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Abstract:
The overall objective of our study is to evaluate the toxicity of ethanolic extracts of Hexalobus monopetalus leaves on the liver and kidney functions of rats in its therapeutic use. Thus, an ethanolic extract of the leaves was made, then a phytochemical screening of this extract. On the one hand, acute toxicity was measured in a 14-day feeding trial in Wistar rats, and on the other hand, a histopathological study of the organs removed was performed. The phytochemical screens of the ethanolic extracts obtained showed the presence of several phytochemical groups with therapeutic activity. Acute toxicity was noted at 3000 and 5000mg / kg PC doses of rats. Histopathological study revealed hepatic pycnosis at a dose of 5000 mg / kg PC in rats. Finally, no renal damage was observed.

Keywords: Hexalobus Monopetalus; Acute Toxicity; Pycnosis; Wistar Rats.


1. Introduction

Flora in general is home to species that provide protection against the environment, habitat for wildlife, wood, fibers and remedies for humans. And Africa is renowned for the richness of its flora, a diversified and valuable abundant resource of plants used for therapeutic purposes by the indigenous population. Indeed, of the 300,000-plant species listed on the planet, more than 200,000 species live in tropical countries of Africa and have proven medicinal virtues (Sofowora, 1993). So, we cannot talk about health in Africa without resorting to medicinal plants. According to WHO estimates in 2012, 80% of the world's population depends mainly on traditional medicine for the treatment of diseases. Medicinal plants constitute a valuable heritage for humanity and especially for the majority of the poor communities in the developing countries who depend on them for their primary health care and subsistence (Salhi et al., 2010). This is due to the weak economic resources of the populations in these countries which limits the purchase of pharmaceutical products (Diallo, 2014).
This said, the Hexalobus monopetalus is a medicinal plant native to tropical Africa whose virtues are not yet fully known. However, it would be effective in the treatment of certain diseases encountered in humans as well as in animals (mainly cattle), such as the treatment of gastrointestinal disorders (stomach pain) and fevers (yellow fever) (Oumar et al, 2014), diabetes in combination with Ficus glumosa (Adjanohoun et al., 2006), anorexia in cattle (Assogba M, 1984). This suspected potentiality on Hexalobus stimulates our interest in the search for possible toxicity in case of overdose of the active substance of the plant and its consequences on the liver and the kidneys which, through their functioning represent essential organs of the body (human). Indeed, the liver is a regulating organ because of its complex metabolic functions and indispensable to life and the main organ of detoxification of the organism. Several drugs used in humans may have hepatotoxic effects. Drug hepatotoxicity can lead to severe hepatic diseases, including acute liver failure, which is associated with a mortality incidence of 45-95% according to etiology (Seide, 2008). Likewise, the kidneys represent the center of purification and regulation of the human body. Each year new chronic kidney disease is detected at some stage. Kidney disease is silent outside of urinary calculi. Often they are not detected early enough to delay the progression to chronic, sometimes severe (and especially irreversible) renal failure. An attack on their functioning, one as well as the other, affects the general state of health of an individual.

Experimental tests are always required to be performed on a new drug or chemical substance that may affect human health. According to the laws, these safety tests are carried out on animals in order to obtain answers concerning the effects on the vital organs such as the heart, the liver, the lungs, the kidneys ... The acute toxicity test is among the Safety tests where animals are administered a single dose of the test compound. The objective of these tests is to define the interval between the dose which does not cause any adverse effect and the lethal dose. The OECD legislative guidelines recommend comparing the effects observed in animal tests with control groups of animals that have not received the compound. The animals are placed under observation for 14 days after receiving the compound; accurate data are collected on possible toxic effects, including the onset of early signs of toxicity and potential recovery. On day 14, all surviving animals were sacrificed and dissected to observe possible signs of toxicity (Animal Research Info).

Moreover, Hexalobus monopetalus is one of the phytotherapeutic species on which extensive studies have not been carried out. In the literature, many studies had not addressed fundamental studies on this plant. However, any biologically active substance is susceptible, at high doses or at low doses, and for prolonged administration to produce adverse or even harmful effects. It is in this perspective that we are interested in the toxicological aspect of the Hexalobus monopetalus, through this work entitled "Evaluation of the toxicity of the ethanolic extracts of the leaves of Hexalobus monopetalus on the kidneys and the liver of the rats" Wistar.

Généralités sur l'Hexalobus monopetalus (A. Rich)

Classification

Taxinomie et noms usuels

La taxinomie est la science qui a pour objet de décrire les organismes vivants et de les regrouper en entités appelées taxons afin de les identifier puis les nommer et enfin les classer. Bien que légèrement variée selon les auteurs, voici une classification plus détaillée de l’espèce Hexalobus monopetalus:
Tableau I : Classification de *Hexalobus monopetalus*

<table>
<thead>
<tr>
<th>Rang</th>
<th>Nom scientifique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Règne</td>
<td>Végétal</td>
</tr>
<tr>
<td>Embranchement</td>
<td>SPERMAPHYTES</td>
</tr>
<tr>
<td>Sous embranchement</td>
<td>ANGIOSPERME</td>
</tr>
<tr>
<td>Classe</td>
<td>MAGNOLIOPSIDA</td>
</tr>
<tr>
<td>Sous-classe</td>
<td>DIALYPETALES</td>
</tr>
<tr>
<td>Ordre</td>
<td>MAGNOLIALES</td>
</tr>
<tr>
<td>Famille</td>
<td>ANNONACEAE</td>
</tr>
<tr>
<td>Genre</td>
<td>HEXALOBUS</td>
</tr>
<tr>
<td>Sous Genre</td>
<td>PLANTAE</td>
</tr>
<tr>
<td>Espèce</td>
<td>H. monopetalus</td>
</tr>
<tr>
<td>Variété</td>
<td>H. monopetalus monopetalus</td>
</tr>
</tbody>
</table>

In Benin, according to the localities, *Hexalobus monopetalus* is designated under various names (in national languages) that are:
Dankokwe (Fon);
Mweswe, Tibaka, Abloggbe (Bariba);
Anambakada, ayetinbaga (Waama);
Akpado, Akpara Lapawe (Yoruba or Nago);
Daimahi (Peuhl)

**Botanical Description**

Of the family Annonaceae, the Hexalobus exists in two varieties the monopetalus and obovatus: they are medicinal plants. H. callicarpus, H. crispiflorus, H. monopetalus, H. mossambicensis, H. salicifolius (Plant and botanical, 2005). To date, six (6) species are counted.

The Hexalobus monopetalus (A. Rich) is actually a leafy shrub or a small tree of 7 to 8m, with a dense and bushy crown and a smooth, gray fibrous and scaly bark.

![Photo 1 : Plante d’*Hexalobus monopetalus*, Source : Cliché de Raïssa TAMÉGNON (2014)]
The simple leaves are alternate, shortly stalked, variable form, generally narrowly oblong, 2 - 10 cm long and 1 - 6.5 cm wide, rounded at the base, attenuated at the top; They are tough green-olive on the top, green-yellow on the reverse. Their petioles are very short and pubescent and about 1-4 cm long.

The axillary subsessile flowers, or placed on the axils of fallen leaves; Solitary or grouped by 2 or 3, open after leaf fall and are yellowish, greenish or cream colored with 3 red to brown sepals (length 6 mm).

The flowering of H. monopetalus variety obovatus occurs between August and September and fruits mature between January and April and are edible by humans and monkeys in the natural state. These fruits are picked at maturity on the tree, but they can also be plucked earlier and stored for ripening. The ripe fruit pulp is sweet and has a pleasant acid taste and gives off a strawberry smell.

The slightly pubescent, fleshy and indehiscent fruits are composed of 1-3 cylindrical to ovoid mericarps (1.5 to 5 cm long and 1.25 cm wide) and contain 2 to 8 seeds; Pulp when they are ripe (Fao, 1984).

The brown, compressed, ovoid seeds are 1.2 to 1.5 cm long, 6 - 9 mm wide and 7 - 8 mm thick (Fao, 1984).

Location and Geographical Description of the Area

Hexalobus is one of the genera of the family Annonaceae which is limited to the Savannah region of tropical Africa (dry forest on dry, rocky and rocky ground), mainly Tanzania, Senegal, French Guinea, Togo, Nigeria, Zimbabwe, Mali, South Africa, Angola, Ivory Coast.

Although there has been no known study of the geographical distribution of Hexalobus monopetalus in Benin, it has already been identified in 4 communes: Kouandé, Parakou and Sakété-Ketou. However, our work has focused on the leaves of Hexalobus monopetalus harvested from Parakou and Kouandé.
Capital of the North Benin, the city of Parakou, is located 407 km from Cotonou. It is an important crossroad of major roads; it is mainly the terminus of the railway that leaves Cotonou, economic capital of Benin. The commune of Parakou is one of the 8 communes of the department of Borgou with an area of 441 km² and is limited to the North by the commune of N'Dali, in the South, East and West by the commune of Tchaourou. It has a rolling appearance, with a succession of hillocks generally having a rounded summit, especially in the formerly cultivated regions. The humid tropical climate is characterized by the alternation of a rainy season (May to October) and a dry season (November to April). Climate change is changing seasons that are no longer distinctly sliced. It is with the harmattan that we record the lowest temperatures in the months of December and January. The average annual precipitation is 1200 mm. The maximum occurs between July and September. The vegetation cover observed in Parakou is dominated by the savanna tree. Fallows are invaded by rather diverse grasses and shrubs (KORA, 2006).

The second town, the commune of Kouandé is located in the eastern part of the department of Atacora and counts among the nine (9) communes of this department. This municipality covers an area of 4,500 km² and is bounded in the north by the commune of Kérou, in the north-west by that of Tanguiéta in the south-west by the commune of Natitingou, in the south by the municipalities of Copargo, Djougou and Boukombé, on the east by that of Pehunco and on the west by the commune of Toucountouna. This municipality enjoys a Sudano-Guinean climate, characterized by a rainy season, from mid-April to mid-October and a dry season from mid-October to mid-April. But altitude sometimes changes the normal parameters of the climate. The municipality belongs to an agro-ecological zone characterized by a rainfall that oscillates between 900 and 1100 mm per year with a peak in August. The average temperature is 27 ° C. This commune is also subject to the harmattan regime, a cold, dry wind that blows between November and mid-March and sometimes causes a thermal amplitude of 9.5 ° C (KORA, 2006).

The commune of Kouandé is part of the dry continental zone. Vegetation cover is the result of pedological skills, climatic variability and human actions on nature. Savannah is the dominant type of vegetation formation with tree and shrub savannahs with strong agricultural holdings, the forest gallery along the rivers, savanna trees and shrub savannah (KORA, 2006).

2. Materials and Methods

Preparation of the Ethanolic Extract of the Leaves of Hexalobus Monopetalus

This extraction method is based on the protocol used in the work of N’Guessan et al. (2007) and Sanogo et al. (2006).

50 g of powder obtained, weighed with an analytical balance Sartorius® is macerated in 500 ml of ethanol at 96 ° and homogenized with continuous stirring for 72 hours at laboratory temperature. Then three successive filtrations were carried out to separate the filtrate from the residue. The filtrate obtained is evaporated at 40. degree. C. using the Rotavapor® rotary evaporator and the mesh is reused for a second maceration in order to increase the yield. The paste deposited at the bottom of the evaporator flask is recovered in jars and dried in an oven at 45 ° C. After drying completely, the dried extracts joined to the bottom of the flasks are scraped with the stainless-steel spatula, crushed in the porcelain mortar and then kept in glass bottles previously labeled.
The yield is determined by the ratio of the weight of the dry extract after evaporation to the weight of the dry plant material used for the extraction multiplied by 100 (BEKHECHI B., 2001) cited by Medane (MEDANE A., 2012).

It is calculated according to the following formula:

\[ R = \frac{\text{mass of the extract}}{\text{mass of the powder of the leaves}} \times 100 \]

Acute Oral Toxicity Test
Choice of doses: gavage

This experimental study was adapted to that described in Guideline 423 (OECD, 2001). Modifications are being made to improve the feasibility of our study. Concentrations of the total ethanolic extract of Hexalobus monopetalus were prepared on the basis of the principle that the concentrations to be administered should be reduced to the body weight of the rats. Thus the doses were expressed in mg / kg of body weight. Different dilutions were therefore carried out to obtain concentrations corresponding respectively to the doses of: 2000 mg / kg, 3000 mg / kg and 5000 mg / kg. The different batches of animals (4 batches of 3 rats) were treated respectively with these different doses against the control receiving distilled water. These animals do not receive any other drug treatment over time outside the extract. The doses are unique, after the first day the rats will follow their normal diet until the 14th day when they will be sacrificed. The liver and kidneys are removed for histopathological observation in order to investigate the impact of extracts on their cells.

The animals of each test batch were gavaged 1 to 1. At the end of the experiment, all the batches were treated and the animals were observed for the first 24 h and regularly for 14 days.

3. Results and Discussions

Phytochemical Analysis
The results of the screening of H. monopetalus leaf powders are given in the table below:

<table>
<thead>
<tr>
<th>Groupes Chimiques</th>
<th>Sous-groupes</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>The polyphenolic compounds</td>
<td>tannins</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Gallic tannins</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Catechic tannins</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Anthocyanin</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Leuco anthocyanin</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Mucilage</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Reducing compounds</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Saponosides</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Alcaloïdes</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Cyanogenic derivatives</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Anthracene Derivatives</td>
<td>Free Anthracenes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O. Heterosides (A. Handsets)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Heterosides (A. Handsets)</td>
</tr>
</tbody>
</table>
Triterpènoides | -
---|---
Steroids | +
Cardenoides | -
Coumarins | -
Quinonic derivatives | +++

Light or weak coloring; (++) strong coloring; (+++) intense coloration; (-) lack of staining.

The phytochemical analysis of the ethanolic extract of the leaves of H. monopetalus made it possible to evaluate their chemical composition qualitatively. So it reveals:

An absence of coumarins, cardenoids, triterpenoids, saponosides, cyanogenic derivatives and combined anthracenics, known as C. heterosides.

The presence of polyphenolic compounds, mucilages, reducing compounds, alkaloids, anthracene derivatives (free anthracenics and O. heterosides), steroids and quinone derivatives.

**Extraction Yield**

\[ R = \frac{16.89}{50} \times 100 = 33.84\% \]

Ethanol extraction was carried out from 50 g of leaf powder of Hexalobus monopetalus. It yielded 16.89 g of a very dark green solid extract (turning black), a fairly good yield of 34%.

**Evolution du poids des rats au cours de la période d’essai (14 jours)**

![Graph showing weight change of rats](image)

Figure 3: Evolution of body weight of rats during the trial period (14 days)

The change in the body weight of the rats during the test period is shown in FIG. 3. The weight constancy is observed approximately for a few days (7 days) before their increase to the end of the test. The body weight of rats fed at the representative dose of 5000mg / kg of body weight, on the other hand, dropped before growing.
Histological Study
Comparative Histology of Rat Liver

Figure 4: Normal control rat liver (liver) (HES X 40)

The lobular architecture is marked by hepatic spans separated by capillaries and arranged radially around the centro-lobular vein

Figure 5: Livers of rats treated at 2000 (A), 3000mg / kg (B) and 5000mg / kg (C) body weight (HES X 40)

The hepatic architecture remains globally conserved in A and B, however, in C, although the lobular architecture is still recognizable, we must note a pycnosis of the nuclei and an effacement of the cellular limits in the hepatocytes.
Comparative histology of the kidneys of rats

Figure 6: Normal control rat kidney (HES X 40)

Renal cortex showing renal glomeruli and kidney ducts.

Figure 7: Rats kidney treated at 2000 (A), 3000 (B) and 5000 mg / kg body weight (C) (HES X 40)

The renal cortex shows a normal glomerulus (A). In (B), renal architecture remains quite normal with two renal glomeruli and normal renal canals. The histological structure of the kidney remains globally conserved (C).

4. Discussion

For a long time, natural substances derived from medicinal plants formed the basis for the treatment of many conditions (Ridtitid W et al., 2008). However, evaluation of the pharmacological activity of natural substances and their toxic effects is essential for a more secure use.

Hexalobus monopetalus is a plant with therapeutic virtues used in the world mainly in Africa to treat several ailments. In Benin, this plant is used more in veterinary medicine (OUMAR et al., 2014). In humans, it has not yet been demonstrated, strictly speaking, a therapeutic action, except for the work done by Adjanohoun et al. (2006), which showed that the aqueous decoctate of the roots in association with the trunk bark Of Ficus glumosa (orally) would treat diabetes. Following the results of the memory studies of (B. Affo, 2014) and (E. Houze, 2014), it was considered useful to evaluate the toxicity of the plant of Hexalobus monopetalus for its valorisation.
Our work consisted in the search for the acute toxicity of the ethanolic extracts of *Hexalobus monopetalus* in a single dose of between 2000 and 5000 mg/kg of body weight (OECD, 2001) by oral gavage of the wistar rats. This operation was carried out on four batches of rats, the first batch of which was used as control. The last 3 lots received successively 2000, 3000 and 5000 mg/kg PC extracts.

Acute toxicity is defined as that resulting from the single, massive exposure (or doses picked up in time) to a chemical causing bodily harm that can lead to death. It introduces the concept of "absorbed" dose (by ingestion, inhalation or dermal contact) and is measured by the LD50 (lethal dose, or dose causing the death of 50% of the animals exposed to a single dose of the product in question) Mg/kg of the experimental animal retained (Bourra, 2008). It is this dose that is used to classify chemicals according to their toxic, corrosive, harmful or irritating regulatory labeling. The lower the LD50, the higher the toxicity of the product tested, the greater the toxicity.

From the analysis, the qualitative phytochemical study of the total ethanolic extract of the leaves of *Hexalobus monopetalus* showed that the plant contains several groups of compounds including alkaloids, steroids, quinone derivatives, polyphenolic compounds, anthracene derivatives, mucilages, reducing compounds. The richness of this extract in active chemical compounds could explain the traditional use of *Hexalobus monopetalus* to treat many diseases such as diabetes (Adjanohoun et al., 2006) (Tumins, alkaloids, sterols, triterpenes and flavonoids possess anti-tuberculous and antiplasmodial properties (Limmattavapirat et al., 2004), which would explain the results of Joseph, 1993) on the plant resulting in an activity of bark extract on strains of *Plasmodium falciparum*.

The animals in general have supported the accepted doses. No deaths have been recorded. All animals survived the 14 days of observation, which did not determine the LD50. This would imply that the LD50 is greater than 5000 mg/kg bw. These results are therefore similar to those of Lebri, (2015), which by the same method demonstrated that the LD50 of *Abrus pectorius* is greater than 5000 mg/kg bw.

The histological study showed that at the concentrations of 2000, 3000 and 5000 mg/kg PC, the ethanol extract showed no negative effects on the kidneys. However at the concentration of 5000 mg/kg PC, hepatic pycnosis of the nuclei with an effacement of the cellular boundaries at the level of the hepatocytes without modification of the architecture was observed. Our results are different from those observed by Chabi and Al, 2015 who studied the evaluation of the toxicity of *Hemizygia bracteosa*, a plant traditionally used in the treatment of diabetes in Benin. They reported that necrosis of the renal cortex with glomerular hyalinization already occurred at the 2000 mg/kg PC concentration and the same observation was made on the liver lobules with hepatic necrosis occurring by acidophilic cytoplasm and disappearance of the nucleus.

5. Conclusions

The qualitative phytochemical analysis carried out on the total ethanolic extract of the leaves of *Hexalobus monopetalus* revealed the presence of several chemical groups, whose presence in the extract would be responsible for many therapeutic properties attributed to this plant. With regard to the acute toxicity test, we noted after oral gavage that for three days, rats fed at concentrations.
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