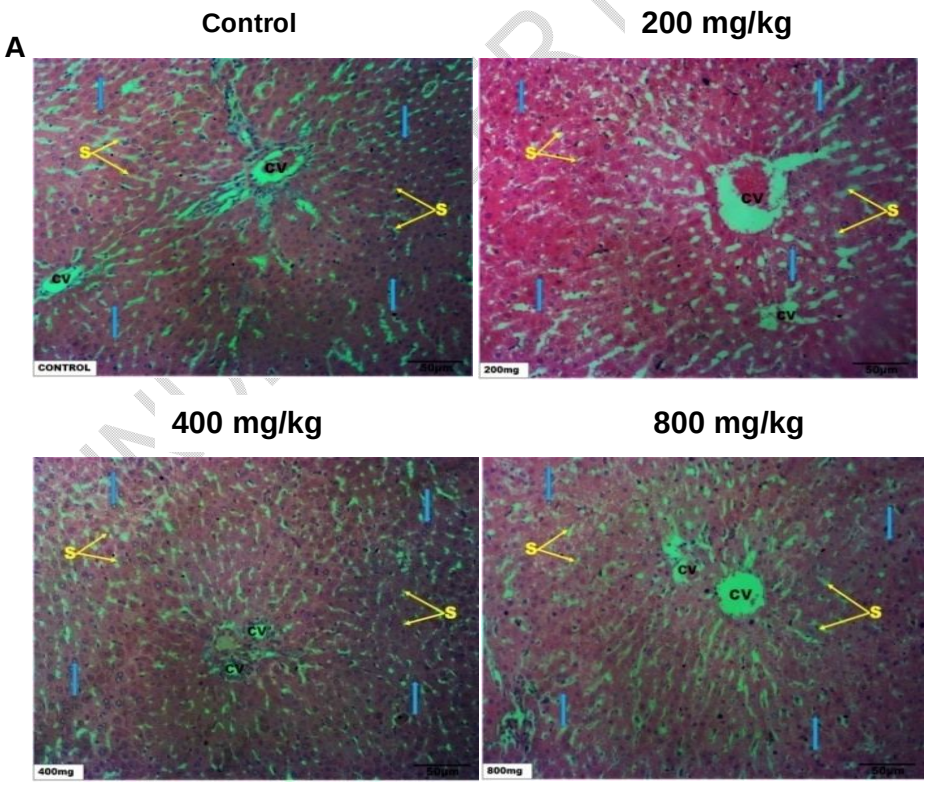


Figure 3: Photomicrographs from Kidneys of male (A) and female (B) rats in the sub-acute group treated with 200, 400 and 800 mg/Kg of HA. G: Glomerulus; T: Renal tubules; Arrows: Renal capillaries. (Stains: Heamatoxylin& Eosin. Mg: x100)

The histology of liver sections from rats treated with 200, 400 and 800 mg/kg body weight showed normal hepatocellular architecture, visible central veins as well as well-preserved liver cells with no histological abnormalities. This is shown in Figure 4:



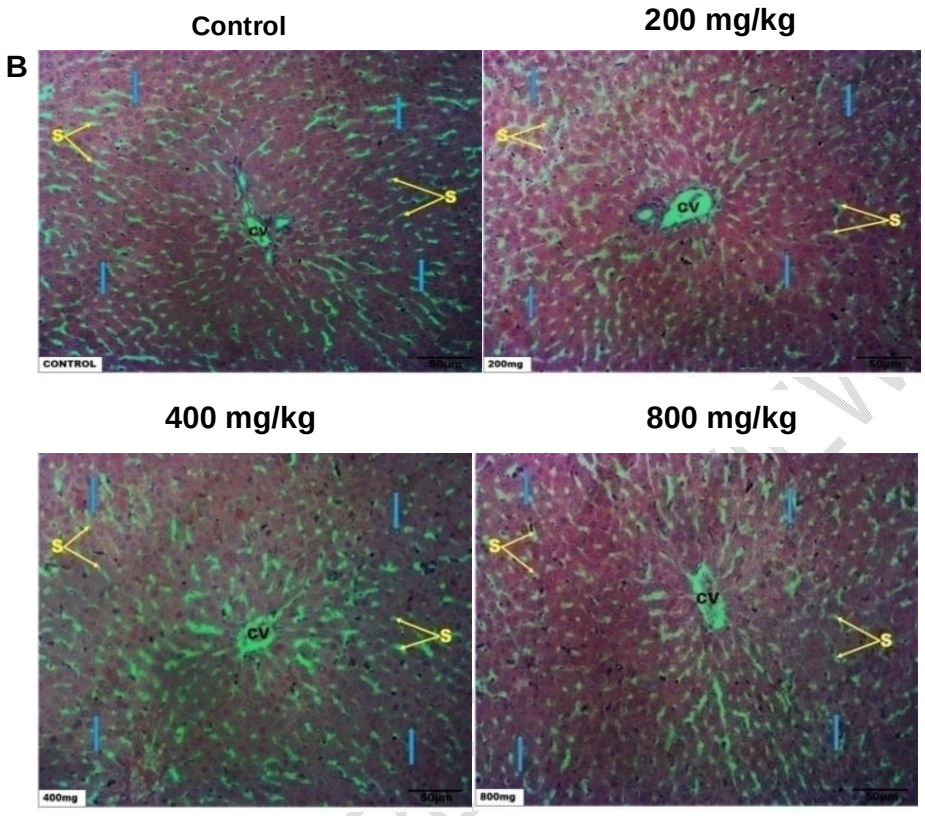


Figure 4: Photomicrographs from Livers male (A) and female (B) rats in the sub-acute group treated with 200, 400 and 800 mg/Kg of HA. CV: Central vein; S: Sinusoids; Arrows: Hepatocytes. (Stains: Heamatoxylin& Eosin. Mg: x100)

4. DISCUSSION

Herbal medicine has been the most adopted remedy for health issues in developing countries due to the high cost of medications and medical consultations [16]. Even though medicinal plants have been reported to produce several biological activities in human and animals, their toxicity have not been fully studied. The impact of a substance on the tissues and organs of laboratory animals determines the toxicity of the substance [14]. In this present study, experiment was carried out in two phases (acute and sub-acute toxicity study) to investigate the safety of aqueous extract of corn husks on Wistar albino rats. The acute toxicity test which is also referred to as single dose toxicity test was used to quantitatively and qualitatively assess the toxicity of the extract within a short period as described by Mukinda [16]. Sub-acute toxicity test evaluated the toxicity changes due to repeated administration of the extract to the experimental animals.

The acute toxicity study revealed that the aqueous extract of corn husks induced hypo-activity and lethargy on the rats in the treated group for few hours which was more pronounced in the group treated with 8000 mg/kg. Groups treated with 4000 mg/kg and 8000 mg/kg were also physically observed to experience diarrhoea which was self limiting after 48 hours. However, no death was recorded throughout the experiment even at the highest dose. This implies that corn husks extract has a median lethal dose (LD_{50}) greater than 8000

Comment [G4]: Please discuss the increase in Cholesterol, Calcium and A/G ratio which occurs with administration of 8000mg/kg.

Comment [G5]: Please discuss why diarrhoea occur? What the active substance in HA that caused diarrhoea?

mg/kg and the high concentration could have posed a laxative effect on the rats. The administration of the extract induced a dose dependent weight gain on the rats which was highly significant in the 8000 mg/kg treated group. Serum biochemical analysis showed various changes in the parameters especially in the group treated with 8000 mg/kg while histopathological examination revealed normal morphological and microscopic architecture of the kidney and liver of the test rats as well as the control.

In the 28 days sub-acute toxicity study, the extract significantly induced weight gain in the rats with no observable change in the behaviour and physical activities of the rats throughout the experiment. Slight changes were observed in the hematological parameters especially the Red and White Blood Cells, eosinophils, neutrophils, heamoglobin concentration and monocytes of treated groups compared to the control. However, both the treated and control rats appeared healthy throughout the 28 days period of study. This proves that the haematopoietic system is not affected as this is one of the key targets for toxic substances usually used to assess pathological and physiological status in animal and man [17].

Biochemical parameters did not show consistent changes according to the dose administered. The absence of significant changes in the levels of cholesterol, ALT, glucose, albumin and globulin especially in 800 mg/kg treated group are indicators of good functioning kidney and liver [18]. Furthermore, most of the biochemical parameters that are indicators for liver damage such as ALT, cholesterol, AST and glucose were not significantly altered. This is in line with the study of Okokon*et al*. [6] who reported the hepatoprotective activity of corn husks extract. The hepato protective ability may be due to the presence of phytoconstituents such as stigmasterol, sitosterol, anthocyanins, octadecanoic acid and p-hydroxycinnamic acid coupled with other antioxidant properties of phenolic compounds present in the extract [19]. This imperatively means that the ingestion of aqueous extract of corn husk at the sub acute doses did not alter the normal functioning of the organs.

Comment [G6]: What the active substance in HA that caused remarkable weight gain? Please explain the mechanism. Thank you

5. CONCLUSION

Generally, no mortality or abnormal findings was recorded in the acute and subacute studies. Therefore, aqueous extract of corn husks was found to pose no toxic effect on the rats when administered in doses up to 8000 mg/kg. Findings in this study validate the essence of using corn husks in the traditional treatment of malaria and pain as it is relatively safe when used at a reasonable dose. However, further study is required to ascertain the safest doses for the treatments of illnesses.

REFERENCES

1. Ogundare AO. Antimicrobial Effect of *Tithonia diversifolia* and *Jatropha gossypifolia* Leaf Extract. Trends in Applied Sciences Research. 2007;2(2):145-150
2. Ogundare OA. The antimicrobial pattern and phytochemical properties of the leaf extracts of *Senna podocarpa*. *African Journal of Microbiology Research*. 2009;*3*(7):400–406.
3. WHO. Global Centre for Traditional Medicine. https://www.who.int/initiatives/who-global-centre-for-traditional-medicine, accessed 4 April, 2023
4. Mouokeu RS, Ngono RAN, Lunga PK, Koanga MM, Tiabou AT, Njateng GSS et al. Antibacterial and dermal toxicological profiles of ethyl acetate extract from *Crassocephalumbauchiense* (Hutch.) Milne-Redh (Asteraceae). *BMC Complementary and Alternative Medicine.*2011;11:43.
5. Mann A, Yahaya Y, Banso A, John F. Phytochemical and antimicrobial activity of Terminalia avicennioides extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases. *Journal of Medicinal Plants Research*. 2008;*2*(5):094–097.

6. Okokon JE, Antia BS, Mohanakrishnan D, Sahal D. Antimalarial and antiplasmodial activity of husk extract and fractions of Zea mays. *Pharmaceutical Biology*. 2017;*55*(1):1394–1400.
7. Tandzi NL, Mutengwa CS. Estimation of Maize (*Zea mays* L.) Yield Per Harvest Area: Appropriate Methods. *Agronomy*. 2020;10(29). https://doi.org/10.3390/agronomy10010029.
8. Sani UM. Anti-Diabetic Potential of Methanol Extract of Cooked Corn Silk (*Stigma maydis*) on Alloxan-Induced Diabetes in Albino Mice. *Journal of Pharmaceutical and Pharmacological Sciences*. 2016;1(1):68–72.
9. Owoyele BV, Negedu MN, Olaniran SO, Onasanwo SA, Oguntoye SO, Sanya JO et al. Analgesic and anti-inflammatory effects of aqueous extract of *Zea mays* husk in male Wistar rats. *J Med Food*. 2010;13:343–347.
10. Solihah MA, Rosli WWI, Nurhanan A. Phytochemicals screening and total phenolic content of Malaysian *Zea mays* hair extracts. *International Food Research Journal*. 2012;19(4):1533–1538.
11. Loha M, Mulu A, Abay SM, Ergete W, Geleta B. Acute and Subacute Toxicity of Methanol Extract of *Syzygiumguineense*Leaves on the Histology of the Liver and Kidney andBiochemical Compositions of Blood in Rats.*Evidence-Based Complementary and Alternative Medicine.* 2019;15. https://doi.org/10.1155/2019/5702159.
12. Kifayatullah M, Mustafa SM, Sengupta P, Sarker MMR, Das A, Das SK. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c mice. *Journal of Acute Disease.* 2015; 4(4): 309–315. http://dx.doi.org/10.1016/j.joad.2015.06.010.
13. Olukunle JO, Ajayi OL, Adenubi OT, Okediran BS, Akinkuotu OA. Evaluation of the toxic effect of aqueous extract of the bark of *morindamorindoides* root in male Wistar rats. *Niger J Anim Prod*. 2012;39:163–71.
14. Ha AW, Kang HJ, Kim SL, Kim MH, Kim WK. Acute and Subacute Toxicity Evaluation of Corn Silk Extract. *Prev. Nutr. Food Sci.* 2018;23(1):70-76 https://doi.org/10.3746/pnf.2018.23.1.70.
15. Fonseca AG, Dantas LLSFR, Fernandes JM, Zucolotto SM, Lima AAN, Soares LALet al. *In Vivo* and *In Vitro* Toxicity Evaluation of Hydroethanolic Extract of *Kalanchoe brasiliensis*(Crassulaceae) Leaves.Please correct
16. Mukinda JT, Syce JA. Acute and chronic toxicity of the aqueous extract of *Artemisia afra*in rodents. *Journal of Ethnopharmacology*. 2007;112:138–144. doi:10.1016/j.jep.2007.02.011.
17. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musangacecropioides* in rats. *Journal of Ethnopharmacology*. 2006;105:374–379.
18. Hilaly EJ, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of *Ajuga Iva* in experimental animals. *Journal of Ethnopharmacology*. 2004;91:43–50.
19. Dong J, Cai L, Zhu X, Huang X, Yin T, Fang H, et al. Antioxidant activities and phenolic compounds of cornhusk, corncob and Stigma maydis. *J Braz Chem Soc*. 2014;25:1956–1964.