Toxicological effects of Opilia amentacea Roxb, a medicinal plant used in traditional African medicine

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ABSTRACT

Opilia amentacea Roxb, is a medicinal plant widely used in traditional African medicine. However, data on its safety remain limited in the scientific literature. The purpose of this study was to evaluate the toxicity of Opilia amentacea Roxb. The work was carried out in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals using Method 423 on Wistar rats. The ethanolic extract of the leaves of Opilia amentacea Roxb was administered at a single dose of 2000 mg/kg. The different clinical signs observed after administration and over 14 days were recorded. As results, no mortality was observed. Only a general state of sleep was observed in animals. The ethanolic extract of the leaves of Opilia amentacea Roxb was not have significant toxic effects on liver, kidney and blood tissue at a dose of 2000 mg/kg. From these data, it can be concluded that the ethanolic extract of Opilia amentacea Roxb does not exhibit acute toxicity to human health at the doses tested. Its use in traditional medicine may be recommended if efficacy data are established.

Keywords: Opilia amentacea Roxb, Toxicity, Benin, Traditional medicine, Africa.

INTRODUCTION

The use of medicinal plants is a common practice worldwide and is part of human culture in some parts of the world [1]. In sub-Saharan Africa in particular and in the world in general, traditional herbal medicine is an alternative to modern chemical and industrial medicines [5]. It is widely used in rural and even urban areas without difference of sex and religion either among children and adults [1]. The increasing use of medicinal plants in the therapeutic practices of populations is mainly due to the prohibitive cost of modern medicine and the low incomes of a large part of the population [4,5]. In addition, the literature is well documented on the effectiveness of many of these traditional herbal medicines [2]. These treatments include body washes, massages, ingestions and others [6].

In Benin, some ethnopharmacological studies have shown the increasing use of medicinal plants as an important and essential practice of culture and the traditional health system [7-9]. Benin has ten phytogeographical zones and is characterized by a diversity of geomorphological, geological, hydrographic, climatic and demographic traits that justify the diversity of plant formations and the variability of the floristic composition of plant groups [10]. Opilia amentacea Roxb formerly called Opilia celtidifolia (Guill. Perr.) is a plant used widely in Africa and Benin in the treatment of various pathologies. In particular, the leaf and root are used to treat dermatological conditions, wound healing, abdominal pain, internal worms and also as an appetite [12]. Flavonoid-rich fractions of O. amentacea have also been shown to exhibit potent antioxidant and antidiabetic activity by inhibiting key enzymes such as α-amylase and α-glucosidase related to type II diabetes, which has been considered an effective strategy for controlling blood sugar [13,14]. The bark is used for the treatment of snakebites, fever and the fruits are consumed by the Malayali tribes of Tamil Nadu, India [17]. The plant is also involved in the treatment of malaria [18] liver diseases and in the treatment of sexually transmitted diseases [19]. According to the work of Aman et al. [20] carried out in Cameroon, Opilia amentacea is used in the treatment of jaundice.

The biological activity of this medicinal plant is due to the diversity of biological properties of the secondary phytochemicals it possesses. Although medicinal plants are presented as natural and harmless products, therefore without toxic effects. However, some evidence suggests otherwise and some studies showed that plants may be associated with health hazards. Medicinal plants can contain many active chemical compounds as well as other substances of great complexity such as mucilages, polyphenols, polysaccharides, etc. [22]. These substances can modulate and modify the effects of the active ingredient.
of the plant. Thus, some herbal recipes can be toxic or can act as agonists or antagonists of the active ingredients. Therefore, the study of toxicity is an essential prerequisite for evaluating the efficacy of plant extracts [24]. Thus, for the well-being of the population, research focused on knowledge gaps on medicinal plants and their potential toxicities is strongly encouraged by many medical organizations and by researchers in complementary and alternative medicine [5]. The toxicity of a plant has been shown to depend on a variety of factors including the strength of secondary metabolites, the amount consumed and the exposure time. *O. amentacea* leaves contain several chemical compounds such as saponins, polyphenols, flavonoids and polysaccharides including arabinose, galactose, rhamnose, mannose, glucose, glucuronic acid and 4-O-methyl glucuronic acid [23].

Despite their interesting chemical characteristics and data on the biological properties of this plant in the world, rare scientific work has addressed the toxicity of this plant on human organs and the side effects it could have. The present study aims to determine the toxicological profile of the extracts of this plant. The general objective is to evaluate the toxicity of *Opilia amentacea* Roxb in order to provide scientific data on the safety of the plant for a better and safe use.

**MATERIAL AND METHODS**

**Plant material**

The plant material used consisted of the leaves of *Opilia amantacea* harvested in the classified forest of Pahou in February 2021. Leaves were certified at the National Herbarium of Benin of the University of Abomey-Calavi by Professor Hounakpon Yédémony.

**Animal material**

The albino rats (*Rattus norvegicus*) of the Wistar strain were acquired at the pet store of the Laboratory of Physiology and Experimental Pharmacology of the Faculty of Science and Technology (FAST) of the University of Abomey-Calavi (Benin). They were acclimatized in the same pet store to a constant temperature of 22 ±1°C with a photoperiod of 12 hours of light and 12 hours of darkness. Their food was mainly granulated feed and water administered *ad libitum*. They were made up of two batches (control and test) of 3 rats by randomization in accordance with the guidelines of Protocol 423 of the Organization for Economic Co-operation and Development [27].

**METHODS**

**Preparation of ethanolic extract**

The leaves were cold dried under air conditioning and the ethanolic extract of *Opilia amantacea* (EEOa) was obtained by maceration of the powder from the leaves. Thus, 200 g of powder were weighed using a Sartorius® analytical balance and introduced into an Erlenmeyer vial to which 2 liters of ethanol were added. Mechanical stirring (cold) was performed for 72 hours [29]. The macerate was filtered at the end of each 24 hours and the deposit was macerated again until the end of the 72 hours. The filtrate obtained is evaporated using rotavapor at 40°C. The recovered extracts were dried in the oven at 45°C. After complete drying, the dry extract at the bottom of the dishes was scraped using a stainless-steel spatula. The resulting extract is stored in glass vials, sterile and tightly closed.

**Ethical Approval**

This experimental protocol received approval of the Scientific Ethics Committee of the Doctoral School (Life and Earth Sciences) of the Faculty of Technical Sciences at the University of Abomey-Calavi (UAC).

**In vivo toxicity tests**

The tests were carried out in accordance with the Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals using Method 423 [27]. At the beginning of the experiment, rats aged 13 weeks, female and weighing between 190 g and 233 g were chosen at random. They were labeled by numbers and kept in their cages for acclimatization to laboratory conditions for two weeks before the experiment. Each step required three animals. Two batches (control and test) of 3 rats were constituted. The ethanolic extract of the leaves of *Opilia amentacea* was administered orally to the animals in the test batch at 2000 mg/kg, using a gastric tube. The control rats were given distilled water extract. The control batch received distilled water. The animals were observed individually at least once for the first 30 minutes and at least twice for 24 hours after treatment. Special attention was given to them daily for 14 days after administration of the substance and clinical and behavioral manifestations such as tremors, convulsions, salivation, diarrhea and lethargy were recorded.

**Parameters measured**

**Body weight**

The individual weight of each rat was determined one hour before administration of the test substance and thereafter at least once a week [29].

**Hematological and biochemical examinations**

During the experiment, blood samples were taken on the 14th day after administration of the extracts to all rats by retro-orbital puncture in dry tubes using a non-heparinized hematocrit micropipette for biochemical examinations and EDTA tubes using a heparinized hematocrit micropipette for hematological examinations [29]. The hematological and biochemical examinations were carried out at the Research Unit in Microbiology and Pharmacology of natural substances respectively using hematology automaton (Sysmex) and spectrophotometer (Sysmex).

**Data analysis**

All collected data was entered into Microsoft Excel 2010 and analyzed using Minitab version 16.fr. Analysis of variance (ANOVA to a comparative factor) was performed for the comparison of means. The significance level was 5%.

**RESULTS**

**Clinical manifestations**

The animals were observed individually and daily with particular attention during the first 30 minutes, at least twice during the first 24 hours after administration of the ethanolic extract of the plant and for 14 days after administration. The various clinical manifestations were systematically recorded and summarized in Table I. A general state of sleep was noted in both the control and test animals.
Table 1: Clinical signs observed during the 14 days after administration of EEOa

<table>
<thead>
<tr>
<th>Clinical Signs</th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Accelerated breathing</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tremors</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Sleep</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lethargy</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paralysis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Abdominal constrictions</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Comma</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Legends: - absence of signs  +: Presence of signs

Body weights of the animals

Figures 1 and 2 below show respectively the evolution of the body weights of the animals during the experiment and the relative average weights of vital organs such as liver and kidneys removed at the end of the experiment. There was a non-significant weight growth from Jo to D7 and from D7 to D14 in test and control batches. It can be deduced that the ethanolic extract of Opilia amantacea does not affect the weight growth of the animals tested. On the other hand, we note a significant difference between the weight growth from D0 to D14 in the two batches.

Figure 1: Variation in the average weight of the control and test animals during the experiment

Analysis of Figure 2 shows that the mean liver and kidney weights for the control and test batches are 8.85 g and 8.60 g for the control batch and 0.78 g and 0.82 g for the test batch respectively. The relative mean weight of the vital organs (liver and kidney) in the control and test batches showed no significant difference (p > 0.05). It can be said that the extract did not affect the weight of vital organs.

Figure 2: Effects of the ethanolic extract of Opilia amantacea on the average organ weight

The histograms with the same letters show that there is no significant difference in the average organ weight.

Figure 3: Effects of ethanolic extract of Opilia amantacea on uricemia, conjugated and total bilirubin

Legends: Au: Uricemia; BT: Total bilirubin; BC: Conjugated bilirubin

Figure 4: Effects of ethanolic extract of Opilia amantacea on urea and creatinine
For the hematological parameters, the hemoglobin (Hb), the number of Red Blood Cells (RBC), the number of White Blood Cells (WBC), the platelets (PLT), the hematocrit (Hte), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin content (MCHC), lymphocytes, neutrophils, and monocytes. For all the parameters evaluated, no significant variation was found between the control and the experimental batch treated with 2000 mg/kg weight of the ethanolic extract of *Opilia amantacea* (p > 0.05). The results are presented in figures 6, 7, and 8.

**DISCUSSION**

Medicinal plants and their derivatives are used as an alternative form of health care mainly by people in less developed countries. Despite the frequent use of plant species for therapeutic purposes, very few toxicological studies have been conducted on these medicinal plants. This study was aimed to evaluate the toxicity of *Opilia amantacea* Roxb in order to provide scientific data on the safety of the plant for a better and safer use. Wistar rats were used to assess the in vivo toxic effect of the ethanolic extract of *Opilia amantacea* Roxb at a single dose of 2000 mg/kg rat weight. A limit test using a dose of 2000 mg/kg was chosen because information is available that *Opilia amantacea* extract is likely not toxic, i.e., the toxicity is above the regulatory dose limit. After administration of the extracts, the animals were observed individually for 24 hours and signs of toxicity were noted. A general state of sleep was noted in both the control and test animals. It was also noted that the rats were slightly agitated for the first 10 minutes after gavage with the ethanolic extract of the test plant.

During the experiment, a non-significant weight growth was observed from Jo to D7 and from D7 to D14 in both batches (test and control). It can be deduced that the ethanolic extract of *Opilia amantacea* does not affect the weight growth of the animals tested. However, there was a significant difference between the weight growth of D0 at D14 observed in both batches may be related to the nutritional quality of the feed consumed by the animals. In addition, a good assimilation of secondary metabolites may be linked to the presence of bioactive substances in the ethanolic extract of *Opilia amantacea*, which may favor weight growth. The change in body weight is used as a general indicator of the adverse effects of chemicals on a living organism [30]. However, weight loss is correlated with the physiological state of the animal and can be explained not only by anorexia but also by impaired metabolism in animals [31]. In this study, weight gains were observed in both the control and experimental batches. It could be inferred from these data that the ethanolic extract of *Opilia amantacea* does not affect the weight growth of the animals. This growth could be attributed to animal feed mainly to the nutritional composition of the feed (granules). According to Guemah [32], rat granules are rich in protein, fat, fiber, and calcium. These nutrients present in the feed justify the weight gains observed in the control batch and the experimental batch.

In toxicological studies, the weight of organs such as the liver, kidney, spleen, testicles, heart, pancreas, brain, and tongue are very important indices used to assess the toxic effects of the substance being studied. The relative weights of the organs provide information on a possible hypertrophy, atrophy, or swelling of these organs [33]. In this study, there was no significant difference in the weight of the liver and

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**Figure 5:** Effects of the ethanolic extract of *Opilia amantacea* on transaminases (ALAT and ASAT) and alkaline phosphatases

**Figure 6:** Effects of the ethanolic extract of *Opilia amantacea* on leukocytes, red blood cells and blood platelets

**Figure 7:** Effects of the ethanolic extract of *Opilia amantacea* on hemoglobin and mean corpuscular hemoglobin concentration

**Figure 8:** Effects of the ethanolic extract of *Opilia amantacea* on hematocrit, neutrophils, lymphocytes, and monocytes
kneys of the rats between the control batch and the batch treated with 2000 mg/kg of the ethanolic extract of Opilia amentacea Roxb. These data show that the extracts did not cause any hepatotoxic effects on the liver or pathological conditions on these organs.

The hematological parameters studied showed no significant difference between the test and control batches (p>0.05). From the absence of a significant decrease in hemoglobin levels between the test and control batches, it could be deduced that the extract did not cause anemia in the animals. A similar analysis obtained at the level of the mean corporcular hemoglobin concentration justifies that the extract does not have an anemic effect on the blood tissue. The absence of a significant parallel decrease or increase between hemoglobin and hematocrit leads us to understand that the extract did not cause hemodilution or hemocoagulation in the blood tissue.

Concerning the results on the leucocyte formula, we notice that the neutrophils and lymphocytes of the test batch do not present any significant difference between the test and control batch. From these findings, it could be concluded that the oral administration of the ethanolic HE of the studied plant did not induce an inflammatory reaction that could significantly increase the WBC count in the animals of the experimental batch. It could be concluded that no infectious disease state was observed. The eosinophilic polynuclear also did not show a significant difference between the test and control batches, which justifies that the extract did not cause allergies in the animals.

The biochemical parameters investigated, ALAT/GPT and ASAT/GOT transaminases, total and conjugated bilirubin, alkaline phosphatase, creatinine, urea and uricemia showed a non-significant statistical difference (p > 0.05) between the test and control batches. From these data, it could be deduced that the administration of the ethanolic extract of Opilia amentacea has no effect on organs such as liver, heart and kidneys. The bibliographic synthesis on the toxicity of Opilia amentacea reveals that very few studies have been devoted to the safety of this plant. The scientific data obtained are mostly related to the evaluation of some pharmacodynamic properties. No animal deaths were recorded during the experimentation and at the end. These results attest to the scarcity of toxicological studies on Opilia amentacea despite its multiple uses. The results obtained can be compared with those of Konaté et al., who found a DIs of 636.2 mg/kg body weight with some signs of reversible toxicity in mice following intraperitoneal administration of the aqueous extract of Opilia amentacea. The lethality observed by these authors may be related to the route of administration and the physiological state of the animals which certainly differs. EEOa therefore has no toxic effects on liver and kidney function studied at 2000 mg/kg. These results corroborate those of Ezegwü et al., who showed that the aqueous extract of Cnidoscolus aconitifolius (Mill.,) plant of a botanical family different from Opilia amentacea is not toxic to the liver and kidneys. Considering these results obtained, we can deduce that the ethanolic extract of the leaf powder of Opilia amentacea was found to be non-toxic, therefore does not have a negative influence on the blood tissue, nor on vital organs such as the liver and kidneys, at the tested doses.

**CONCLUSION**

The evaluation of the acute oral toxicity of the ethanolic extract of the leaves of Opilia amentacea attests that the plant is without toxic effects on blood tissue, liver and kidneys at the dose of 2000 mg/kg. These results justify that the use of Opilia amentacea in an alternative way in the treatment of various pathologies is not subordinated to an immediate intoxication. Further work such as subchronic or chronic toxicity tests and histopathological studies must be carried out to confirm the medium and long-term toxicity of this plant.

**Conflict of Interest**

None declared.

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