Available online at http://www.sjbas.com.ng
Savanna Journal of Basic and Applied Sciences (December, 2025), 7(2): 174-181
https://doi.org/10.5281/zenodo.17220835 P:ISSN 2705-3164 | E:ISSN 2695-2335

Toxicological Assessment of Okra Varieties (Clemson spineless, Ex-Samaru, and NHAE-74) in Wistar Rats

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ABSTRACT
Since plants make up the majority of the food consumed by living things, their metabolisms generate a variety of poisons that can cause illness and occasionally even death. In order to determine the safety of three improved okra seed variants, this journal assesses their toxicity. Twenty-eight albino rats weighing between 132 and 136 g were split into four groups at random for the investigation. For twenty-eight days, group 1 (control) was fed their regular diet of chicken feed, while groups 2, 3, and 4 were fed a mixture of a 1:3 ratio of chicken feed and (75% okra seed), a variety of okra seed (Clemson spineless, Ex-samaru, and NHAE-74, respectively). The mass difference of each rat was measured at the end of each seven days. After twenty-eight days, the rats were killed and their blood was extracted by cardiac puncture. The kidney function parameters, creatinine, urea, Na^+, K^+, Cl^- and HCO_3^- are within their normal range. The liver function parameter of Total protein (3–6.5), albumin (2.87–2.93), and globulin (2.23-2.85) concentrations (g/dL) are all within the normal range. The levels of direct bilirubin (0.14-0.17), total bilirubin (0.39-0.43), and AST, ALT, and ALP (mg/dL) are all within the normal range. In haematological studies, every parameter that was examined fell within the normal range. The seeds are safe to eat because feeding on them causes an increase in body weight without causing anaemia, kidney, or liver problems.

Keywords: Toxicity**,** Liver Function Test, Kidney Function Test, Bilirubin, and Aspartate Aminotransferase.

INTRODUCTION
Food, which comes from either plants or animals, is one of the essentials for human survival. According to the Royal Botanic Gardens, humans eat more than 7000 different types of vascular plants (Urugo and Tringo, 2023). Many varieties of secondary metabolites, classified as poisons, are produced by plant crops that are eaten as food (Hajšlová *et al*., 2004). Plant secondary metabolites can have acute or long-term harmful effects on human health (Madariaga-Mazón *et al*., 2019). While chronic toxicity causes irreversible harm to some important human organs and systems, such as the kidney, reproductive, and immune systems, and can occasionally be found to be carcinogenic and even fatal in severe cases, acute toxicity manifests as symptoms such as nausea, vomiting, skin allergies, and upset stomach, among others (Dridi *et al*., 2017). Food safety is one of the key elements of food security; however, in poor nations, food safety regulations are often lax, increasing individuals' ongoing exposure to foodborne pathogens (Delia, 2015). The ability of the liver to carry out its physiological function in the body in relation to the diet consumed by a patient with liver illness (Elsebaie *et al.*, 2023) and the possible involvement of diet in increasing chronic kidney disorders (van Westing *et al*., 2020) have recently attracted scientific attention. Therefore, it is crucial to look into the potential health risks associated with newly developed plant types developed by Agricultural Research Centres. This study aims to identify the biochemical factors (Kidney and others) that influence the toxicity of three enhanced okra seed varieties.

MATERIALS AND METHODS
The Institute of Agricultural Research at Ahmadu Bello University in Zaria, Nigeria, provided the three improved okra (*Abelmoschus esculentus*) seeds (Clemson spineless, NHAE 74, and Ex-Samaru). The seeds were ground up and left to dry in the sun for a week (Ndangui *et al.*, 2010). Each sample's powder was kept in a plastic bag. Twenty-eight male albino rats (*Rattus norvegicus* var. albus) weighing between 132 and 136 g were bought from the biological garden of Usmanu Danfodiyo University in Sokoto, Nigeria.

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Kidney and liver function tests were performed using reagent kits that were bought from Randox Laboratories in England.

Toxicological Studies

Twenty-eight male albino rats (*Rattus norvegiccus var. albus*) weighing between 132 and 136 g from Usmanu Danfodiyo University's biological garden in Sokoto, Nigeria, were used. After a week of acclimatization to laboratory settings, the rats were kept in room temperature iron cages. The animals were given clean tap water and chicken feed at that time (Ajayi and Adeshina, 2014).

Feed formulation and animal divisions were in accordance with Umar (2010):
Four groups of seven rats each—three treatment groups and one control group—were randomly assigned to the rats. The numbers assigned to the four groups were 1, 2, 3, and 4. Below is a description of each group's feed formulation.

Group 1(Control group): Fed with 100% livestock feed

Group 2: Fed a mixture of 25% livestock feed and 75% Clemson spineless.

Group 3: Fed a mixture of 25% livestock feed and 75% Ex-Samaru

Group 4: Fed a mixture of 25% livestock feed and 75% NHAE

Before the experiment started, each animal's initial weight was determined, and it was recalculated at the conclusion of every seven days. Each group's average mass gain or loss was calculated at the conclusion of each seven-day week by deducting the group's average mass from the week prior from the week ending. By deducting the average starting weight of a group from the weight at the conclusion of the fourth week, the total gain or loss in body weight was calculated.

Blood Sample Collection from Animals

The animals' access to food and water was cut off overnight at the end of the twenty-eight-day period. All of the animals were taken to the Usmanu Danfodiyo University Teaching Hospital's Pharmacology and Toxicology Laboratory in Sokoto, where they were put to sleep in a desiccator with cotton wool drenched in chloroform vapor. Each rat's heart was punctured to obtain blood samples, which were then placed in two separate sterile containers. One container contained ethylene diamine tetraacetic acid (EDTA), which was used as an anticoagulant for haematological investigations, while the other container was used for chemical pathological analysis (Ajayi and Adeshina, 2014).

Biochemical Analysis

For biochemical analysis, the obtained blood samples were spun in a refrigerated centrifuge at 3500 rpm for 15 minutes to separate the serum, which was then decanted and stored in clean bottles in a deep freezer at -40 ^{0}C (Sathya *et al*., 2012).

The sera obtained from the blood of rats were used to measure the following parameters: creatinine, bilirubin, urea, uric acid, glucose, electrolytes, albumin, aspartate aminotransferase activity, alanine aminotransferase activity, alkaline phosphatase activity, and serum total protein. Assay kits obtained from Randox laboratories Ltd., UK, were used to measure the activities of serum total protein (Biuret method), albumin (Bromocresol Green (BCG) method), creatinine, glucose, bilirubin (total and direct), urea, uric acid (Phosphotungstate Method), aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP). By deducting albumin from the total protein, the globulin content was determined. The mercurimetric method described by Schale and Schales (1941) was used to quantify chloride, whereas the flame emission spectrophotometer described by Cheesbrough (1991) was used to detect serum Na+ and K+.

Haematological Analysis

Haematological indices were assessed from the anticoagulant blood using a particle counter (model PCE-210, Erma Inc., Tokyo, Japan, at the Haematology Laboratory of Usmanu Danfodiyo University Teaching Hospital, Sokoto) in accordance with the procedure outlined by Enyuma *et al*. (2015). The parameters analysed are: total red blood cells, white blood cells and differentials, Hb level, and packed cell volume.

RESULTS AND DISCUSSIONS

Feed Intake and Weight Gain/Loss in the Sample Treatment to the Animal Body Weight

The impact of feed intake and weight gain/loss on the control and treated animals is displayed in Table 1. The animals' starting weights did not differ significantly ($p > 0.05$). In the first and second weeks, there was a significant difference ($p < 0.05$) in the feed intake of the treated groups compared to the control group. During the first week, there was a significant difference ($p < 0.05$) in the intakes of groups 3 and 4, and by the conclusion of the second week, there was a significant difference ($p < 0.05$) between group 2 and group 4. The control group's feed intake at the end of week three was substantially different ($p < 0.05$) from that of groups three and four, and at the end of week four, it was considerably different ($p < 0.05$) from that

of group three. At the end of the first week, the treated groups lost weight, whereas the rats in the control group gained weight on average. The average mass of every group rose at the conclusion of the second, third, and fourth weeks.

Body weight changes of rats fed with *M. corchorifolia* leaves

The average feed intake of rats fed chicken feed as the control group (group 1) and rats fed 75% sample in the first and second weeks of the experiment differed significantly ($p<0.05$) from that of treatment groups 2, 3, and 4. This suggests that members of groups 2, 3, and 4 refused to accept the newly introduced feed intake at the start of the experiment, which resulted in a general decrease in the groups' weight. However, at the conclusion of the experiment, the total body weight of every group generally increased, suggesting that the seeds contained enough nutrients for the animals' growth and development.

Kidney Function Parameters

The kidney function test results for the three okra types are displayed in Table 2. There is no discernible difference ($p > 0.05$) between the samples according to the parameters that were examined (creatinine, bicarbonate, sodium, and potassium). However, there is a significant difference ($p < 0.05$) in the urea content between group 3 (the group that received the Ex-Samaru treatment) and the control group.

Table 1: Feed Intake and Weight Gain/Loss on Wistar Rats Fed with Okra Seeds

Group	1	2	3	4
Initial Body Weight	133.65±0.95^{a}	132.55±3.32^{a}	136.80±0.99^{a}	135.05±0.07^{a}
Week 1 Feed Intake (g)	174.71±2.82^{a}	151.94±3.42^{bc}	157.97±1.89^{b}	145.49±7.88^{c}
Week 2 Feed Intake (g)	179.41±1.48^{a}	166.66±3.12^{b}	161.21±1.72^{bc}	162.73±4.01^{c}
Week 3 Feed Intake (g)	183.54±1.74^{a}	178.06±4.8^{ab}	174.17±4.53^{b}	172.96±4.91^{b}
Week 4 Feed Intake (g)	199.24±2.00^{a}	187.84±12.83^{ab}	168.36±24.32^{b}	188.73±8.61^{ab}
Week 2 Weight Gain/Loss (g)	2.50	-1.40	-1.20	-1.97
Week 3 Weight Gain/Loss (g)	1.53	5.75	6.70	3.22
Week 4 Weight Gain/Loss (g)	2.77	1.70	3.50	1.50
Overall Weight Gain/Loss (g)	6.80	5.98	8.80	3.30

The result is expressed as mean ± standard deviation of triplicate results. Values in the same row that do not share a letter are significantly different ($p<0.05$).

Table 2: Kidney Function Parameters of Albino Rat Fed with Okra Seeds Varieties

Parameter	**Control**	**C.spineless**	**Ex-Samaru**	**NHAE-74**
Creatinine (mg/dL)	0.90±0.31	0.70±0.00	0.70±0.00	0.70±0.00
Urea (mmol/dm^{3})	28.45±3.93^{b}	29.92±4.37^{b}	30.90±2.42^{ab}	39.30±6.42^{a}
K^{+} (mmol/dm^{-3})	5.38±0.75	5.52±0.59	5.23±0.55	5.23±0.42
Na^{+} (mmol/dm^{-3})	108.33±7.53	110.00±6.32	110.00±00	106.67±5.77
HCO_3^{-}(mmol/dm^{-3})	23.83±2.32	26.33±3.61	27.00±3.61	25.00±1.00

The mean ± standard deviation of the triple values is used to express the outcome. Significant differences exist between values in the same row that do not share a letter ($p<0.05$).

Both the control and treatment serum creatinine levels fell between the recommended range of 0.5 to 1.5 mg/dL (Satyanarayana and Chakrapani, 2013). Rats fed with the sample have lower serum creatinine due to a decrease in synthesis or an increase in the functional capacity of tubular excretion (Ullah *et al*., 2008).

The blood serum urea levels of the treated rats and control rats (28.45 to 39.30 mmol/dm^{3}) are higher than the usual range (2.1 to 8.5 mmol/dm^{3}) (Mayo Clinic, 2021). This implies that eating all of the seeds boosts the serum urea level due to their high protein content (Waikar and Bonventre, 2006). Serum urea cannot be used to accurately measure kidney function. Serum urea levels can be easily affected by additional variables unrelated to glomerular filtration rates, such as severe gastrointestinal bleeding, consuming too much protein, tissue deterioration, and ageing (Wang *et al*., 2014).

Fluid and acid-base balance are maintained by serum electrolytes (K^+, Na^+, Cl^-, and HCO_3^-). According to Nssien *et al*. (2002), they are also essential for the transmission of muscle and neuromuscular impulses. Variations in these ions' concentration have an impact on the amount of extracellular fluid and the transmission of neuromuscular impulses.

The treated rats' sodium concentration (106.67–110.00 mmol/dm^3) is lower than the reference (normal) range (135–145 mmol/dm^3) of sodium concentration in blood (Opoku-Okrah *et al*., 2015) and higher than that of the control rats (108.33 mmol/dm^3). In addition to causing cellular *oedema*, which affects the central nervous system and causes in depression and cerebral *oedema*, sodium levels below 135 mmol/dm^3 produce *hyponatraemia* (Sun *et al*., 2014). In addition to kidney failure, low serum sodium levels can also be caused by an excess of bodily water relative to the body's total sodium content (Golestaneh *et al*., 2017). According to Anthony *et al.* (2015), the potassium serum concentration of treated rats (5.23 - 5.52 mmol/dm^3) and control rats (5.38 mmol/dm^3) is marginally higher than the reference range (3.5 - 5.0 mmol/dm^3). According to the results, a high blood K^+ level could cause cardiac arrest, so all seeds should be consumed carefully (Yao *et al*., 2022).

The average serum bicarbonate of treated rats ranges from 25.00 to 27.00 mmol/dm^3, while the average of the control group is 23.83 mmo/dm^3. The results fall between 21 and 32 mmol/dm^3, which is the typical range for serum bicarbonate (Kanda *et al*., 2013). Accordingly, eating the seeds can lower the risk of heart failure and may successfully stop the course of chronic kidney disease (CKD) (Kanda *et al*., 2013).

Liver Function Parameters

The liver function parameters of the control and treated rat groups using three different types of okra (NHAE-74, Ex-Samaru, and Clemson spineless) are displayed in Table 3. There was no discernible difference ($p > 0.05$) between the control and treated groups in any of the parameters examined. The liver is responsible for the processes of detoxification and the metabolism of proteins, lipids, and carbohydrates (Helal *et al*., 2008). Certain enzymes and the metabolic pathway's byproducts harm or impair liver functioning during the detoxification process (Gowda *et al*., 2009). Tests for liver function are used to keep an eye on liver damage or illnesses. The tests quantify the blood's concentrations of specific proteins and enzymes. Liver issues are indicated by liver function indicators that are higher or lower than the usual range (Limdi and Hyde, 2003).

Table 3: Liver Function Parameters of Albino Rat Fed with Okra Seeds Varieties

Parameter	Control	C. spineless	Ex-Samaru	NHAE-74
Total protein (g/dL)	5.78±0.83	5.63±0.29	5.33±0.81	5.17±0.42
Albumin (g/dL)	2.73±0.45	2.93±0.16	2.87±0.12	2.93±0.23
Globulins (g/dL)	3.05±0.80	2.85±0.31	2.47±0.92	2.23±0.64
Total Bilirubin (mg/dL)	0.24±0.04	0.39±0.27	0.43±0.29	0.43±0.19
Direct Bilirubin (mg/dL)	0.14±0.00	0.17±0.06	0.14±0.00	0.14±0.00
AST (UL^{-1})	41.50±17.96	32.50±19.74	21.00±8.66	22.33±16.17
ALT (UL^{-1})	24.00±12.47	27.00±14.81	22.33±14.74	27.70±19.60
ALP (UL^{-1})	37.00±11.58	41.00±23.26	45.33±10.69	53.33±12.90

The mean ± standard deviation of the triple values is used to express the results.

Both the control and treatment groups' total protein contents fall between 3 to 6.5 g/dL, which is the normal range (Zaias *et al*., 2009). According to Nazifi *et al*. (2011), elevated serum total protein concentrations are indicative of a relative decrease in albumin and an increase in globulin concentration. According to Don and Kaysen (2004), chronic inflammation is linked to a drop in albumin concentration (below the normal range).

According to Zaias *et al*. (2009), the serum albumin concentrations of the control and treatment groups fall between the rats' reference range of 1.2 and 2.7 g/dL. Numerous factors, including dietary status, catabolism, hormones, and losses in the gastrointestinal tract and urine, affect albumin levels (Limdi and Hyde, 2003). *Hyperlipemia*, a sign of inadequate nutritional condition and an unusually high blood lipid concentration (Vincent *et al*., 2014), is caused by low albumin levels (Bishop *et al*., 2010).

The globulin concentrations in the sample and control treatments fall between 2.5 and 3.5 g/dL, which is the normal range (Kumar and Gill, 2018). Globulins are crucial for blood coagulation, liver function, and infection prevention (IOM, 2018).

Total and direct serum bilirubin levels in the control and treatment groups are both within the normal range (0.2–1 and <0.4 mg/dL, respectively) (Kumar and Gill, 2018). The results indicate that the samples can be used to prevent metabolic syndromes such as *dyslipidaemia*, hypertension, obesity, and glucose intolerance, which increase the risk of diabetes and cardiovascular disease (Li *et al*., 2017).

When compared to their respective control values, the treatments' serum levels of alkaline *phosphatase* (ALP), *aspartate aminotransferase* (AST), and *alanine aminotransferase* (ALT) do not differ significantly ($p>0.05$) (Table 3). According to Limdi and Hyde (2003) and Huang *et al*. (2006), both fall within the normal ranges of 5 to 40 UL^{-1} for AST, 5 to 35 UL^{-1} for ALT, and 30 to 120 UL^{-1} for ALP. As a result, the seeds are safe to eat.

Haematological Studies

The results of the haematological analyses of the control and treated rat groups fed three different types of okra are displayed in Table 4. There was no discernible difference ($p > 0.05$) between the control and treated groups in any of the measures examined.

Table 4: Haematological Indices of Albino Rat Fed with Okra Seeds Varieties

Parameter	Control	Clemson spineless	Ex-Samaru	NHAE-74
WBC (L^{-1}).	4.34±2.46 x 10^9	5.57±1.85 x 10^9	8.95±3.04 x 10^9	7.07±1.20 x 10^9
RBC (L^{-1})	6.55±2.55 x10^{12}	7.41±1.31 x10^{12}	8.88±0.04 x10^{12}	7.32±0.51 x10^{12}
Hemoglobin (gdm^{-3})	11.85±4.09	13.23±1.55	14.50±0.14	13.07±0.50
PCV (%)	37.50±12.40	40.67±5.51	46.00±0.00	40.67±1.53
Neutrophils (%)	13.00±3.56	18.00±8.66	20.00±8.49	30.00±2.65
Lymphocyte (%)	83.50±3.87	78.33±11.72	77.00±8.49	69.00±3.61
Monocytes (%)	2.00±2.00	3.00±2.65	3.00±0.00	1.00±1.00
Eosinophils (%)	2.50±0.71	2.00±0.00	-	-
Basophils (%)	-	-	-	-

The mean ± standard deviation of the triple values is used to express the results.

Red blood cells (RBC) and *haemoglobin* (Hb) contents in the control and okra seed treatments did not differ significantly ($p>0.05$), according to the study's *haematological* analysis. For RBC and Hb, the values fall within normal ranges of 6.76 and 9.75 X 10^{12} L^{-1} and 115 and 16.10 g/dm^3, respectively (The Rat Fan Club, 2014). According to Kanu *et al.* (2016), this suggests that none of the samples may result in *anaemia*.

The normal range for packed cell volume (PCV), sometimes referred to as *haematocrit*, is 37.6 to 50.6% (The Rat Fan Club, 2014). A low *haematocrit* measurement implies either overhydration or a reduction in the quantity of red blood cells in circulation, which may deliver oxygen. A high *haematocrit*, on the other hand, indicates either a drop in plasma volume or an absolute increase in *erythrocytes* (RBC) (Wennecke, 2004). A normal number of circulating red blood cells is indicated by eating the seeds.

White Blood Cell (WBC) counts in the control (4.34 X 10^9 L^{-1}) and okra seed treated rats (5.57 to 8.5 X 10^9 L^{-1}) fall between the normal range of 4.5 and 11.0 X 10^9 L^{-1} (Dean, 2005), demonstrating the samples' capacity to strengthen the immune system against infections caused by bacteria, fungi, and viruses (Agiang *et al.*, 2017). The differentiation of white blood cells (WBC) into five distinct components—*lymphocytes*, *monocytes*, *neutrophils*, *eosinophils*, and *basophils*—occurs during a complete blood count (CBC). More details on the underlying issue are provided by each type of WBC. For instance, the majority of the rise in WBCs during the early phases of an infection can be attributed to the rise in *neutrophils*. As the infection continues, *lymphocytes* grow. *Eosinophils* rise in worm infections, while *basophils* rise in allergic reactions such high fever (Dean, 2005). Every parameter analysed falls within the normal range: 0–7% for *eosinophils* (O'Connel *et al*., 2015); 1–10% for monocytes (Stanojevic, 2019); and 70–80% for *lymphocytes* (Liu *et al*., 2013 and O'Connell *et al*., 2015).

Rats fed Clemson spineless (18.00%) and Ex-Samaru (20.00%) did not exhibit a significant difference ($p > 0.05$) in *neutrophils* compared to the control group (13.00%), while rats fed NHAE-74 (30.00%) did exhibit a significant difference ($p<0.05$). According to O'Connell *et al*. (2015), the results of Ex-Samaru and NHAE-74 fall within the normal range of 20 to 30%.

The control group and the rats given Clemson spineless, Ex-Samaru, and NHAE-74 did not have *basophils*. Rats rarely have *basophils* (O'Connell *et al*., 2015). However, *basophils* multiply in response to allergic and parasitic reactions (Folgueras *et al*., 2008).

From the study's results, it can be stated that the safety of the seeds was demonstrated by the gain in weight of the experimental animals, as well as the results of liver and renal function tests. Additionally, the biochemical research shows that the seeds are harmless. The results of the haematological investigation showed no evidence of infection or *anaemia.* Therefore, it is safe to eat the seeds.

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