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Insecticidal performance of *Cnidoscolus aconitifolius* (Euphorbiaceae) in the control of crop and stock pest of cowpea: exploratory acute oral toxicity studies

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1 ABSTRACT

Cowpea (*Vigna unguiculata* F) is a favoured host for pest life cycle. Many recourses control methods have been promoted leaving questions on the integrated management of pests in stocks. The general objective of this survey is to evaluate the in vivo acute oral toxicity of the ethanolic extract of *C. aconitifolius* (EECa), a plant species with insecticidal potential for cowpea pest management. Acute toxicity tests were performed in vivo at the Laboratory of Physiology and Experimental Pharmacology (LPEP/FAST) according to OECD Guidelines 423 on the Wistar albino rats (*Rattus norvegicus*). The EECa was administered at a single dose of 5000 mg/kg to rats using a stomach tube. The control lot received distilled water. Several clinical signs following the administration of EECa leaf powder over 14 days were noted and no mortality was observed. Apart from white blood cells, with statistically significant difference in control ($p<0.05$), the statistical difference for other haematological parameters was insignificant ($p>0.05$). The biochemical parameters showed statistically insignificant difference for test and controls batches ($p>0.05$), except creatinine and uricemia of the control batch, which showed a significant statistical difference after 14 days ($p<0.05$). Also, there is no significant statistical difference in the weight variation of animals. The EECa leaf powder presents no harmful effect on human health and can be used as an alternative in cowpea pest control in Benin.

2 INTRODUCTION

The conservation of food crops and mainly of cowpea (*Vigna unguiculata*) is a serious problem in tropical regions due to insect pests. Indeed, bruchids are insects' pests that attack cowpea seed stocks, leading to quick crop degradation. Post-harvest losses caused by the latter are important (Kayombo *et al.,* 2014). Many means of control have since been considered. Among them, chemical control is the most used. These have negative impacts on human health, environment and prove ineffective against insects over time. In addition, their high cost poses real problem of accessibility to small farmers whose income positively influence global food security (Lindquist, 2000). The search for alternatives without major side effects on the environment was the option experimented by research for a few decades. As a result, plant-based pesticides are nowadays of great interest in integrated pest management (Kpatinvo *et al.,* 2017). Plants are nowadays a rich source of bioactive principles. Much effort has then been focused on plant extracts used as commercial pest control agents. Many extracts of plants belonging to several families are used as botanical insecticides. *C. aconitifolius* is one of the plants used in traditional medicine and known for its therapeutic properties (Pérez-

González *et al.,* 2018). Akachukwu (2014) showed that aqueous extract of *C. aconitifolius*, at concentrations of 100,200 and 400mg/kg body weight has no toxic effect on albino rats. Despite the virtue of this plant in traditional medicine in the management of many diseases, very little work has been done on toxicity tests. Furthermore, our researches showed that the powder and ethanolic extract of *C. aconitifolius* has an insecticidal effect on adults of *C. maculatus*. This study evaluated the acute oral toxicity of (EECa) at a limit dose of 5000 mg/kg with a view to using this extract as alternative in cowpea seed pest control.

3 MATERIALS AND METHODS

3.1 Materials: The animal material consisted of Wistar albino rats (*Rattus norvegicus*) acquired at the (LPEP/FAST). They were acclimatized in the same animal house photoperiod of 12: 12 hours. The rats were fed with pelleted feed and water served *ad libitum*. Two batches (control and test) of 3 rats were formed by randomization in accordance with the guidelines of (OECD) (OECD, 2001). The plant material was the EECa. The plant was collected in Abomey-Calavi in the subequatorial climate zone and certified under the number YH 504/HNB at the National Herbarium of Benin.

3.2 Methods

3.2.1 Preparation of the ethanolic extract: The ethanolic extract was obtained by maceration of the powder of leaves after drying under shade. Thus, 200 g of powder were weighed with a Sartorius® analytical balance and introduced into an Erlenmeyer flask to which 2 litres of ethanol were added. The mixture was mechanically shaken (cold) and brought to maceration. The macerate was filtered at the end of each 24h for 72h. The deposit was each time put back in maceration until the end of the 72h. The filtrate obtained was evaporated with rotavapor at 40° C. The recovered extracts were placed in oven at 45°C for drying. After complete drying, the extract obtained was stored in sterile, hermetically sealed glass vials.

3.2.2 Ethical notice: The execution of this research work has received the favourable opinion of the scientific committee of Doctoral School (FAST/UAC) under the number (UAC/ FAST/ EDSVT/ 574601).

3.2.3 Exploratory tests of *in vivo* toxicity: The EECa was served orally to the test batch animals at 5000 mg/kg. At the beginning of the test, 12-week-old female rats weighing between 175g and 220g were randomly selected. They were marked with numbers to allow individual identification and then kept in their cages to acclimate them to laboratory conditions for 2 weeks prior to the experiment. The previously preserved ethanolic extract was dissolved in physiological water and administered to the rats at a rate of 10 ml/Kg body weight. A single dose was given using a gastric tube. The test lot received the dose of 5000 mg/kg extract while the control lot received distilled water. Each step required 3 animals. A limit test at a dose level of 5000mg/kg was chosen because information is available indicating that the EECa is probably not toxic. After treatment, the animals were observed individually during the first 30 minutes and twice the first 24 hours. Close attention was paid to them daily for 14 days after administration. All observations were recorded systematically. Particular attention was paid to behavioural signs such as tremors, convulsions, salivation, diarrhoea and lethargy.

3.3 Parameters assessed

3.3.1 Body weight: The individual body weight of each rat was determined 1h before administration of the test substance and then at least once a week. Weight changes were calculated and recorded. At the end of the test, animals were weighed and then sacrificed by overdose of anaesthesia.

- **Haematological and biochemical parameters:** Blood samples were taken on day 14 after administration of the extract and from all rats by retro-orbital puncture in dry tubes using non-heparinized haematocrit micropipettes for biochemical examinations and tubes containing anticoagulant (EDTA) using heparinized haematocrit micropipettes for

haematological examinations at the (LPEP/FAST).

- **Haematological and Biochemical examinations:** Haematological examinations (counting of red blood cells, white blood cells, platelets and the determination of haemoglobin level, haematocrit, mean globular volume, average corpuscular content in haemoglobin, average corpuscular concentration in haemoglobin) and Biochemical examinations (transaminases (ASAT, ALAT), bilirubin (free and conjugated), uricemia, urea, creatinine) were performed using a SYSMEX KXN21 automaton and Semi-Automate of the brand RAYTO. Two rats per group were sacrificed for further evaluation of the histological configuration of the liver and kidneys.

3.4 Statistical analysis: All data were entered in Microsoft Excel 2010 and processed using Minitab version 16.FR. This one was used for analysis of variance (one-factor comparative ANOVA) for comparison of the averages. Threshold of significance is 5%.

4 RESULTS AND DISCUSSION

4.1 Body weight of animals: The observation of variation in body weight of animals during experiment shows no significant difference from Do (Day 0) to D7 (Day 7) and from D7 to D14. However, there was a significant difference between the weight gains from Do to D14. This significant difference may be due to the diet and by the presence of secondary metabolites in the EECa, responsible for a good assimilation of food, which may promote weight growth. This study concluded that EECa had no effect on variation in the animals' weight. These results corroborate those of (Ebeye *et al.,* 2015) who had shown that aqueous extract of *C. aconitifolius* has no effect on the body weight of rats (*Rattus norvegicus*). Other studies have also shown a decrease in the weight of rats after oral giving of ethanolic extract of *Chicococca alba* and *Stryphnodendron adstringens* (Gazda *et al.,* 2006). This reduction in weight was explained by a probable reduction in food consumption, but also by the possibility of dose/absorption interactions.

4.2 Weight of organs (liver and kidney): The average liver and kidney weights for control and test are 8.85g and 10.96g; 0.78g and 0.80g respectively. The relative mean weight of the latter shows no significant difference ($p > 0.05$). The extract has no effect on the weight of the target organs.

4.3 Clinical signs observed: Animals were observed singly at least once during the first 30 minutes and twice during the first 24h after giving the EECa. For 14 days after administration, close attention was paid to them daily. The various clinical manifestations were systematically recorded and summarized in Table 1.

Table 1: Clinical signs observed during 14 days after EECa administration

Batch Clinical Signs	Control	Test
Salivation	-	-
Accelerated breathing	-	-
Tremors	-	+
Sleep	+	+
Diarrhoea	-	-
Lethargy	-	+
Paralysis	-	-
Abdominal constrictions	-	+
Comma	-	-

-: absence of signs +: Presence of signs

The following Table presents the haematological parameters of animals of batches (Control and Test) (Table 2).

Table 2: Effect of EECa on haematological parameters.

Batch Parameters	Control	Test
GB (10^9/L)	5.33 ± 0.04	6.4 ± 0.01 b
HGB (g/dl)	13.9 ± 0.15	13.6 ± 0.20 a
GR (10^{12}/ L)	7.01 ± 0.155	7.60 ± 0.15 a
HCT (%)	42.500 ± 2.53	40.08 ± 1.17 a
VGM (fL)	53.250 ± 1.06	54.65 ± 2.15 a
TMH (pg)	16.100 ± 1.314	17.900 ± 1.21 a
CCMH (g/dL)	31.850 ± 1.061	33.4 ± 1.344 a
IDR-CV (%)	16.200 ± 1.370	18.50 ± 2.192 a
IDR-DS (fL)	23.500 ± 0.114	23.200 ± 3.536 a
PLT (10^9/L)	554.00 ± 12.80	564 ± 40.31 a
VMP (fL)	7.900 ± 1.007	8.300 ± 2.121a
IDP	12.750 ± 0.15	12.800 ± 2.121a
PCT (%)	0.412 ± 0.12	0.48 ± 0.0912 a

a = Statistical difference not significant ($p > 0.05$); m ± esm = mean ± standard error ; b:=Statistically significant difference between test and control batch for the considered parameter ($p<0.05$) on the mean, n = 3, GB= white blood cells ; HGB= Haemoglobin ; GR= red blood cells ; HCT= Haematocrit ; VGM=mean corpuscular volume ; TMH=average haemoglobin content ; CCMH= average corpuscular haemoglobin concentration ; PLT=platelets ; IDR=red blood cell distribution index ; VMP=mean platelet Volume ; IDP=platelet distribution index.

The Table 2 presents haematological parameters of animals of batches (Control and Test). Statistical analysis of blood counts of the control and test batches reveal that white blood cells are the only cells that show a significant difference compared to the control ($p<0.05$). The other haematological parameters show a non-significant statistical difference ($p> 0.05$). These results are similar to those of (Kone *et al.,* 2009) who showed that total aqueous extract of *Sacoglottis gabonensis* does not cause changes in the erythrocyte and leukocyte lineages. Indeed, the elevation of white blood cell rate leads to think that EECa stimulates functions of the immune system. The various biochemical parameters explored provided information on the probable effects of EECa on the liver and kidney. Transaminases (ASAT, ALAT), bilirubin (free and conjugated) are liver parameters while urea, uricemia and creatinine are kidney parameters.

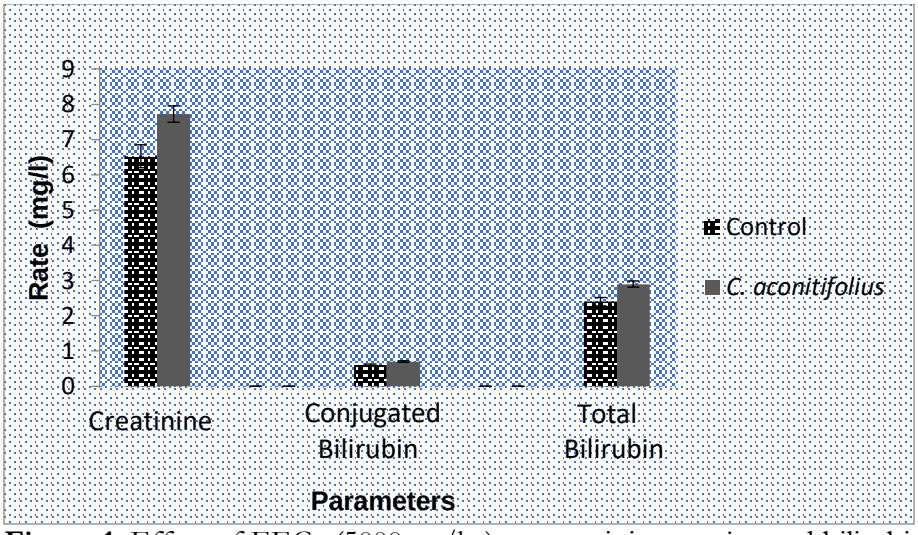

Figure 1: Effect of EECa (5000mg/kg) on creatinine, conjugated bilirubin and total bilirubin.

The values of creatinine, conjugated bilirubin and total bilirubin in the control and test batches are 6.5 mg/l and 7. 7 mg/l; 0. 6 mg/l and 0.7mg/l; 2.4 mg/l and 2.9 mg/l respectively. There was no significant difference between the control and the test batches ($p > 0.05$) for conjugated bilirubin and total bilirubin after 14 days. However, creatinine of the test batch treated with EECa show significant difference compared to the control ($p < 0.05$).

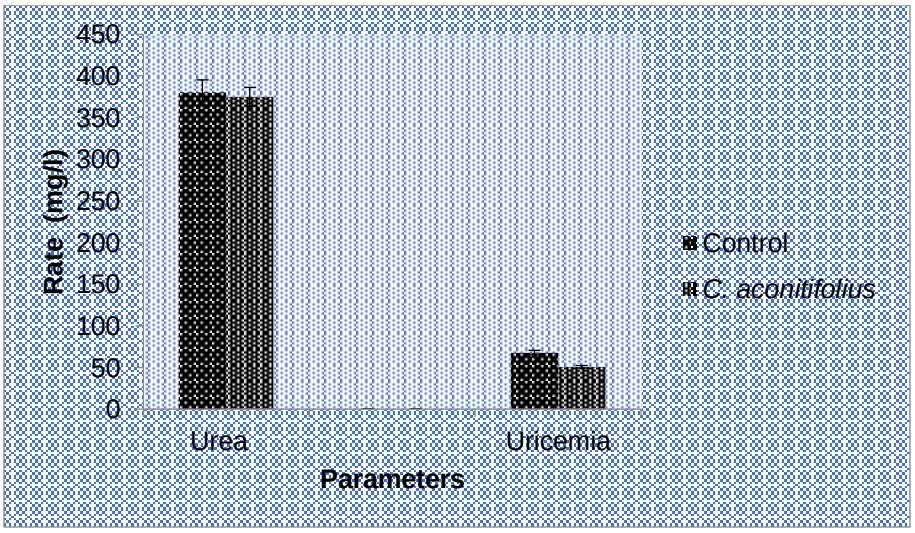

Figure 2: Effect of EECa (5000mg/kg) on urea and uricemia

The values of urea and uricemia in the control and test batches are 0.38 g/l and 0.37g/l; 67.9 mg/l and 50.8 mg/l respectively. Urea of the test batch treated with EECa did not show significant difference compared to the control ($p > 0.05$) after 14 days. However, uricemia of the test batch treated with EECa show significant difference compared to the control ($p < 0.05$). The transaminases (ALAT and ASAT) of the control and test batches are 95.4UI/l and 31.39Ul/l; 92.2UI/l and 31.7UI/l respectively. Transaminases (ASAT and ALAT) of test batch treated with the EECa did not show significant difference with the control after 14 days ($p > 0.05$). All the biochemical parameters studied, except (creatinine and uricemia) showed a non-significant statistical difference ($p > 0.05$) between the test and control batches. The

extract has no toxic effect on these biochemical parameters. Among the three renal parameters, two showed a significant statistical difference ($p< 0.05$) between the test and control batches. We can deduce that the EECa could have an effect on renal function. The EECa leaf powder has no significant effects on biochemical and haematological parameters. The extract tested at 5000mg/kg of body weight does not cause a significant increase in transaminases. From these results obtained, we can deduce that the EECa leaf powder was found to be non-toxic for the tested parameters, thus not having any negative influence on the blood tissue, nor on the vital organs such as liver and kidneys. These results are similar to those of (Ezeigwe *et al.,* 2020) who showed that the aqueous extracts of *F. capensis* and *C. aconitifolius* did not have any form of harmful effect on kidneys and liver of rats. Also, these results corroborate those obtained by Akachukwu *et al.* (2014) who showed that the aqueous extract of *C. aconitifolius* leaves is not toxic. These results attest to the safety of this plant, which can therefore be used in agriculture to protect crops in Benin. The risk of intoxication related to the consumption of crops treated with *C. aconitifolius* is therefore very low. The realization of histological sections will confirm these observations.

5 CONCLUSION

The exploratory tests of acute toxicity of EECa by the oral route attest that it is without toxic effects on biochemical and haematological parameters studied at dose of 5000 mg /kg. These results justify the use of *C. aconitifolius* in control of cowpea pests. It will not negatively affect the quality of crops since ethanolic extract of this plant does not show an immediate toxicity. However, other studies on chronic toxicity tests of the extract by the oral route deserve to be carried out in order to confirm its non-toxic character in long term.

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