Biochemical and histopathological assessment of transgenic cowpea in male Wistar rats

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Abstract

Cowpea is one of the most consumed legumes in Africa. Recombinant DNA technology has been used to insert foreign genes into plants genome thereby creating a new generation of plants with desired traits. Such plants possess improved seed quality, yield and resistance to pests and pathogens. The aim of this work is to assess the safety profile of an improved variety of cowpea by examining its effect on the liver and kidney.Native cowpea and improved variety of cowpea, IT89KD, were fed to Wistar rats which were divided into 3 groups. One group was fed poultry feed with 50% native cowpea and the second group was fed poultry feed with 50% improved cowpea variety while the control group was fed standard rat chow. At the end of 40 days feeding period, improved varieties group were found to have higher protein content than normal variety. Also, there was elevation in the following liver enzymes, Alanine Transaminase (ALT), Aspartate transaminase (AST) and bilirubin in the group fed with improved variety. The presence of black arrowheads and inflammation in liver and kidneys of rats fed with Genetically Modified (GM) cowpea was observed. This suggests that caution

should be applied in the use of GM Cowpea as feed for Wistar rats and food for man.

Keywords: Bilirubin, Inflammation, Arrowhead, cowpea, genome.

Introduction

Since the discovery of the three dimensional structure of the DNA and advances in the knowledge of genetic engineering, there has been a marked increase in the utilization of genetic engineering to improve food production through recombinant DNA technology. Besides its application in agriculture, other essential medicinal items like the human growth hormone, insulin, have also been made possible through recombinant DNA technology (James, 2014; Onuh*et al*., 2022). Plants are designed to possess improved seed quality, yield and resistance to pests as well as pathogens (Boldura and Popescu, 2016; Onuh *et al*., 2022). Although there is a great deal of scientific evidence supporting the direct application of genetic modification (GM) technology to food security, there have been concerns about the safety profile of GM food in humans, animals and the environment (Sateesh, 2008).The emergence of antibiotic-resistant pathogens through unintended transgenesis via pollination, the recent increase in various cancer types, the introduction of known and new trans-species allergens as well as toxins are just a few of the problems relating to the use and consumption of GM foods in society (Amiri*et al.,* 2013). Millions of people in Africa and other developing nations depend on cowpea as a primary dietary protein source to supplement their diets of low-protein staples like grain and tubers. Cowpea is also a valuable and stable commodity that generates cash for farmers and dealers (Singh, 2002; Langyintuo *et al.,* 2003). Cowpea, one of Africa's basic crops, is crucial to preserving food security. Making sure that the population's health is not negatively impacted by genetically engineered cowpea is necessary. Consequently, it is necessary to do a risk

assessment(Osuji *et al,* 2016). Evaluation of the safety profile and nutritional impact of transgenic cowpea will be required for policy decision that will be based on reliable experimental data on the continued use of genetically modified food.

Materials and Methods

Diet Formulation

The experimental feed for the nutritional assessment in rats was formulated consisting of a standard rat chow with 50% cowpea composition. The major nutritional contents of the laboratory diet include 22% protein, 3.48% fat, and 3.71% fiber (Nowicki *et al.,* 2010). Another feed formulation containing wheat, with the same nutritional value as the laboratory diet, was used as the non-GM diet.

In vivo Nutritional Assessment using Wistar Albino Rats.

Twenty-one male Wistar albino rats were purchased from the animal house of the National Veterinary Research Institute, Jos immediately after weaning. These were divided into 3 groups of seven rats each. Group 1 (GM formulated diet group) were fed with a GM-tested and formulated laboratory diet for 40 days. Group 2 (Non-GM formulated diet group) were fed with normal diet for similar periods of time and group 3 served as the control. All groups of animals were housed in standard cages and under standard conditions and exposure to light was maintained for 12h. Diet and water were provided ad libitum. Animals were euthanized at 40 days. Serum samples were analyzed for biochemical markers. The kidney and liver were harvested and analyzed for histological changes. The protocol applied throughout this study complies with the NRC guidelines on the handling of laboratory animals.

Biochemical Studies

At the end of each of the experimental intervals (40 days), blood sample was collected from the retro-orbital venous plexus of dissected rats and centrifuged for biochemical analyses.

Blood Serum Analysis

The blood sera were separated and Alanine amino transferase (ALT) and Aspartate amino transferase (AST) enzymes activity were evaluated according to Kasarala *et al.* (2016). Concentrations of both creatinine and uric acid were also evaluated.

Histopathological and Histochemical Investigations

Histopathological investigations were carried out according to Kittel *et al.* (2004). The collected specimens of liver and kidney were dissected immediately after death, washed thoroughly with saline, and fixed in 10% neutral-buffered saline for 72 h. All specimens were washed in tap water for 30 min, dehydrated in ascending grades of alcohol (70%, 90%, 95%, and absolute), cleaned in xylene, and embedded in paraffin wax Serial sections of Liver and Kidney were cut to a thickness of 5µm and stained with hematoxylin (Hx) and eosin (E) for histopathological investigation and bromophenol blue for demonstration of protein content in the tissues. Total protein content was determined by measuring the optical densities (OD) of the bromophenol blue stained sections using a Leica Qwin 500 image analyzer system (Kasarala *et al*., 2016).

Statistical Analysis

Data analysis was performed with SPSS version 23. Data was presented as Mean ± STD. The effects of GM diets on liver and kidney of the Wistar albino rats was determined using One-way ANOVA and the differences was considered significant at $p \leq 0.05$.

Results

Biochemical analysis of genetically modified cowpea and native cowpea (*Vigna unguiculata*)on Albino Rats

The results of the biochemical analysis are presented in Tables 1, 2, 3 and 4. The analysis of the blood biochemical parameters in most cases showed significant effect of transgenic cowpea diet on the metabolism of the animals in comparison to

the control and local cowpea varieties at ($p \leq 0.05$). Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) levels were significantly ($p \leq 0.05$) elevated in the group fed with GM formulated diet compared with the control and local cowpea variety fed groups. The transgenic cowpea diet had significant impact on the serum uric acid, creatinine, albumin, total bilirubin and protein levels as seen in table 2 and 3. Uric acid, creatinine and albumin of GM formulated diet fed group are significantly different from control and local varieties groups but similar in protein and total bilirubin levels.

The electrolyte contents of group fed with GM formulated diet are significantly different from the group fed standard rat chow and the one fed with local cowpea variety. All three (3) groups were significantly different from each other in terms of effect on electrolyte contents.

Histopathological Analysis of liver and Kidney tissues

The liver tissue sections of rats fed GM (group B) cowpea showed slight scarring and inflammation of cells whereas these were not observed in the rats fed with native cowpea. The kidney sections of rats in group B showed hypertrophy of cells blocking glomeruli space which was not observed in rats fed with native cowpea variety. Figures 1, 2,3 and 4 show the micrographs of liver and kidney sections of rats fed with native and GM cowpea varieties respectively.

Table 1: Effect of genetically modified cowpea and native cowpea the activities on serum enzyme in albino rat.

Group	Treatment	ALT(U/L)	AST (U/L)	ALP(U/L)
A	Control	29.23 ± 0.029	41.22 ± 0.118	88.77 ± 0.078
B	GM cowpea	33.78 ± 0.121[a]	72.28 ± 0.161[a]	101.94 ± 0.014[a]
C	Native cowpea	31.09 ± 0.085[ab]	66.61 ± 0.206[ab]	92.98 ± 0.035[ab]

ALT= Alanine transaminase, AST=Aspartate transaminase, ALP=Alkaline phosphatase values are expressed as mean ± STD, n=3.

[a] and [b] implies values are significantly different when compared with control and improved variety ($p \leq 0.05$).

Table 2: Effect of genetically modified cowpea and native cowpea on urea and creatinine albino rats.

Group	Treatment	Ur(mmol/L)	Cr(µmol/L)
A	Control	4.01 ± 0.031	65.40 ± 0.100
B	GM cowpea	6.82 ± 0.029[a]	76.45 ± 0.136[a]
C	Native cowpea	5.85 ± 0.076[ab]	73.65 ± 0.187[ab]

Ur = urea and Cr = creatinine. Values are expressed as mean ± STD, n=3.
[a] and [b] implies values are significantly different when compared with control and improved variety ($p \leq 0.05$).

Table 3: Effect of genetically modified cowpea and native cowpea the activities on total protein, albumin, total bilirubin in albino rat

Group	Treatment	Protein (g/L)	Albumin (g/L)	Total bilirubin (µmol/L)
A	Control	53.92 ± 0.033	39.787±0.4208	0.93 ± 0.310
B	GM cowpea	49.49 ± 0.325[a]	32.877±0.1317 [a]	1.31 ±0.166[a]
C	Native cowpea	47.93 ±0.190[a]	38.763±0.1794	1.12 ±0.028[a]

Values are expressed as mean ± STD, n=3.

[a] and [b] implies values are significantly different when compared with control and improved variety ($p \leq 0.05$).

Table 4: Effect of genetically modified cowpea and native cowpea on serum electrolytes on albino rats

Group	Treatment	Na^+ (mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	HCO_3^- (mmol/L)
A	Control	144.07 ± 0.054	6.62 ± 0.017	84.01 ± 0.035	32.69 ± 0.648
B	GM cowpea	139.13 ± 0.068[a]	11.25 ± 0.026[a]	81.97 ± 0.040[a]	31.95 ± 0.064[a]
C	Native cowpea	132.08 ± 0.053[ab]	10.23 ± 0.017[ab]	83.01 ± 0.035[ab]	30.04 ± 0.024[ab]

Na^+ =Sodium, K^+=Potassium, Cl^-=Chloride, HCO_3^-=Bicarbonate Values are expressed as mean ± STD, n=3.

[a] and [b]implies values are significantly different when compared with control and improved variety ($p \leq 0.05$).

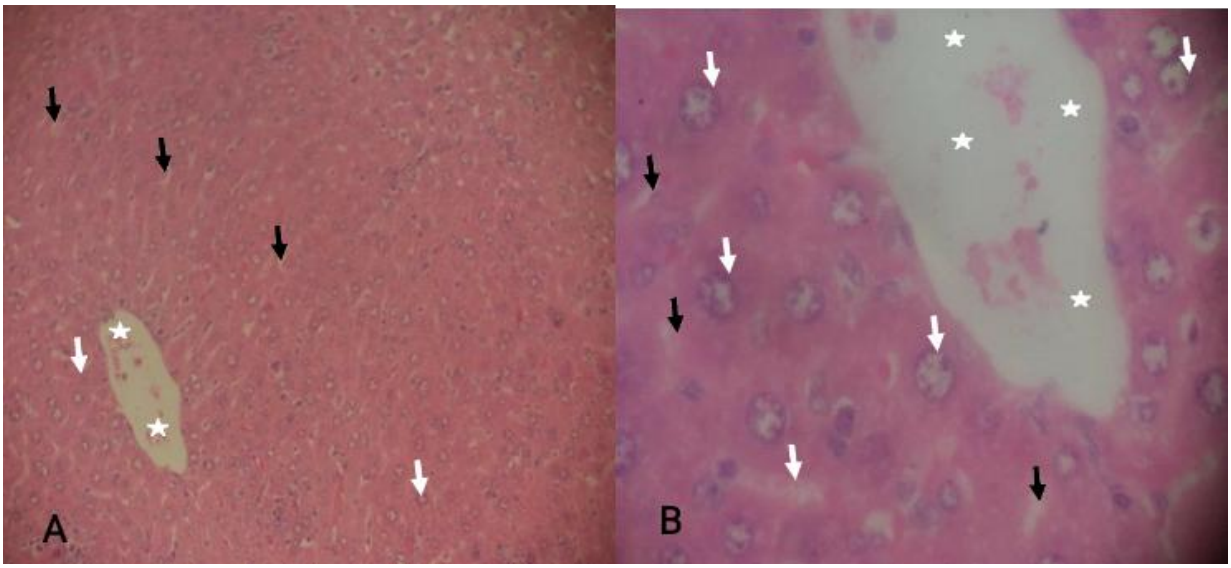

Figure 1C: Liver of an albino mouse administered native cowpea for 40 Days, showing normal hepatocytes morphology as demonstrated by the cells presenting with their characteristic polygonal shapes and interspersed by hepatic sinusoids (black arrows) which are linked to the central vein (white stars). The cells present with intact nuclei (white arrows), surrounded by intact cytoplasmic components. H&E **A**: X100 **B**: X400

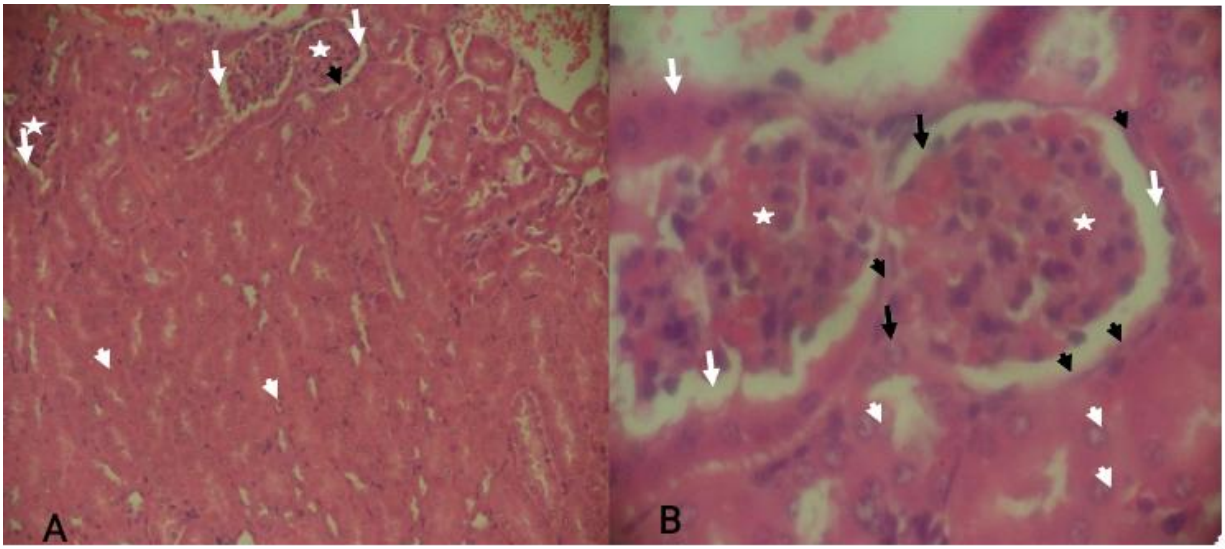

Figure 2 (C):Kidney of an albino mouse administered native cowpea for 40 days, showing normal tissue morphology. The glomeruli (white stars) are surrounded by a clear zone of capsular spaces (white arrows) and the glomerular capsules (black arrowheads) present with their characteristic simple squamous epithelial cells. The cell nuclei (white arrowheads) are intact and surrounded by intact cytoplasmic components. H&E **A**: X100 **B**: X400

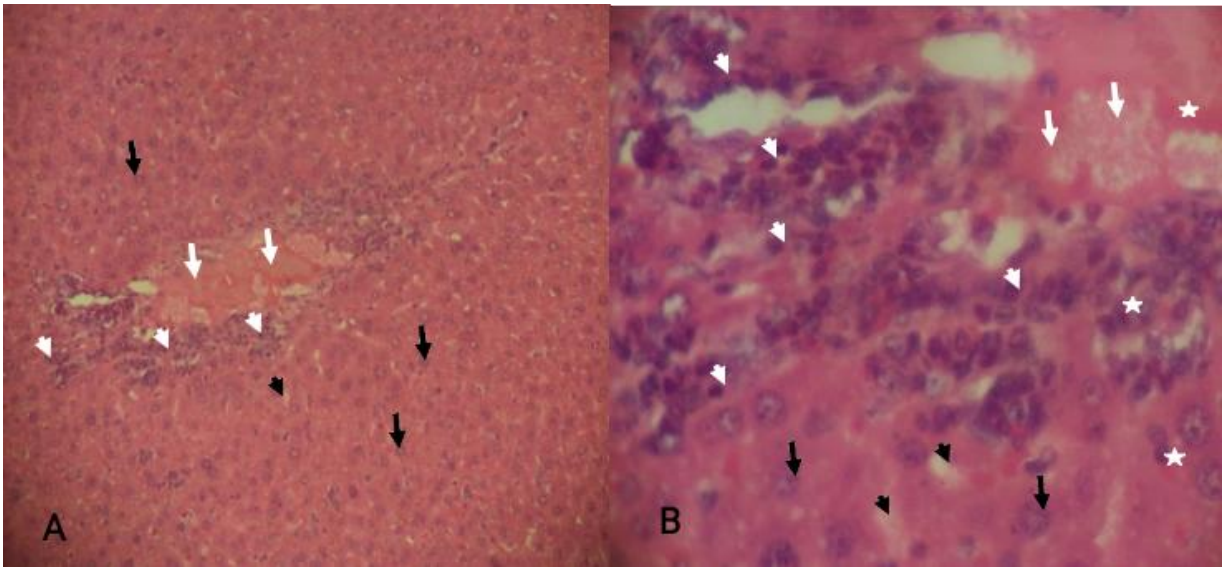

Figure 3 (B): Liver of an albino mouse administered GMO cowpea for 40 days, showing hepatocyte inflammation evident by the presence of inflammatory cells (white arrowheads) within the tissue and necrosis, evident by the complete loss of cellular morphology and components (white arrows) and their replacement by connective tissue. Surrounding the inflamed area are hepatocytes presenting with normal morphology as demonstrated by intact nuclei (black arrows) surrounded by intact cytoplasmic components. The normal hepatocytes are interspersed by hepatic sinusoids (black arrowheads). H&E **A**: X100 **B**: X400

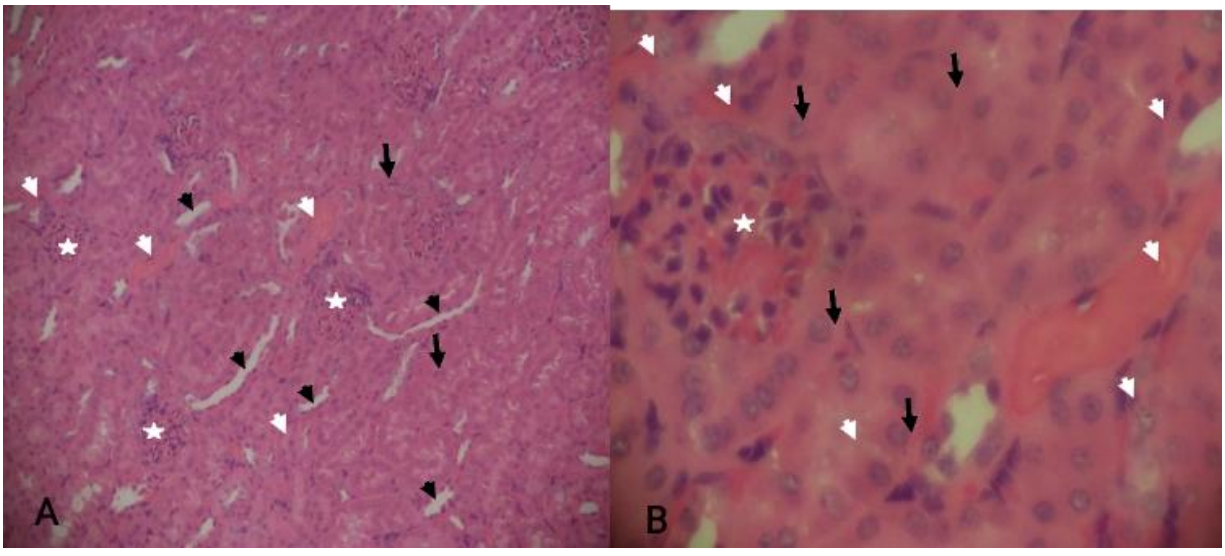

Figure 4 (B): Kidney of an albino mouse

administered GMO cowpea for 40 days, showing glomerular hypertrophy as seen by the complete loss of capsular space. The glomeruli (white stars) are so hypertrophied that they occupy the capsular spaces and are in direct contact with the glomerular capsule, making it difficult to identify the glomerular-glomerular capsule boundary. The kidney is congested with red blood cells (white arrowheads). The nuclei (black arrows) are intact. Black arrowheads= kidney tubules. H&E **A**: X100 **B**: X400

Discussion

Histopathological examinations revealed changes in all samples of the liver and in all preparations of the kidneys of the different animal groups excluding the control group. Liver represents a suitable model for monitoring the effects of a diet, due to its key role in controlling the whole metabolism. The changes in the liver, as a site responsible for bio transformation and detoxification, suggest alterations in the metabolic processes. Histopathological examination of liver sections showed inflammatory changes in hepatocytes of rats fed the with GM diet for 40 days. Disturbed (inflamed and necrotic) liver tissue as well as abnormal loss of cellular morphology and components with subsequent replacement by connective tissues was observed. This agrees with the work of Vandomois *et al.* (2009) who reported that the modified maize varieties MON863, MON810, and NK603 had toxic effects on the liver and kidney in mammals. El-Shamei *et al.* (2012) also reported that rats fed on GM corn for 90 days showed histopathological changes in the liver, kidney, and testis. Similarly, Oraby*et al.* (2015) and Eissa*et al.* (2019) also reported similar observations of inflamed liver tissue with necrotic areas in rats fed with GM diet. The changes in the liver, an organ responsible for bio transformation and detoxification of GM fed-rats showed irregularly shaped nuclei, which generally represent an index of high

metabolic rate, and a higher number of nuclear pores, suggestive of intense molecular trafficking Oraby *et al.,* (2015).

Data from the serum biochemical analysis showed significantly higher activity levels of enzymes used to assess liver function such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in rats fed with improved cowpea variety compared to the control and is significantly different from the local cowpea fed groups. Elevated values of these biomarkers of liver injury were observed in the GM fed group further supporting results of the histopathological analysis. The biochemical results agree with previous reports (Malatesta *et al.*,2002; Walsh *et al*.,2013; Oraby *et al.,* 2015; Eissa*et al.,*2019) who all reported altered (elevated) values of these enzyme biomarkers which are significantly different from the group fed with non-GM diet.

There were signs of focal inflammations (glomerular hypertrophy as seen by loss of capsular space), congestion of red blood cells and hemolysis in the kidney tissues of rats fed with GM diet-improved cowpea compared to those fed non-GM feed (local cowpea). This observation has been reported by previous researchers who worked on other types GM diets (De Vendômois*et al.,* 2009; Oraby*et al.,* 2015; Eissa *et al.,* 2019). Poulsen *et al.* (2007) reported similar findings in kidney histological analysis in rats fed GM rice for 90 days.

The histopathological results were further given credence by the biochemical analysis of the kidney. In this study, the blood creatinine and uric acid concentrations increased significantly in rats fed with GM diet for 40 days. Elevated creatinine and uric acid levels indicated an impaired kidney function or kidney disease (Chawla and Kellum, 2012). Significant changes in creatinine levels have also been reported by Kilic and Akay, (2008), Walsh *et al.* (2013), Oraby *et al.* (2015) and Eissa*et al.* (2019).

The biochemical results of the protein

content of the liver and kidney tissues were consistent with those obtained from the histopathological analysis. Feeding rats with GM diet for 40 days caused a significant drop in the protein and albumin contents of the liver and kidney samples analyzed, which is indicative of dysfunction in these tissues (Oraby*et al.,*2015). The reductions in protein contents agree with Schroder *et al.* (2007), Oraby *et al.* (2015) and Eissa *et al.* (2019). The abnormal protein content results of the organs examined in the GM-fed groups indicated abnormal cellular activities in these groups. It is widely known and accepted that proteins are not only a major structural component of cells but also as enzymes which mediate metabolic processes within cells. Thus, the nature and quantity of proteins present within any individual cell determine the nature and rate of activity of that cell.

The serum electrolytes test panel consists of measurements of sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-). This test is usually carried out to assess water and pH balance in the body and assess kidney injury/ disease amongst several uses (CDC, 2016). Sodium (Na^+) values of GM cowpea fed group are significantly different from control group and the group with fed local cowpea variety, though still within the acceptable range of 135 to 145 mmol/L (CDC, 2016). Potassium (K^+) is significantly higher in group fed with GM cowpea than that of control group and relatively higher than acceptable range of 3.5 to 10.0 mmol/L (CDC, 2016). Serum chloride (Cl^-) for group fed with improved cowpea though significantly different from control group is still within the acceptable range of 45-140 mmol//L.

The electrolyte values provide further clarification to the histopathological, biochemical and histochemical analyses, showing the extent of damage to the liver and kidney. The results of histopathological and biochemical analyses from this study is suggestive that GM cowpea might not be

completely safe and as reported by Goodman *et al*. (2011), they can cause allergic reactions which may be due to other proteins present in them . Furthermore, allergic reactions or certain adverse reactions to GM cowpea may be due to the proteins activated during digestion which may trigger the adverse reactions and not necessarily the transgenes themselves.

Conclusion

There were observed changes in all samples of the liver and in all preparations of the kidneys of the different animal groups excluding the control group. The results of histopathological and biochemical analyses from this study suggests that GM cowpea might not be completely safe due to the potential allergic reactions they can cause in some hypersensitive individuals. This could be due to the proteins activated during digestion and not the transgenes.

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