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Toxicity Assessment of the Crude Ethanolic Pod Extract of *Swartzia madagascariensis* Desv. in Rats

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Abstract

This study assessed toxicity of the crude ethanolic pod extracts of *Swartzia madagascariensis* Desv in Wistar rats. Forty (40) Wistar rats aged 8–10 weeks were orally administered with crude extracts from pods of *S. madagascariensis*. Chemical analysis of serum and histopathology of liver and kidney from test animals were performed to determine the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine in serum. In addition, serum albumin levels were also determined. The levels of ALT, AST and creatinine were found to be high in groups treated with crude extracts compared to the control group, suggesting some damage in liver cells and kidney of treated groups of Wistar rats. The albumin levels in serum samples of crude extract treated groups were found to be low compared to control group, suggesting some leakage to the urine due to damage in the kidney. Histopathological analyses revealed damages in the liver and kidney treated with 1000 mg/kg of crude ethanolic pod extracts of *Swartzia madagascariensis* (CEPES) at days 2 and 14, corresponding to what was observed in the biochemical variables. The findings revealed that *Swartzia madagascariensis* pods are toxic once taken in large quantities (1000 mg/kg).

Keywords: Toxicity, Wistar rats, Histopathology, biochemical parameters.

Introduction

Plants play great roles for human beings, animals and the environment, such as providing food and medicines (Farnsworth and Soejarto 1991, Rasool Hassan 2012) and prevention of soil erosion (Abdallah and Monela 2007). Although plants are beneficial to animals and humans, they may also cause harm when wrongly identified and improperly prepared for consumption, especially when they contain toxic components (Adeneye et al. 2006, Nasri and Shirzad 2013). Plants produce secondary metabolites to adapt to their harsh environment; however, these phytochemicals may have biological activities to other organisms including animals which forage on these plants (Ha et al. 2018).

Swartzia madagascariensis Desv, which is also known as *Bobgunnia madagascariensis* in the word database of legumes, has also common names as "fule fule" or "mukowo" as called by Hehe of Tanzania. The family of this species is Leguminosae, which is among the plants that are used as fodder and medicines (Amna et

al. 2013). Because of its drought-resistant characteristics, *S. madagascariensis* is used as livestock fodder during dry seasons (Dewees et al. 2010, Sidi et al. 2015). The genus *Swartzia* is widely distributed from tropical America to tropical Africa, and so far, about 150 species have been described (Tucker 2003, Bunney et al. 2019). In Africa, *S. madagascariensis* is found in Cameroon, Gabon, Ivory Coast, Angola, Zaire, South Africa, Zimbabwe, Burkina Faso, and Tanzania (Kirkbride and Wiersema 1997, Chingwaru et al. 2020). In Tanzania, *S. madagascariensis* is widely spread in miombo woodlands regions such as Morogoro, Iringa, Katavi, Tabora, Manyara, and Lindi. Apart from being used as fodder for animals, the crushed pods of *S. Madagascariensis* have been reported to be harmful to fish, insects, and mollusks (Hostettmann et al. 1998, Neuwinger 2004, Yang et al. 2004, Stevenson et al. 2010). Also, the silver leaf whitefly (*Bemisia tabaci*), an important agricultural pest, has been controlled using extracts from *S. madagascariensis* (Georges et al. 2008).

There have been reports from livestock keepers from villages within Iringa Rural District at Mlangali, Mgera, Kiwere, and Kipera areas regarding cattle dying allegedly due to consumption of pods from *S. madagascariensis*. The previous studies have established the toxicity profile of *S. madagascariensis* pods in fish, mollusks, fungi, and insects. However, no studies had been carried out to evaluate the toxic status of pods of *S. madagascariensis* in mammals. Thus, the present study aimed at assessing the acute toxicity of crude ethanolic pod extract of Swartzia (CEPES) in Wistar rats to establish its toxicity profile in mammals. Findings from this study will enlighten farmers on toxicity of *S. madagascariensis* and hence develop strategies for obtaining pastures for their animals.

Materials and Methods

Collection of plant materials

Swartzia madagascariensis pods were collected from the study villages, namely; Mlangali (7°78'067"S, 35°67'862"E), Mgera (7°69'723"S, 35°62'096"E), Kiwere (7°64'388"S, 35°60'286"E), and Kipera (7°°66'446"S, 35°54'469"E). All the four villages are found in Iringa Rural District, Iringa, Tanzania. The plant as shown in Figure 1 was identified by the livestock keepers in the field and confirmed by a taxonomist from the Department of Biological Sciences, Mkwawa University College of Education (MUCE). Voucher specimen (AM-MC2021) was prepared and deposited at the department's herbarium. The collected pods were handled carefully, packed in plastic zipper bags and transported to the Department of Biological Sciences laboratory, MUCE for processing.

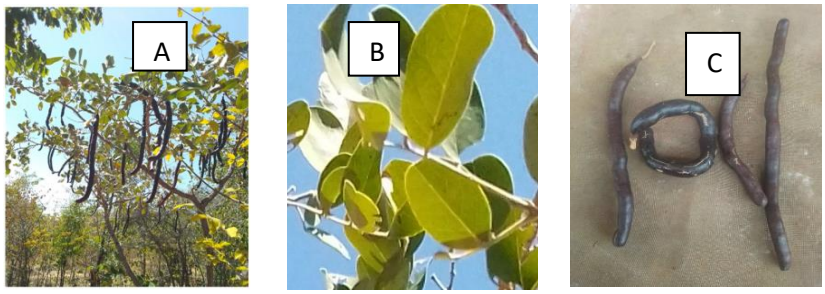

Figure 1: *S. madagascariensis* images; A: tree, B: leaves and C: pods.

Processing of plant materials

The pods were sorted and chopped into small pieces then shade dried at room temperature (22 ± 2 °C) for two weeks on a laboratory bench. The dried pods were packed into plastic zipper bags and transported to the Department of Veterinary Physiology, Biochemistry and Pharmacology, Sokoine University of Agriculture, where further processing was done. The pods were

ground into powder using 500 A multifunction grinder (China). The powder was packed in plastic zipper bags and properly stored till used for extraction.

Extraction of plant materials

Extraction was done following the procedure described by Barbosa et al. (2016) in which ethanol was used for extraction because it produces quality extract, but also is cost-effective. One kilogram of the powdered plant materials was extracted in ethanol at a ratio of 1:3 powder to solvent in a conical flask (5000 ml) to ensure complete soaking of the powder in the solvent. The mixture was left for 72 hours on a laboratory bench with frequent shaking. The mixture was then filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator, followed by complete evaporation of the remaining solvent in a water bath at 50 °C to obtain the crude extract. The obtained crude extract was stored in tight covered bottles and stored in a refrigerator at 4 °C till use.

Assessment of toxicity of pod crude extract in Wistar rats

Forty (40) mixed sexes Wistar rats aged 8–10 weeks obtained from Small Animal Unit, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture were used in acute toxicity assessment of crude ethanolic pod extracts of *Swartzia madagascariensis* (CEPES) via oral gavage. Criteria for selection were age as recommended by the Organization for Economic Cooperation and Development (OECD) test guideline number 423. The rats were allowed to acclimatize for 14 days while being fed normal rat food and given drinking water ad libitum. On the 15^{th} day, food was withheld and the rats were given only water ad libitum. Then, on the 16^{th} day, the rats were grouped into four groups randomly, with ten rats in each group in two replicas of five rats each. Groups were named A, B, C and D, whereby groups B, C and D served as treatment groups, while group A served as a control group. Three doses of CEPES were prepared at 10, 100, and 1000 mg/kg body weight and given to rats of groups B, C and D, respectively, while the control group was given drinking water only. The rats were then observed for any behavioural changes and death for 14 days (Halim et al. 2011).

Analysis of biochemical parameters

Following termination of the experiment, two rats per each group were sacrificed by a combination of ketamine and xylazine. The blood was collected by cardiac puncture in plain vacutainer tubes. The blood samples were then centrifuged at 4000 rpm (MPW M-Diagnostic, model; M-universal, Poland), to obtain serum which was transferred into Eppendorf tubes using micropipette. The serum samples obtained were used in assessment of liver and kidney functions using alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and albumin kits (Erba Lachema s.r.o). Levels of ALT, AST, creatinine and albumin in the serum were determined by reading the absorbance using a spectrophotometer (1100 RS Spectrophotometer, Unico).

Histopathological examination

Dead and sacrificed rats were immediately dissected; liver and kidney were checked for abnormalities particularly gross lesions and collected for histopathological examination (Halim et al. 2011). Organ samples which were collected for the histopathological examination were fixed in 10% neutral buffered formalin (NBF) then trimmed into small pieces and placed in the tissue cassette, and then subjected to manual tissue processing. The sections were prepared using Tat lock Rotary microtome (England), stained by haematoxylin and eosin and viewed under the light microscope (Optika Binocular, Italy) (Al-Afifi et al. 2018).

Data analysis

Means of weight of rats and levels of ALT, AST, creatinine and albumin were entered into Microsoft Excel and analysed using Statistical Package for Social Sciences (SPSS version 25). One way ANOVA was used to determine the differences in toxicity levels between treatment groups and Student

t-test used to compare each treatment group with the control, values at $p < 0.05$ were considered significant.

Results

Effect of CEPES on body weight of Wistar rats

Weight of rats administered 10 mg/kg and rats in a control group increased from day 1 to day 14. Weight of rats administered 100 mg/kg and 1000 mg/kg was reduced from day 1 to day 4. From day 9 to day 14, all the rats with different dose treatments increased in weight as shown in Figure 2. Weight of rats treated with CEPES was significantly reduced as compared to the control group ($p < 0.05$).

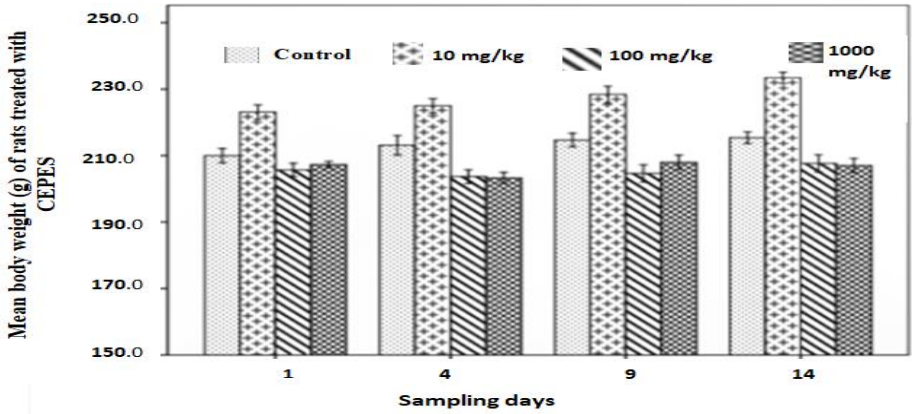

Figure 2: Weight of Wistar rats treated with different doses of CEPES.

Effect of CEPES on biochemical parameters in the serum of Wistar rats

The levels of ALT, AST and creatinine of rats that received CEPES was found to increase as compared to the control group, while the levels of albumin in serum were reduced in comparison to control group as indicated in Figure 3 A-D.

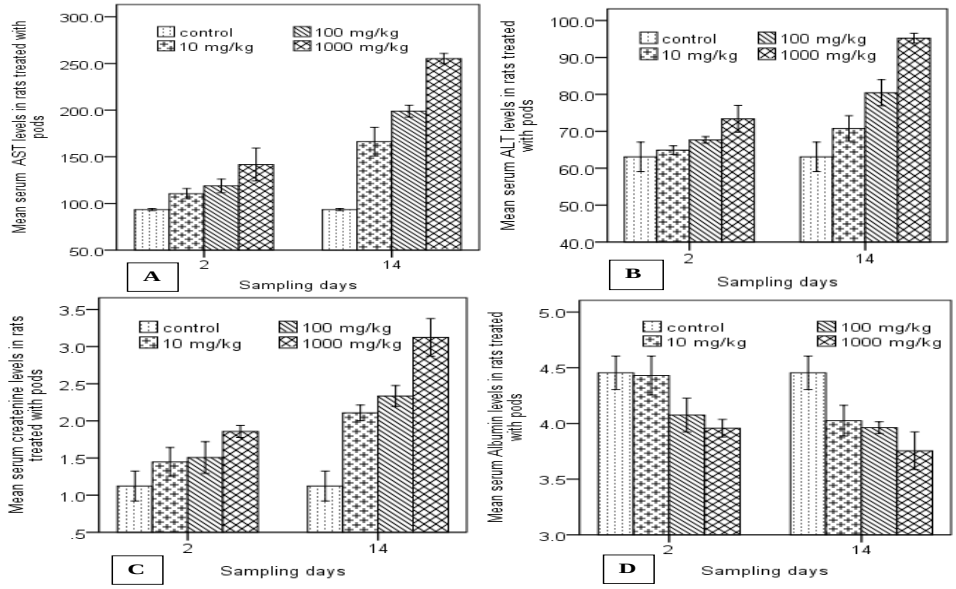

Figure 3: Levels of serum biochemical parameters in CEPES treated rats; **A** = AST (I/U), **B** = ALT (I/U), **C** = Creatinine, and **D** = Albumin.

Effect of CEPES on kidney and liver histopathology

Liver pathological features such as necrosis, vacuolization, inflammatory infiltrate, activation of Kupffer cells and congestion were observed in rats treated with 1000 mg/kg, but not 10 mg/kg and 100 mg/kg of CEPES as shown in Figure 4 A-D. Kidney pathological features such as necrosis, inflammatory infiltrate, distorted glomeruli, rupture of convoluted tubule and expansion of tubular lumina, were observed in rats treated with 1000 mg/kg, but not 10 mg/kg and 100 mg/kg of CEPES as shown in Figure 5 A-D.

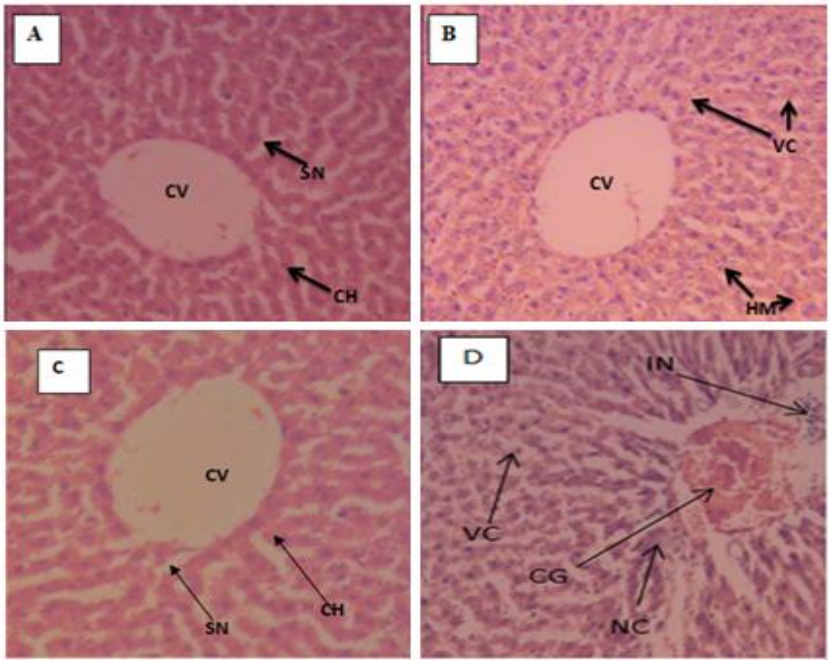

Figure 4 A-D: Liver sections stained with haematoxylin and eosin (400×) under Optika binocular light microscope. **A** is a control and **B** is liver section treated with 1000 mg/kg of CEPES at day 2, **C** is a control and **D** is liver section treated with 1000 mg/kg of CEPES at day 14. **CV** = Central vein, **SN** = Sinusoids, **HM** = Haemorrhage, **VC** = vacuolation, **CH** = cord of hepatocyte cells, **CG** = Congestion, **NC =** Necrosis, **IN =** Inflammatory infiltrate.

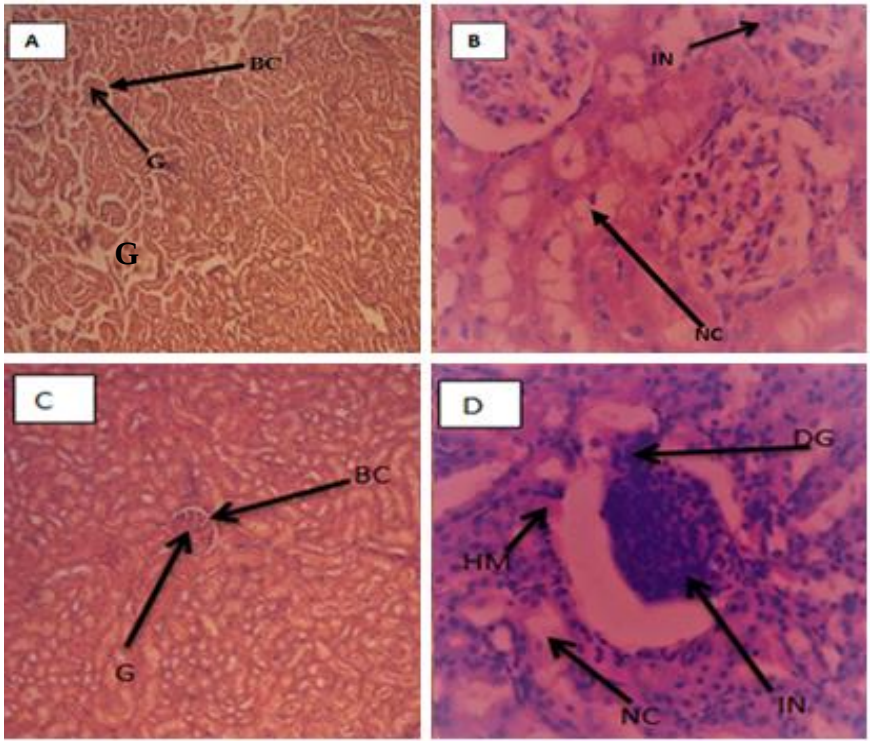

Figure 5 A-D: Kidney sections stained with haematoxylin and eosin (400×) under Optika binocular light microscope. **A** is a control showing normal histology and **B** is kidney section treated with 1000 mg/kg of CEPES showing abnormal histology at day 2, **C** is a control and **D** is kidney section treated with 1000 mg/kg of CEPES showing abnormal histology at day 14 . **G** = Glomerulus, **BC** = Bowman's capsule, **IN** = Inflammatory infiltrate, **NC** = Necrosis, **DG** = Degeneration of glomerulus, **HM** = Haemorrhage.

Discussion

The weight of rats administered with extract decreased significantly at $p < 0.05$ when compared to that of the control group. This could be attributed to the negative effects of the high doses of the extract on feed intake which in turn affected the weight of the rats administered with the extract. This is in agreement with York et al. (2007), who reported decreased weight of rats fed with an herbal extract and the decrease was due to reduced feed intake.

Evident behavioural changes such as ataxia, reduced feed intake, piloerection, difficult breathing and restlessness were observed in rats treated with high concentration of the extract, while rats in the control groups did not show any behavioural changes. These behavioural changes could be attributed to the toxic effects of the extract in study animals. When organisms are exposed to toxic materials, they manifest clinical signs which are observable as behavioural changes (OECD 2001, Nigatu et al. 2017).

Significant elevation of studied biochemical parameters (AST, ALT and creatinine) was observed in the serum of rats treated with the extract compared to the control groups. On the hand, significant decrease in the levels of albumin was observed in extract treated groups compared to the control group. The higher concentrations of AST and ALT in serum of extract treated rats could be due to the fact that, the liver cells were damaged by the phytochemicals present in the extract. The findings are in agreement with those from previous studies (Barbosa et al. 2016, Zhang

et al. 2016, Nigatu et al. 2017). The levels of creatinine in serum were found to be elevated in rats administered with the extract in comparison to that of the control group. This could be as a result of damage of the kidney tubules, while the levels of album in serum of Wistar rats treated with the extract were recorded to be lower compared to that of the control group. The decrease in albumin in serum from rats treated with extract could be due to the leakage of albumin into the glomerular filtrate. These findings are similar to what was reported in other studies (Barbosa et al. 2016). Histopathology of liver and kidney sections revealed toxic evident features such as necrosis, congestion, inflammatory infiltrates, haemorrhage and vacuolation. This confirmed the findings from biochemical analysis on the liver and kidney functions, and are in agreement with the findings other studies (Rangel et al. 2014, Ibrahim et al. 2018).

Conclusion

From the findings of the study, it can be concluded that, the pods of *Swartzia madagascariensis* are harmful to animals when taken in large quantities. Hence, it is recommended that livestock keepers should avoid grazing their animals on *S. madagascariensis* pods. Further studies are needed to corroborate these findings.

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Declaration of Conflict of Interest

Authors declare no conflicting interest regarding the publication of this paper.

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