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Research Article
Acute Toxicity of *Chenopodium ambrosioides* and *Annona muricata* oils with Acaricidal Potentials

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Abstract

Background and Objective: Recently, the acaricidal activities of the essential oil extracted from the leaves of *Chenopodium ambrosioides* L. (EOCA) and the almonds oil of *Annona muricata* (OAMA) have been proven *in vitro* on the invasive tick *Rhipicephalus microplus* in Benin. For a better valorization of these products as substitutes of synthetic acaricidal molecules which have shown their limits, it is recommended to determine the safety of these oils. The purpose of this study was to evaluate the acute oral toxicity of these extracts. **Material and Methods:** To this end, test was performed according to the OECD Test Guidelines 425 at a single dose of 2000 mg kg^{-1} body weight/oral route of each oil. Selected biochemical and hematological parameters were determined at 0, 7 and 14 days. Then, the organ toxicity of the oils was evaluated by histopathological examination at the end of the assay. **Results:** There were no deaths or significant alterations in live weight in the OAMA group. Administration of OAMA caused an increase in transaminase levels (ASAT and ALAT) and urea and a decrease in creatinine. Histopathology showed that there were no alterations in the kidney but some liver disturbances were observed. With respect to EOCA, all rats in the group treated with this extract died at a single dose of 2000 mg kg^{-1} body weight. Histopathology revealed some renal and hepatic changes. **Conclusion:** The single oral dose of OAMA and EOCA induces toxic alterations in Wistar rats suggesting the cautious use of these phytoacaricides externally as animals may tend to lick the products.

Key words: *Chenopodium ambrosioides*, *Annona muricata*, anti-tick, *Rhipicephalus microplus*, toxic substances, histopathology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Benin, livestock production represents the second contribution after crops to the gross domestic product[1]. This production is struggling to face the negative impacts caused by ticks which are harmful for livestock[2]. Among these, ticks, *Rhipicephalus microplus* is one of the most invasive external bloodsucking pest causing heavy losses in affected livestock. This tick was introduced in Benin between 2000 and 2006 when importing cattle from Brazil called Girolando by the Government of Benin through the Pafilav Project which aim was to improve local breed milk production[3,4]. This tick species has a great ability for ecological adaptation to tropical and subtropical conditions and represents one of the most widely distributed and economically important ticks and the major obstacles to the development of cattle breeding[5]. It lowers significantly the production performances in infected cattle through its destructive actions and by transmission of pathogens to livestock, which results in severe economic losses for breeders such as reduction of weight, decrease of meat and milk quality[6]. Besides, tick can transmit pathogens such as viruses, bacteria and protozoa, which cause important diseases, generally termed tick-borne diseases[7,8]. So far a number of synthetic acaricides were used for the control of *Rhipicephalus microplus*. However as a consequence of their intensive use, this tick species have developed a resistance to the major and common classes of acaricides. Regarding its acaricide resistance, *Rhipicephalus microplus* constitutes a threat to livestock in Benin[6]. To face this threat, alternative treatment methods are needed. In recent decades, particular interest has been given to research focusing on the use of plant extracts against pests including *Rhipicephalus microplus*[9]. This interest aims to address a number of problems related to the use of synthetic chemicals[10-12], particularly the resistance of parasites against such products, environmental pollution and the presence of their residues in animal production and the prohibitive cost of these products. As a result, some plant extracts or phytochemicals have been reported to be effective against insecticide-resistant pests[13,14]. The acaricidal properties of these plant materials on different tick species in particular *Rhipicephalus microplus* have also been reported by Borges *et al.*[15]. Satisfactory results have been obtained from these works carried out *in vitro* on engorged females and larvae of tick using *Annona muricata* and *Chenopodium ambrosioides* extracts[16,17]. The plant extracts are then potential candidates for the alternative fight against ticks. However, for a substance having pharmacological effects to possibly be used as a drug, it is first necessary that it is active in doses for which the toxicity is negligible. Similarly, although plant extracts have a promising future in the phyto-acaricide market, registration progress must be made before they occupy a significant share of global markets[18]. The toxicity tests, therefore, accompany the biological activity during the selection of new molecules to contribute to a better valorization of these products. For better valorization of extracts in the fight against *Rhipicephalus*, the present study aimed to determine the safety of the 2 plant extracts *Chenopodium ambrosioides* (L.) and *Annona muricata* whose acaricidal activity has been proven *in vitro* by recent studies against this tick.

MATERIALS AND METHODS

Framework of study: This study was carried out from December, 2017 to October, 2018 at the Polytechnic School of Abomey-Calavi (EPAC) and at the Institute of Applied Biomedical Sciences (ISBA) of the Faculty of Health Sciences (FSS) of the University of Abomey-Calavi within the following structures: Communicable Diseases Research Unit (URMAT), Laboratory of Research and Study in Applied Chemistry (LERCA), Laboratory of Pharmacognosy and Essential Oils (LPEO), Laboratory of Histology of the Unit of Human Biology (LHUHB).

Plant material: The plant material consisted of the almonds of *Annona muricata* belonging to the family Annonaceae collected in different localities of the commune of Abomey-Calavi in Benin and *Chenopodium ambrosioides* (L.) leaves of the family Chenopodiaceae harvested at Dassa-Zoume and Savalou in the Department of Collines. They were sent to the Laboratory for their extractions.

Biological material: The animal material consisted of 9 male Wistar albino rats 7 weeks old. They were divided according to their live weight in metal cages of dimensions $50\times30\times20$ cm^3. They were acclimatized under standard environmental conditions ($27\pm2^{\circ}C$) of the animal house for 21 days. They received water and a standard food distributed *ad libitum*. Principles of animal care laboratory were followed. The experimental procedures were performed in agreement with the Ethical Directors in Animal Research and approved by Ethical Committee of Research Unit in Applied Microbiology of Natural Substances, Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi (EPAC) under the number 167-08, 2018.

Methods

Extraction of oils

Essential oil of the leaves of *Chenopodium ambrosioides*: Extraction of the essential oil was carried out by hydro-distillation in a Clevenger-type apparatus (1928) for about 5 h using methods reported by Dedome *et al.*[19]. The extracted oil was dehydrated with anhydrous sodium sulfate (Na_2SO_4). Its preservation was done in the refrigerator at 4°C in shaded glass bottles, hermetically sealed and covered with aluminum foil, until the time of use.

Vegetable oil of almonds from *Annona muricata*: The vegetable oil of *Annona muricata* was obtained from the almonds derived from the shelled seeds of this plant species. The almonds were crushed using a grinder and weighed. The oil was extracted from the grindstone using Soxhlet hexane[16]. The hexane residue in the oil was evaporated under reduced pressure consisting of a water bath and a rotavapor. The obtained oil was stored in shaded glass bottles.

Acute toxicity study: Acute toxicity studies were conducted in accordance with the World Health Organization for Economic Co-operation and Development (OECD) Guideline 425 for the evaluation of the safety and effectiveness of drug-based of plants[20].

Constitution of batches: After acclimatization, the rats were weighed and divided into 3 batches of 3 animals according to their weight:

- **Batch No. 1:** Rats having received the oil from the leaves of *Chemopodium ambrosioides* at a dose of 2000 mg kg^{-1} of live weight
- **Batch No. 2:** Rats having received the oil resulting from almonds of *Annona muricata* at the dose of 2000 mg kg^{-1} of live weight
- **Batch No. 3:** Rats having received distilled water (control batch)

Acute oral toxicity: About 16-18 h before administration of the extract, the rats were deprived of food. Gavage was performed using an esophageal tube. The behavior of the animals was observed and recorded every 2 h for the first 12 h after treatment and every 24 h during the entire experimental period of 14 days.

The clinical observations concerned skin color changes, mucous membranes, eyes, somatomotor activity, the nervous system and respiratory movements. The number of survivors was recorded during 14 days and their weights were recorded on the 7th and 14th days. The LD_{50} was then determined at the end of the experiment according to the method of Lorke[21].

Study of the weight evolution of the rats: The rats of each batch were weighed on days D0, D7 and D14 by a scale to study the variation of the weight in the time and according to the extracts.

Study of biochemical parameters: For each batch, 3 sets of blood samples were taken on D0, D7 and D14, respectively. Sampling was done by capillarity in dry (EDTA) tubes (without anticoagulant) using a hematocrit tube after anesthetizing of the rats by inhalation of the ether. Each tube was labeled to facilitate identification throughout the experimental period. Blood samples were centrifuged at 2580 rpm for 10 min. The sera collected, stored at -20°C, were analyzed using Bioplus Bio200 semi-automatic equipment (Gold Analysis kits) to evaluate the concentration or activity of certain serum parameters that are indicative of hepatic and renal pain: Blood glucose, urea, creatinine, transaminases: Aspartate aminotransferase (ASAT) and Alanine aminotransferase (ALAT)[22,23].

Histopathological study: This study was performed according to method reported by Chabi *et al.*[24] consisted in observing of possible lesions that could be induced by the extracts on the liver and the kidneys. The observations were made at 100X magnification and the images were captured and transferred in JPEG format.

Statistical analysis: The statistical analysis of the results were carried out using the Statistica version 7.1 software. The values were expressed as mean±standard error. Variance analysis were performed by ANOVA one way followed by the student-Newman Kerls test. The $p<0.05$ was considered the level of statistical significance.

RESULTS

Acute toxicity of *Annona muricata* essential oil: After administration of the phytoacaricide at a single dose of 2000 mg kg^{-1} body weight, no signs of toxicity such as mortality, behavioral disorders, neurological disorders, etc. were observed during the 14 days.

Evolution of live weight: Table 1 shows the change in weight of the rats during the toxicity test. It revealed a non-significant

Table 1: Evolution of live weight of rats

	Days			
Groups	0	7	14	Significance
Control	169.0±46.77	174.00±46.77	182.33±52.54	NS
A. muricata	180.0±71.97	176.67±53.13	188.33±64.67	NS

NS: No significance

Table 2: Study of the biochemical parameters of the rats after the toxicity test

Biochemical parameters	Control	*A. muricata*	Significance
Glucose (g L^{-1})	0.99±0.38	0.99±0.38	NS
Urea (g L^{-1})	0.47±0.10	0.49±0.09	NS
Creatinine mg L^{-1})	2.50±1.61	1.36±0.79	NS
ALAT (UI L^{-1})	244.65±45.67	257.28±45.69	NS
ASAT (UI L^{-1})	74.83±18.98	94.29±31.86	NS

ALAT: Alanine aminotransferase, ASAT: Aspartate aminotransferase, NS: No significance

decrease between the days D0 and D7 and a non-significant increase between the days D7 and D14 in weight in the subjects treated. Moreover, no significant difference was observed in the change in weight of the subjects treated and that of the controls (p>0.05).

Study of biochemical parameters: The safety of *Annona muricata* oil has been assessed through the study of certain biochemical parameters. The measurement of these parameters (glucose, urea, creatinine, transaminases (ASAT and ALAT) was performed on blood samples from rats dosed at 2000 mg oil per kg body weight. The values obtained are shown in Table 2. This table showed that *A. muricata* oil caused an increase in transaminases and urea and a decrease in creatinine. However, the Chi-square (χ^2) test revealed no significant difference (p>0.05) between the biochemical parameters values of the subjects who received this extract compared to those who received the distilled water.

Histological study

Histological aspects of the liver and kidneys in normal rats: On healthy liver, the hepatic lobule had well individualized bays which were arranged in rays around the centrilobular veins. These bays were separated by sinusoidal capillaries (Fig. 1).

On the cortex of a healthy kidney, glomeruli and renal tubules were identifiable and well individualized (Fig. 2).

Histological features of liver and kidney in rats treated with *Annona muricata* essential oil: After gavages at the single dose of 2000 mg kg^{-1} live weight of the oil from *Annona muricata* almonds, it was observed a disorganization of the hepatic architecture with filling of the vascular spaces, a

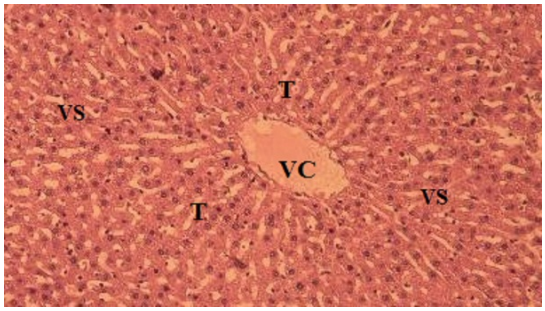

Fig. 1: Normal rat hepatic lobule (HE X100)
T: Hepatic trabeculae VC: Centrilobular vein, VS: Sinusoidal capillaries

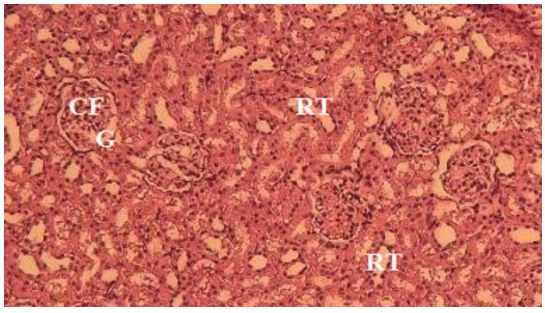

Fig. 2: Normal rat kidney cortex (HE X100)
G: Glomeruli , RT: Renal tubes

disappearance of the radial arrangement of the bays. Hepatocytes showed homogenization of the cytoplasm, pyknosis or even peri-lobular hepatic necrosis (Fig. 3). As regards the renal cortex, the histological architecture of the cortex is almost normal (Fig. 4).

Acute toxicity of the essential oil of *Chenopodium ambrosioides*: After administration of the phytoacaricide at a

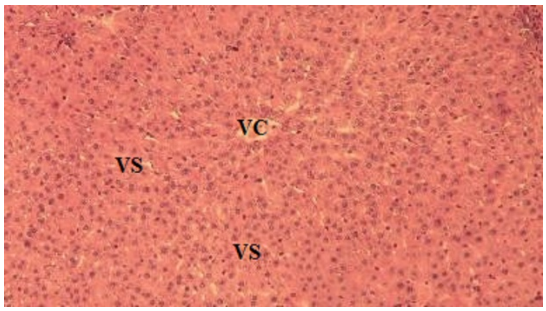

Fig. 3: Rat hepatic lobule treated with *Annona muricata* (HE X100)
VC: Centrilobular vein, VS: Sinusoidal capillaries

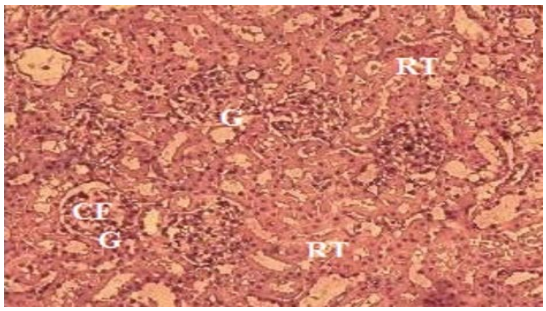

Fig. 4: Renal rat cortex treated with *Annona muricata* (HE X100)
G: Glomeruli, RT: Renal tubes

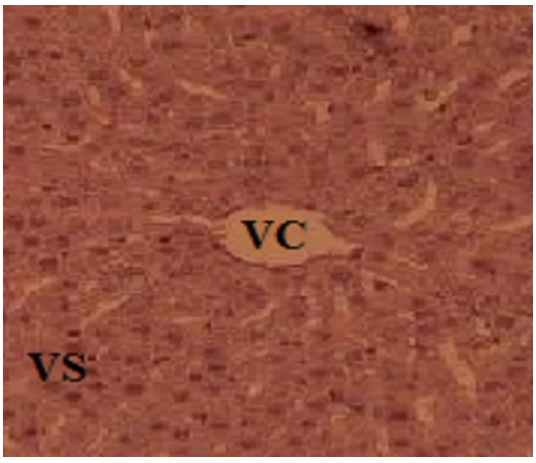

Fig. 5: Rat hepatic lobule treated with *Chenopodium ambrosioides* (HE X100)
VC: Centrilobular vein, VS: Sinusoidal capillaries

single orally dose of 2000 mg kg^{-1} body weight, all rats died within 24 h. The histopathological study revealed that the lobular architecture of the liver had been conserved overall. However, a slight filling of the vascular spaces and a

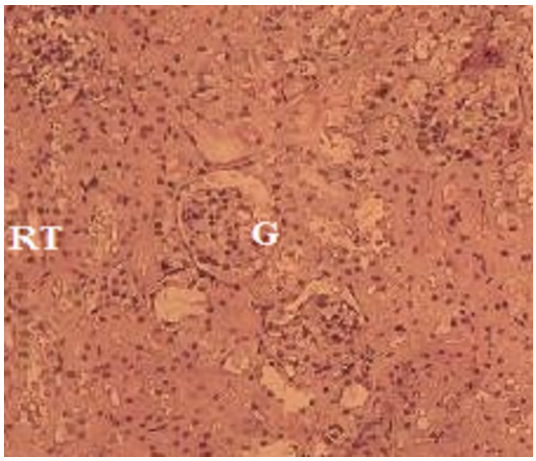

Fig. 6: Renal rat cortex treated with *Chenopodium ambrosioides* (HEX100)
G: Glomeruli, RT: Renal tubes

predominantly peri-lobular pyknosis (cell necrosis) had been noted (Fig. 5). Regarding the renal cortex, the general structure of the renal parenchyma was generally conserved, the glomeruli appearing almost normal, nevertheless, the renal tubules were unrecognizable with signs of cellular degeneration (Fig. 6).

DISCUSSION

Oral administration of vegetable oil from *Annona muricata* almonds at a single dose of 2000 mg kg^{-1} body weight clinically revealed no sign of toxicity (mortality, behavioral disturbances and neurological disorders) after careful observation of rats during the 14 days of experiment. Moreover, this acute toxicity study showed no significant decrease in the weight of treated animals compared to controls in the first days after gavage. This non-significant decrease in weight may be related to anorexia induced by administration of the extract in rats, which would result in a decrease in their consumption of food influencing their health status. Values from biochemical parameters showed a non-significant increase in transaminases and urea followed by a non-significant decrease in creatinine which suggested that this oil had no negative impact on liver and kidneys of treated rats. These results were similarly to those obtained by Syahida *et al.*[25], who revealed that the pulp of *A. muricata* administration for 28 days to rats showed no effect on blood hematology and serum biochemistry. Nevertheless, the histopathology showed a disorganization of the hepatic architecture with filling of the vascular spaces, a disappearance of the radial arrangement of the bays.

Hepatocytes showed homogenization of the cytoplasm, pyknosis and even perilobular hepatic necrosis, the histological architecture of the renal cortex being almost normal. These results showed that the administration of high doses of the whole oil of *Annona muricata* almonds (OAMA) is likely to cause liver damage. The effect of this plant on the liver is probably due to the presence of alkaloid. In fact, the seed extract of this plant is rich in alkaloids such as acetogenins and annonacin[26,27]. A study that evaluated the toxicity of crude leaf extract and enriched acetogenin extracts showed that acetogenin extract enriched was more toxic than the others[28]. Studies of Moghadamtousi *et al.*[29] revealed that annonacin had negative effect on some healthy cells. Lannuzel *et al.*[30] reported in a study in Guadeloupe on the toxicity of Annonaceae for dopaminergic neurons that the annonacin it contains can cause neuronal degeneration and lead to an atypical form of Parkinson's disease. Arthur *et al.*[31] reported that doses greater than 5 g kg^{-1} of aqueous extract of *Annona muricata* could cause kidney damage, in contrast to the 1 g kg^{-1} dose that had hypoglycaemic and hyperlipidemic properties.

Regarding the results of the acute toxicity study at a dose of 2000 mg kg^{-1} live weight, a mortality of all the rats treated with this extract was observed. This mortality suggests that the LD_{50} of this oil was less than 2000 mg kg^{-1} body weight. According to Diezi[32], substances with an LD_{50} between 50 and 500 mg kg^{-1} body weight are toxic and those with an LD_{50} of more than 5,000 mg kg^{-1} are virtually non-toxic. By referring to this classification, EOCA was orally toxic. Moreover, the chemical composition of the EOCA could provide serious indications that could allow the identification of the chemical compounds at the origin of its toxicity. Indeed, EOCA was mainly composed of monoterpenes: ascaridole (terpene peroxide) which represents up to 70% of gasoline), ascaridole glycol, aritasone, b-pinene, limonene, myrcene, cymene, phellandrene, camphor, -terpinene, α-terpineol, associated with small amounts of methyl salicylate and butyric acid[33]. Ascaridole is toxic, has an unpleasant pungent flavor and is a pure shock-sensitive explosive[33]. The effect of this plant on the liver and kidney proved by histology in this study was therefore due to the presence of ascaridole[34,35]. Although the toxic character of ascaridole was known, it represents the main active principle of the biological properties of this plant[36]. Rigorous compliance with the therapeutic dose and the route of administration of the extracts of this species plants can take advantage of its many biological properties.

CONCLUSION

The present study revealed the hepatorenal toxicity of essential oil of *Chenopodium ambrosioides* and vegetal oil of *Annona muricata* almonds and suggested the caution regarding the use of these extracts. However, since the route of administration of phytoacaricides is external, the likelihood of its use having consequences for users and animals would be minimal. Nevertheless, in-depth studies of ocular and cutaneous irritability, chronic toxicity, nontoxic therapeutic dose and residues research in production will refine their use.

SIGNIFICANCE STATEMENT

This study discovered that OAMA and EOCA at single dose of 2000 mg kg^{-1} body weight induces renal and hepatic alterations in Wistar rats and can be beneficial for farmers in the search for active phytoacaricides to fight against *R. microplus*. This study will help the researchers to uncover the critical areas of OAMA and EOCA uses as phytoacaricides that many researchers were not able to explore. Thus a new theory on biological potential of these extracts may be arrived at.

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