

*Full Length Research Paper*

# **Acute toxicity profile of ethanolic extracts of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC in rats: Medicinal plants used in the treatment of arterial hypertension in Beninese traditional medicine**

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***Croton gratissimus* Burch. and *Schrankia leptocarpa* DC. are two plants from the Beninese flora which are traditionally used for the treatment of arterial hypertension. The present study investigated the acute toxicity of ethanolic extracts of these plants. The experiment was conducted according to OECD guideline 423 categories 5 using a single dose of 5000mg/kg of the extracts. The average lethal doses (LD<sub>50</sub>) of the extracts are higher than 5000 mg/kg body weight. The oral administration of *C. gratissimus* and *Schrankia leptocarpa* extracts to the rats of all groups provoked a significant decrease in the plasma levels of AST ( $P < 0.05$ ) compared to the control batch between day 1 and day 14. There was no significant alteration in the creatinine levels in the all treated groups. The authors results showed that acute treatments with *C. gratissimus* and *Schrankia leptocarpa* extracts significantly ( $p < 0.05$ ) elevated serum total protein. However, the administration of the *Schrankia leptocarpa* extract to rats resulted in a statistically significant decrease ( $p < 0.05$ ) in WBCs, GRA in the different batches between day 1 and day 14. The *Schrankia leptocarpa* extract caused a significant increase ( $p < 0.05$ ) in MCV, in haematocrit, in blood platelets between day 1 and day 14. The *C. gratissimus* extract caused a significant increase ( $p < 0.05$ ) in blood platelets, in neutrophil in the different batches between day 1 and day 14. It appears that the extracts can be used therapeutically and that *C. gratissimus* may have hepatoprotective and immunostimulatory effects. Finally, *Schrankia leptocarpa* in addition to an immunostimulant effect could prevent microcytic anaemia.**

**Key words:** *Croton gratissimus*, *Schrankia leptocarpa*, acute toxicity, lethal doses.

## INTRODUCTION

In all regions of the world, and more specifically in Africa and in some less developed countries in Asia, plants are used to treat both communicable and non-communicable diseases (Diallo et al., 2003; Gurib-Fakim et al., 2006; Boukandou et al., 2015; Lawaly et al., 2019). For this reason, for some years now in Africa, the public authorities have been looking into the formalization of so-called traditional medicine. Also, several steps have been taken to experiment with the preventive and curative powers of different medicinal plants from the African flora. In several countries, plants such as *Moringa olifera*, *Kaya senegalensis*, *Annona muricata* and others are used for their therapeutic virtues. Indeed, most of these natural products used in traditional medicine have solid scientific evidence regarding their biological activities (Prado-Ochoa et al., 2014; Mbiri et al., 2017; Tang et al., 2017; Kognou et al., 2018). However, the great challenge of this medicine is to provide sustainable scientific data on the degree of toxicity of its products in order to establish the conditions for their safe use. No drug should be used clinically without clinical trials and toxicological studies (Anisuzzaman et al., 2001; Kasthuri and Ramesh, 2018). The OECD guidelines allow toxicological studies to be carried out in relation to the use of different doses in order to estimate LD<sub>50</sub> values. This line allows toxicity tests to be carried out according to well-defined categories and related to specific doses. The limit dose of 5000 mg/Kg corresponds to category 5, the so-called danger category. Such a test is only possible when there is a strong probability that the results will be important for the protection of human and animal health or the environment (OECD, 2001). *Schrankia leptocarpa* DC and *Croton gratissimus* Burch are two plants of the Beninese flora traditionally which are used in the treatment of arterial hypertension. The present study is conducted in order to evaluate the degree of toxicity of these two plants according to category 5 of the OECD guideline 423. The results will have the advantage of removing any ambiguity regarding their medicinal application.

## MATERIALS AND METHODS

### Plant material

The two plants were selected in July 2018 in the agro-ecological zone of Abomey-calavi in southern Benin. They were certified at the National Herbarium of Benin by comparison with reference samples kept under the numbers AA 6744/HNB for *C. gratissimus* Burch and AA 6745/HNB for *Schrankia leptocarpa* DC.

### Drying and spraying

The leaves of *C. gratissimus* Burch and the leafy stems of *Schrankia leptocarpa* DC after harvesting were taken to the Physiopathology/Pharmacology and Molecular Toxicology laboratory of the University of Abomey-calavi for drying at about 23°C for seven weeks and then ground. Greenish coloured powders were obtained.

### Preparation of extracts

Ethanolic extracts were made following the method used by Danda et al. (2017):

**Ethanolic extract of *Croton gratissimus* Burch.:** 208.7 Grams of *C. gratissimus* Burch. powder was macerated in 1000 ml of 95° ethanol, and passed through sonicator before filtering. The residues obtained after filtration were then successively macerated and sonicated two more times after adding 750 ml of 95° ethanol twice. The filtrates were passed to the rotary evaporator in order to isolate the solvent from the crude extract. The crude extract obtained was placed in an oven at 45°C.

**Ethanolic extract of *Schrankia Leptocarpa* DC:** 130 grams of *Schrankia Leptocarpa* DC. powder was macerated in 400 ml of 95° ethanol and passed through a saponifier before being filtered. The residues obtained after this filtration were then subjected to a series of macerations, sonification and filtrations with decreasing volumes ranging from 400 to 150 ml. The filtrates were passed to the rotary evaporator to separate the crude extract from the solvent. The crude extracts are placed in an oven at 45°C.

### The animals in the experiment

The animal material consisted of male albino rats of the Wistar strain. The rats were bred at the animal house of the Institute of Applied Biomedical Sciences (ISBA) of the Faculty of Science and Technology of the University of Abomey-Calavi. They were about 3 months old and they had an average weight of 211g. The rats are acclimatized in the Laboratory of Physiopathology/Molecular Pharmacology and Toxicology (Faculty of Science and Technology of the University of Abomey-Calavi) for two weeks before the beginning of the experiment at a constant temperature of 22±1 °C with a cycle of 12h of light and 12 h of darkness. They are fed with granulated feed and libitum water without discontinuity in feeding bottles.

### Ethical notice

The experimental protocol was approved by the Scientific Ethics Committee of the Doctoral School (Life Sciences) of the Faculty of Science and Technology (FAST) at the University of Abomey Calavi (UAC) under the number (UAC/FAST/EDSV/10489712 has authorized this study.

### Acute toxicity study

The tests were performed in accordance with the guidelines of the

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Organization for Economic Cooperation and Development (OECD) for the testing of chemicals substances through Method 423 (OECD, 2001). The acute study lasted 14 days with an increase in the number of animals to 6 per batch. Three batches were made up. Batch 1 was the control lot. Lots 2 and 3 were the experimental lots.

The toxicity assessment consisted of administering the limit dose of 5000 mg/Kg to the experimental batches as a single dose and observing certain parameters such as mobility, respiratory rate, appetite, somnolence, asthenia, body mass and mortality over the experimental period. Prior to the administration of the extracts, the animals were subjected to a 12h fast during which they had no access to food. Gavage was performed per site with the aid of an endogastric tube. Before dose administration, the body weight of each animal was determined and the dose was calculated according to the body weight. The ethanol extract was dissolved in distilled water and administered to the rats at a ratio of 1 ml/100 g of body weight. After administration, the batches were subjected to a period of observation for the first six hours in order to record changes in the above-mentioned parameters. This observation continued on a daily basis until the end of the experiment on day 14.

### **Weight and blood sampling**

Weight measurements were taken on D0, D4, D8 and D12 to assess the evolution of the animals' mass. Two samples were taken from the rats of the different batches during the experiment. The first sample was taken 24 h after the administrations and the last one on day 14. The authors followed the protocol described by Danda et al. (2017) with some modifications for each sample. Blood was collected from the retro-orbital sinus after anaesthesia. The animals were held in lateral decubitus with one hand and held by the skin of the neck. Thumb pressure on the neck, behind the angle of the jaw, allowed compression of the jugular vein, and thus venous stasis towards the head, favouring the filling of the retro-orbital sinus. By gently pulling on the upper eyelid with the index finger, it created an exophthalmos facilitating blood sampling with a non-heparinised haematocrit tube. The tip of the tube was slowly inserted into the lateral angle of the eye. The progress through the tissue is facilitated by rotating the pipette slightly. As soon as the venous plexus is reached, the blood gushes into the periorbital space and rises by capillary action into the tube. For each animal, the blood is collected in a dry tube and in an EDTA tube. Before the tube is removed, the compression is released and the bleeding stops spontaneously when the eye pressure normalises. The recovered blood samples were used for the determination of different biochemical parameters on the one hand and haematological parameters on the other.

### **Biochemical and haematological analyses**

Many biochemical markers of toxicity were evaluated. The authors measured transaminases (AST and ALT), creatinine, total bilirubin, total protein and serum urea. The Spectrum brand pack was used for the different assay.

The blood count was made up of: White Blood Cells (WBC), Lymphocyte, Neutrophil (Mid), Granulocyte, Red Blood Cells or RBCs, Haemoglobin (HGB), Haematocrit (HCT), Mean Cell Volume (MCV), Haemoglobin Corpuscular Content (HCM), Mean Corpuscular Concentration of Haemoglobin (MCHC), RDW-CV, RDW-SD, Platelet (PLT), Mean Platelet Volume (MPV), PDW and PCT. The hematological examinations are carried out using a SYSMEKX-N21 automaton according to the method used by Sodipo et al. (2012).

### **Histopathological examination of the liver and kidney tissues**

At the end of the experimental period, two rats were randomly selected from each batch for histological analysis. Macroscopic examination of vital organs was carried out soon after sacrifice. Liver and kidneys were surgically removed and immediately forwarded to the histopathological processing. The fragments of livers and kidneys were fixed in a 10% buffered formalin solution, included in paraffin wax, cut into 3-4  $\mu\text{m}$  sections, and stained with hematoxylin and eosin. The histological sections were then visualized under optical microscopy (Optika Microscopes, Italy) and captured by an Infinity 1 camera microscope with  $\times 40$  magnifications. Histopathological analysis consists of the observation of tissue integrity, searching for injuries such as degeneration, necrosis, apoptosis, and infiltration of leukocytes which could indicate signs of toxicity.

### **Statistical analysis**

The Mitab 16 software under Windows was used to carry out the statistical analyses. The results were expressed as mean $\pm$ standard. The one-way analysis of variance (one-way ANOVA) with Fisher's test was used to express the degrees of significance of the different treatments in relation to the control and between the experimental batches. Differences were significant for  $P < 0.05$ .

## **RESULTS**

### **Extraction yields**

The yields were calculated using the following formula:

$$R = (Me \times 100) / Mv$$

R=Yield

Me=Mass of extract obtained

Mv = Vegetable matter used

Yields of 8.56% were obtained for *C. gratissimus* Burch. and 6.78% for *Schrankia leptocarpa* DC.

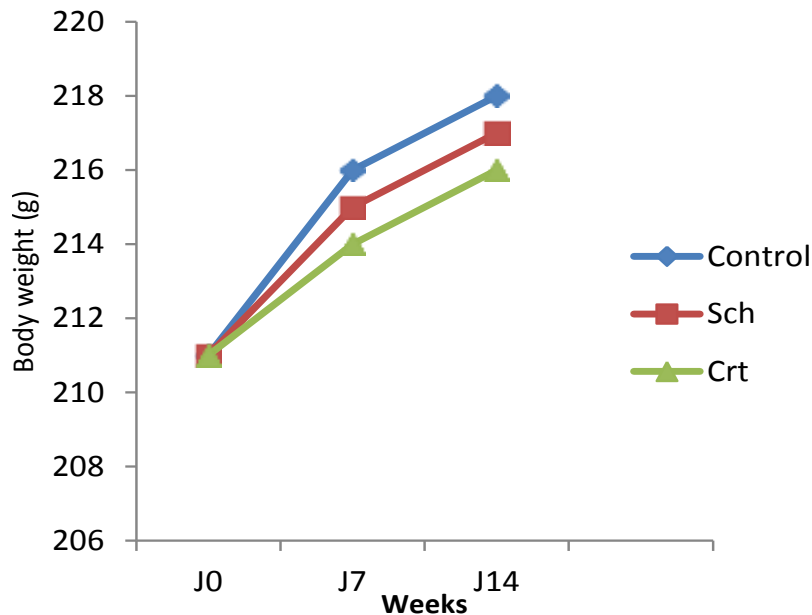
### **Acute toxicity**

#### **Clinical signs observed**

Observations made within the first six hours after administration of the extract showed no signs of intoxication in the control rats. On the other hand, in the experimental batches, we were able to observe some slight signs of drowsiness, respiratory effort and poor motor skills. The authors also noted a relative fatigue in the rats as well as a loss of appetite. However, one death was observed in the lot given 5000 mg/kg of the ethanolic extract of *C. gratissimus* Burch. 48 h after administration. None of the rats were given an equal dose of the ethanolic extract of *Schrankia leptocarpa* DC.

### **Body weight**

A relative increase in body weight was noticed in the control and experimental batches (Figure 1). Statistical



**Figure 1.** Body weight changes of rats have been treated with ethanolic extract of *Croton gratissimus* and *Schrankia leptocarpa* in the acute toxicity study. The results are presented as the means  $\pm$  standard deviation ( $n=6$ ).

analysis revealed that these differences were not significant ( $p>0.05$ ) in all cases.

### Biochemical parameters

The results of the clinical biochemistry parameters assessed in this work, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, total protein, total bilirubin and serum urea are summarised in Figure 2 and Figure 3. Figure 2a and b shows the evolution of the serum AST and ALT level between day 1 and day 14 in the different batches. The daily oral administration of *C. gratissimus* and *Schrankia leptocarpa* extracts to the rats of all groups provoked a significant decrease in the plasma levels of AST ( $P < 0.05$ ) and ALT ( $P < 0.05$ ) compared with the rats of the control group. After 14 days of treatment, there was no significant alteration in the creatinine and serum urea levels in the all treated groups compared to control group (Figure 3a and d). Figure 3b shows the evolution of the serum total protein level. There was a highly significant increase ( $p < 0.05$ ) in the serum total protein compared with the rats of the control group. Figure 3c shows the evolution of the serum total bilirubin level. It shows a significant decrease ( $p < 0.05$ ) in the batch tested with *C. gratissimus* Burch.

### Hematological parameters

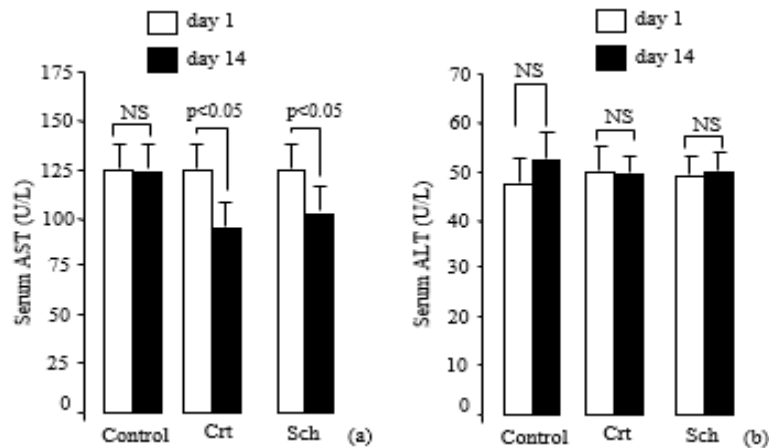
The analysis of hematological parameters, which included Hb, RBCs, Gran, Lymph, MCH, MCHC, MPV in

rats treated with *C. gratissimus* and *Schrankia leptocarpa* extracts did not differ significantly in the different batches between day 1 and day 14 (Figure 4a to g).

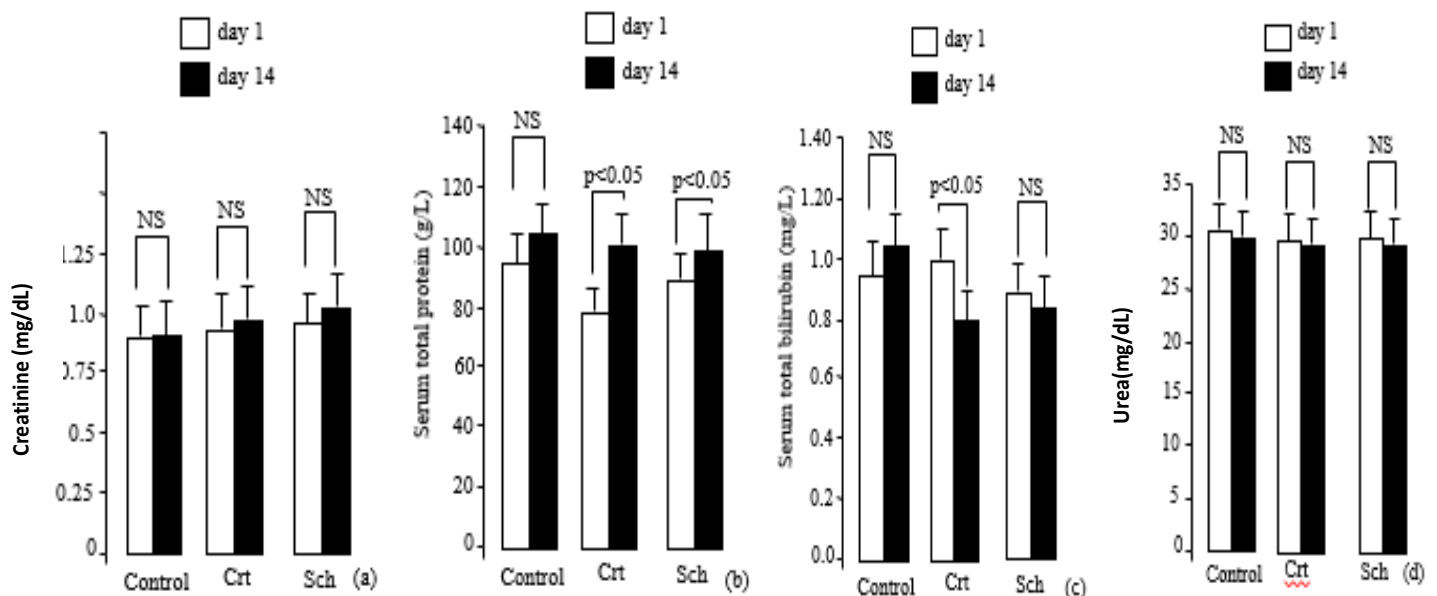
However, the administration of the *Schrankia leptocarpa* extract to rats resulted in a statistically significant decrease ( $p < 0.05$ ) in WBCs, GRA in the different batches between day 1 and day 14. The *Schrankia leptocarpa* extract caused a significant increase ( $p < 0.05$ ) in MCV, a significant increase ( $p < 0.05$ ) in haematocrit, a significant increase ( $p < 0.05$ ) in blood platelets and a highly significant increase ( $p < 0.05$ ) in the different batches between day 1 and day 14. Hematological parameters show significant differences between control and rats treated with *C. gratissimus* extract on day 14 after the administration. The *C. gratissimus* extract caused a significant increase ( $p < 0.05$ ) in blood platelets, a highly significant increase ( $p < 0.05$ ) in neutrophil in the different batches between day 1 and day 14. The other parameters did not reveal any significant differences.

### Histopathological changes

Histology of the liver sections of control rats showed normal hepatocellular architecture along with well-preserved hepatic cells, and visible central veins and no histologic abnormalities (Figure 5a). In rats treated with the extract of *C. gratissimus* (5000 mg/kg) and the extract of *Schrankia leptocarpa* (5000 mg/kg), there was not found adverse effect on the histoarchitecture of hepatocytes (Figure 5b and c).



**Figure 2.** Effect of acute oral administration of *Croton gratissimus* and *Schrankia leptocarpa* extracts on (a) AST (U/L), (b) ALT. Parameters were determined as described in research methods and procedures section. Values are means  $\pm$  SEM,  $n = 6$  per group of animals. NS insignificant differences. AST: aspartate aminotransferase; ALT: alanine aminotransferase; Crt: *Croton gratissimus* Burch; Sch: *Schrankia leptocarpa* DC.

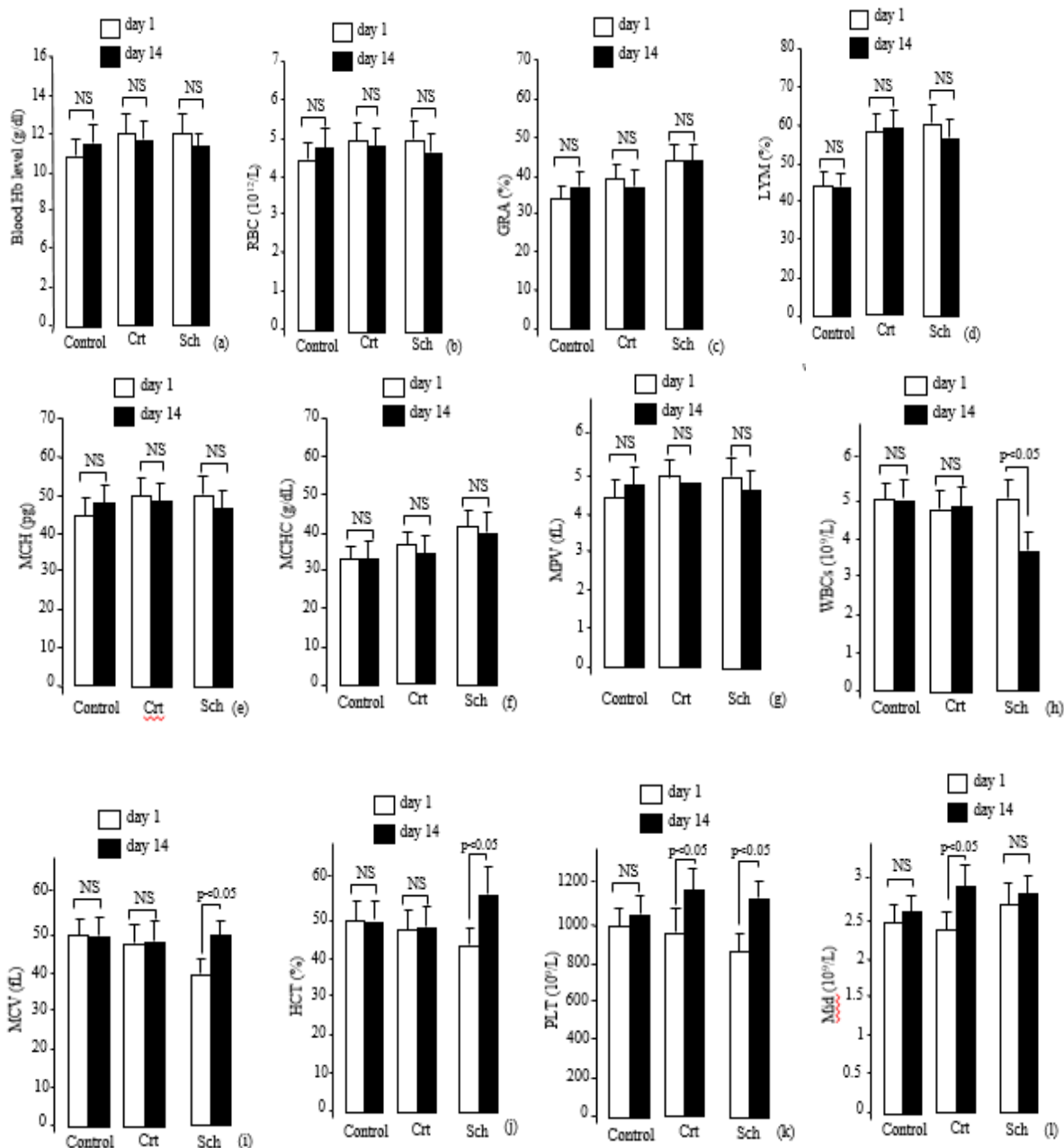


**Figure 3.** Effect of acute oral administration of *Croton gratissimus* and *Schrankia leptocarpa* extracts on (a) Creatinine, (b) Serum total protein, (c) Serum total bilirubin and serum urea (d) of rats. Parameters were determined as described in research methods and procedures section. Values are means  $\pm$  SEM,  $n = 6$  per group of animals. NS insignificant differences. Crt: *Croton gratissimus* Burch; Sch: *Schrankia leptocarpa* DC.

The liver parenchyma of the rats treated with the different extracts retained the typical appearance as in the control rats. The hepatocytes (arrows) are typical and organised in cords around the centrilobular veins (CV). Between the hepatocyte cords are the clearly visible venous sinusoids (S).

Normal histology of rat kidneys (glomeruli, tubules, interstitium, and blood vessels) was found in the control

group (Figure 6E), and the group treated with the extract of *C. gratissimus* (5000 mg/kg), and rats treated with the extract of *Schrankia leptocarpa* (5000mg/kg) (Figure 6 (F) and 6 (G)). The renal parenchyma of the rats treated with the different extracts has the normal architecture as in the control rats. The renal glomeruli (G), proximal and distal renal tubules (TR) and collecting ducts (CC) are without visible atypia.

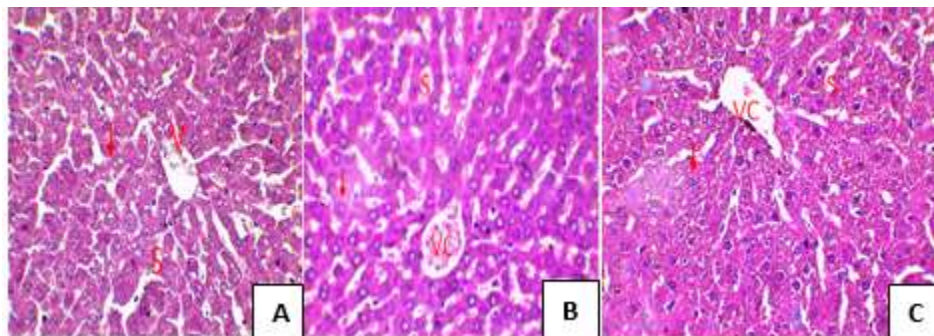


**Figure 4.** Effect of acute oral administration of *Croton gratissimus* and *Schrankia leptocarpa* extracts on hematological parameters of rats. Values are means  $\pm$  SEM, n = 6 per group of animals. NS insignificant differences. Crt: *Croton gratissimus* Burch; Sch: *Schrankia leptocarpa* DC. Hb: hemoglobin, RBC: red blood cells, GRA: granulocytes, LYMP: lymphocytes, MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MPV: mean platelet volume; WBCs: white blood cells, MCv: mean corpuscular volume, HCT: hematocrit, PLT: platelets, Mid: neutrophils.

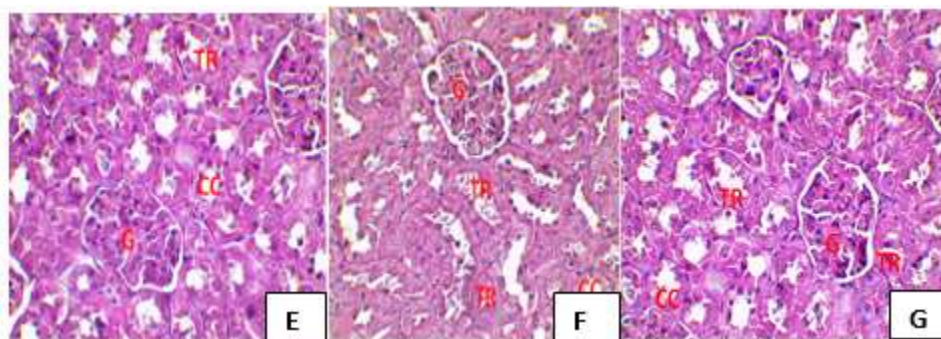
## DISCUSSION

The present study showed that ethanolic extracts of *Schrankia leptocarpa* DC. and *C. gratissimus* Burch. did not induce weight depressions compared to the control. There was indeed a non-significant increase in body weight in the experimental batches. This non-significant

increase shows on the one hand that the extracts do not have an anorexic effect on the rats. On the other hand, the non-significance of this weight increase also reveals that the extracts do not induce a hyper acceleration of the activity of the adipocytes. Indeed, it has been reported that abnormally excessive growth of adipose tissue due to the biological effects of chronic stress can lead to



**Figure 5.** Histopathology of the liver. Light micrographs of the liver sections from different treatment groups. The numbers on the images represent the treatment groups. (A) Control rats, (B) rats treated with the extract of *C. gratissimus* (5000 mg/kg), (C) rats treated with the extract of *Schrankia leptocarpa* (5000mg/kg).



**Figure 6.** Effect of the extract on kidney histology in rats. Histological sections were visualized by staining with hematoxylin and eosin (H and E) and observed by optical microscope (OPTIKA Microscopes, Italy) with magnification  $\times 40$ . (E) Control rats, (F) rats treated with the extract of *C. gratissimus* (5000 mg/kg), (G) rats treated with the extract of *Schrankia leptocarpa* (5000 mg/kg).

obesity, which is caused by an imbalance between food intake and energy expenditure (Schulte et al., 2007; de Santé et al., 2011). Furthermore, the change in body weight is used as an indicator of the adverse effects of chemical compounds (Hilaly et al., 2004; Boussahel et al., 2018). Ethanolic extracts of *Schrankia leptocarpa* and *C. gratissimus* are reported to have no adverse effects.

The assessment of liver and kidney activity in toxicity studies is of paramount importance as the liver is the site of biotransformation of ingested substances and works in conjunction with the kidneys to remove toxic substances from the blood (Rhiouani et al., 2008). Khan and Zafar reported in 2005 that changes in blood parameters were related to stress, infection or intoxication (Khan and Zafar 2005; Mwale et al., 2014).

Among the biochemical parameters evaluated, AST, ALT are considered markers of liver function (Feres et al., 2006; Arneson et al., 2007; Obici et al., 2008). Hepatocellular damage is characterized by a mutual rise in serum levels of AST and ALT. But since ALT is

localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than AST and within limits can provide a quantitative assessment of the degree of damage sustained by the liver (Bowman et al., 1980).

No significant effects have been observed in creatinemia and urea recorded in the different batches tested. In fact, an increase in serum creatinine can be seen several days after the onset of the first renal lesions (Cheyron et al., 2008). This result is confirmed by the appearance of the renal parenchyma.

ALT is an enzyme synthesised by hepatocytes and released in the event of hepatocellular injury or necrosis. ALAT is expressed in very low concentrations in other tissues and is considered specific to hepatocellular injury (Bragança et al., 2017). In the present study, administration of *Schrankia leptocarpa* resulted in a significant increase in ALT, which may reflect hepatotoxicity. However, the appearance of the hepatocytes revealed by histological examination of the

liver shows that this hepatotoxicity was reversible as the liver parenchyma did not show atypia. Serum bilirubin is also considered a true test of liver function, since it reflects the liver's ability to take up, process, and secrete bilirubin into the bile. Administration of *C. gratissimus* resulted in a significant decrease in total bilirubin and AST. It can therefore be inferred that the extract did not have any deleterious effects on hepatic metabolism or biliary excretion. The same can be said of serum bilirubin in extract-treated groups. These observations seem to display that *C. gratissimus* may have some hepatoprotective effects. *C. gratissimus* could help treat jaundice by offering protection on the liver, improving its bilirubin conjugating property and helping clear bilirubin from circulation. The following mechanisms could be suggested for the bilirubin-lowering potential of *C. gratissimus*. It could be suggested that *C. gratissimus* activated the Constitutive Androstane Receptor (CAR), a key regulator in the bilirubin clearance pathway (Huang et al., 2004), increasing the activity of glucuronyl transferases (Ostrow et al., 2003), synthesis of ligandin, a transporter of bilirubin, increasing its transport to the liver for conjugation (Greige-Gerges et al., 2007). Also, *C. gratissimus* could inhibit the activity of haem oxygenase, the rate limiting enzyme of the bilirubin pathway, reducing serum total bilirubin in treated animals.

Nevertheless, further studies need to be carried out to scientifically establish this speculated hepatoprotective effects.

There was a significant increase in the level of ALT of rats administered with ethanolic *Schrankia leptocarpa* extract compared to control. ALT is a hepatospecific enzyme that is principally found in the cytoplasm in rats (Benjamin et al., 1978; Ringer and Dabieh, 1979) and is a specific marker for hepatic injury. The increase in the level of ALT therefore indicates hepatic injury (biochemical or pathological) which has not reflected on the histology of the liver.

Significant levels of serum total protein were observed in the study. Serum protein largely regulated via synthesis in the liver and reflects synthetic ability of the liver (Mabeku et al., 2007). An increase in serum concentration of total protein resulting from treatment with the extract may suggest stimulation of their synthesis or inference in feedback or in mobilization pathways associated with this organ. However, these increases can not be attributed to liver damage as no histological lesions were observed in the hepatic tissue during the administration of this extracts.

An abnormally high level of protein in the blood (hyperproteinemia) may indicate too little water in the blood (dehydration) or an increase in the gamma globulin (antibody) fraction, as in chronic inflammatory diseases, certain viral infections or bone marrow disorders (multiple myeloma) (brion.com). The extracts could therefore have an immunostimulatory effect. Apart from biochemical parameters, the haematopoietic system is one of the

most sensitive targets of toxic substances (Hajjaj et al., 2017). Therefore, any change in haematological parameters has predictive value for human intoxication, when the data are translated from animal studies (Rhiouani et al., 2008; Hajjaj et al., 2017). White blood cells are a group of immune cells that play a crucial role in the efficiency of the immune system. Following the administration of an extract, an increase in the number of white blood cells reveals the stimulating effect of the extract on the immune system (Allouni, 2018). Similarly, granulocytes are the main cells involved in non-specific immune responses after infection and thus in phagocytosis of bacterial pathogens (Sivagurunathan et al., 2011). The significant decreases in white blood cells and granulocytes observed with the administration of *Schrankia leptocarpa* show that the extract weakens the immune system to some extent.

However, knowing that neutrophils constitute an important category of granulocytes, their highly significant increase with the same extract is rather in the direction of strengthening the immune system through an immunostimulant effect.

It is also possible that the extract contains agents that stimulate the bone marrow to produce neutrophils and release them into the blood. Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms (Ganong., 2005). The granules of the neutrophil contain many enzymes, which makes it a powerful and effective killer machine and, hence, deficiency of neutrophils in the body leads to myriad defects, including conditions such as chronic granulomatous disease. This effect on neutrophil count may be partly responsible for the claim that *C. gratissimum* has antibacterial actions (Akinyemi et al., 2005; Lopez et al., 2005).

The decrease observed would therefore be that of other granulocyte families such as basophilic granulocytes and eosinophilic granulocytes (Dupont et al., 2018). Indeed, eosinophilic granulocytes are involved in allergic reactions and basophilic granulocytes in immediate hypersensitivity reactions (Cloutier et al., 2014). Blood platelets are cells that play a major role in the processes of primary haemostasis and play a key role in both innate and adaptive immunity (Nguyen, 2013). They are now recognised for their role in immunity, particularly in antiviral immunity (Flaujac et al., 2010; Berthet et al., 2010). Their significant increase following the administration of *Schrankia leptocarpa* indicates a beneficial effect in the maintenance of primary homeostasis and specific immune defence. Furthermore, decreases in mean corpuscular concentration (MCHC) and mean corpuscular volume (MCV) reflect iron deficiency anaemia states (de Santé et al., 2011). From the results obtained, the extracts can induce iron deficiency anaemia. However, the significant increase in MCV in the different batches would indicate the



occurrence of macrocytic anaemia. Macrocytic anaemia is a type of anaemia where the average red blood cell volume is greater than normal (Maner and Moosavi, 2019).

The administration of *Schrankia leptocarpa* caused an increase in the erythrocyte count.

This was confirmed by the increased hematocrit. In normal circumstances, local tissue anoxia apparently leads to the formation of a glycoprotein called erythropoietin, which stimulates increased production of erythrocytes (Arneson et al., 2007). It is very likely that *Schrankia leptocarpa* leaves extract contains erythropoietin-like agent(s) which is/are responsible for the increased production of erythrocytes. Similar results were obtained for thrombocytes (platelets). There was thrombocytosis in the group that received *Schrankia leptocarpa* dose of the extract. Therefore, it would seem likely that the extract also contains some compounds that are capable of causing the release of a thrombopoietin (Erslev et al., 1979).

This condition is confirmed by the significant increase in haematocrit with *Schrankia leptocarpa* extract. Indeed, the measurement of haematocrit makes it possible to objectify a possible anaemia and to evaluate the haemoconcentration of the blood (Brakch et al., 2011; Boudras, 2020). The extracts would thus induce an increase in the production of red blood cells.

Finally, the present study enabled the authors to determine the LD<sub>50</sub> of the two plants. The LD<sub>50</sub> is in its simplest form and the dose of a compound that causes 50% mortality in a population of animals tested. This means that they have received a single administration of a product under well-defined experimental conditions (Boussahel et al., 2018).

According to OECD guideline 423, when a test at 5000 mg/kg is required, only one step (with three animals) is required. If only one of the three animals dies, the LD<sub>50</sub> value is estimated to be higher than 5000 mg/kg (OCDE, 2001).

The authors therefore were able to establish that the LD<sub>50</sub> of their two plants is greater than 5000 mg/kg. According to Diezi's (1989) classification, both plants are non-toxic and could therefore be used therapeutically.

## Conclusion

The results suggest that acute administration of ethanolic extracts of *Schrankia leptocarpa* and *C. gratissimus* are not endowed with any signs of toxicity. Biochemical and haematological tests and histological study showed that the extracts have beneficial effects on the liver, kidney and immune system.

They can therefore be used in the treatment of hypertension.

Finally, studies on therapeutic doses should be continued, particularly on *Schrankia leptocarpa* because of the increase in ALT levels that it induces.

## CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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