RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND TOXICOLOGICAL STUDY OF THE AQUEOUS EXTRACT OF STEM BARK OF MITRAGYNA INERMIS (WILD) O. KUNDZE (Rubiaceae), A TRADITIONAL MEDICINE PLANT.

Maxime Machioud Sangare¹ ³*, Jean Marc Ategbo¹ ², Eugene Selidji Attakpa¹, Jean Robert Klotoe¹ ², Felix F. D. Guinnin¹ ³, Abdoulayeslottina Zibrila³ and Karim Laye Dramane¹ ³.

1. Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou (Benin).
2. Laboratory of Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi (LARBA / EPAC / UAC), Benin.
3. Laboratory of Animal Physiology and experimental pharmacology, University of Abomey-Calavi 01 BP 526 Cotonou (Benin).

Manuscript Info

Abstract

**Background:** The use of plants gained widespread as well in developing countries as in developed countries for different purposes and both as mixture or single formula. That widespread use does not guarantee their safety and efficiency. The aim of the present study is to assess the phytochemistry and subacute toxicity of *Mitragyna inermis*, an alleged nephroprotective properties plant acclimated in Benin.

**Methods:** By complexion and/ or precipitation reactions, the different groups of secondary metabolites in these plants were highlighted. The crude aqueous extract of *M. inermis* has been force-fed to rats for seven days. They were observed for toxicity signs and some blood biochemical parameters have been evaluated before and after treatment. We used Microsoft excel and Minitab 1.6 to process data.

**Result:** Different phytochemicals were found and crude aqueous extract of *M. inermis* doesn’t induce toxicity signs on rats after seven-day force-feeding up to 2500 km/kg of body weight using oral route. Biochemical analysis shows a significant decrease in blood level of urea and creatinine up to 2500 km/kg of body weight. The significant increase in ALAT by D2 (1000 km/kg dose) and both in ALAT and ASAT by D3 (2500 km/kg dose) after 7 days is decreased and normalized during the observation period.

**Conclusion:** Crude aqueous extract of *M. inermis* stem back is less toxic up to 2500 km/kg of body weight and should be taken during consecutively less than 7 days.

**Introduction:**
The use of plant for nutritional and therapeutic purposes dates back to the ‘old world’ where trial and error methods were employed in the folklore means to treat ailments and diseases. Traditional medicine has proved to be a key element among the rural communities in developing countries for the provision of primary health care especially...
where there are inadequate primary health care systems (Tijani et al., 2012). The existence of traditional medicine depends on plant species diversity and related knowledge of their use as herbal medicine (Ssegawa et al., 2007). Nowadays, many plants have been studied and some of their attributed empirical properties in healing proved to be true. Most of these plants often display a wide range of biological and medicinal properties such as anti-inflammatory, antihemorrhagic, fertility enhancer, laxative, sexual enhancer, anti-microbial, anti-fungal (Tijani et al., 2012). Thus, the use of these plants gained widespread as well in developing countries as in developed countries. Today, due to the side effects of western medicines (i.e. chemical drugs), drug tolerance, rise of the complications of the disease (despite careful management), and high financial implications imposed on society and individuals; the use of medicinal herbs and traditional medicine has become a priority globally (Zareiet et al., 2015). Conversely, the widespread use and increasing popularity of herbal medicines does not guarantee their efficacy and safety (Shafaei, 2011).

*Mitragynainermis* is a savannas, forest and forest galleries shrub of 3–14 m in height and it is widely distributed in West to Central Africa (Senegal, Cameroon, Ghana, Ivory Coast, Mali, Chad, DR Congo and Sudan), where it is termed Nkiwu, lènin FonorNago, kabe in dendi (Akouegninouet et al., 2006). Well known for its ornamental and medicinal purposes, *Mitragynainermis* medicinal applications however still depends as practiced by inhabitants of different geographical areas. In Benin, the aqueous decoction of root barks and leaves is used orally against anorexia, constipation and in steam bath against leprosy (Akouegninouet et al., 2006). *Mitragynainermis* is reported to be used for the treatment of diabetes, ulcer, piles, dysentery and bone pain among the Hausa/Fulani tribes of northern Nigeria (Adoum et al., 2012). The hypotensive, cardiothoracic and vasodilatory properties of the aqueous extracts of the plant has also been reported (Ouedrago et al., 2004). Beyond its medicinal use, crude extract mixtures of leaves and stem from *M. inermis* showed good biopesticide activity against *Lepidopterous caterpillars* infesting plants of cotton (Ndiaye et al., 2008).

The effects on haematological and biochemical parameters should be assessed in order to determine the safe and unsafe use of medicinal plants. Change from normal physiological levels of these parameters after administration of a chemical agent to an experimental animal is an indication of adverse effects of such agent on living organisms (Kolawole et al., 2011). The present study is a preliminary work which contributes to a better knowledge on properties of medicinal plants used in traditional medicine in Benin for their alleged hepatoprotective and nephroprotective properties, especially *Mitragynainermis* of which phytochemical and toxicity are reported here.

**Material and Methods:**

**Preparation of plant extract:-**

For the purpose of this study, the plant material used is composed of stem bark *Mitragynainermis*. Plant material was air-dried to constant weight at room temperature afterwards it was grounded into powder using an electric blender. The powder was then dissolved in distilled water for three days. After every 24 hours, the mixture was filtered through a Whatman filter paper and water was then added for the following 24 hours. Crude extract was collected by removal of water in oven at 40°C. The concentrated extract obtained was used for the trials. Phytochemical examinations of the extracts were carried out for detection of alkaloids, phenols, flavonoids, tannins, saponins, phytosterols, terpenes, glycosides and steroids following the method reported by Koudoro et al. (2014).

**Experimental Animals:-**

Male and female Wistar strain albino rats weighing between 140-200g were used for the purpose of this experiment. The animals were obtained from the Institute of Applied biomedical sciences (ISBA-FSS/FAST, Cotonou, Bénin, from Prof.Laleye) and kept in well ventilated and hygienic animal house of Animal Physiology and experimental pharmacology laboratory under constant environmental and nutritional conditions (18 to 22°C). All the rats were kept in cages and given food and water ad libitum.

**Experimental Design:**

Animals were randomly divided into four groups of three rats each after body weight measurement to ensure homogeneity of the batches. The experimental groups (labelled group D1, D2, and D3) were administered 100 mg/kg of body weight, 1000 mg/kg of body weight and 2500 mg/kg of body weight of the extract respectively each day. The control group received distilled water. The administration was carried out for a period of 7 days and data was collected up to the 37th day.
**Biochemical essays:**
The biochemical assays were performed using blood from the eyes of the rats and samples were collected three times. The first batch of samples were collected five days before drug administration (D0) from the rats and the second batch was done on the eighth day after the start of drug administration (D+7); with the third and last batch of samples collected on 37th day from the start of drug administration (D+30). Blood samples were collected into a clean dry test tube without an anticoagulant. The blood then was centrifuged at 3000 rpm for 10 minutes and the collected serum was used for estimation of biochemical parameters. Estimation of biomarkers for kidney (alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT)) and liver (urea and creatinine) functions were done using spectrophotometer KENZA MAX BioChemisTry Photometer according to standard procedures provided along with the kits supplied by Biolab (France).

**Data Analysis:**
The values of parameters were compared between groups using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. All the data analysis was performed using Minitab 16. Data was presented as mean ± SD and significance was taken at p<0.05

**Results:**
**Phytochemical group:**
Table 1 showing phytochemical group identified into aqueous crude extract of Mitragynainermis reveal the presence of catechic tannins, alkaloids, flavonoids, saponosides, terpenes and sterols. However, compounds such as gallic tannins, anthraquinones and coumarins were absent.

**Toxicity study of crude extracts of M. inermis:**
Along this part of our paper D0 means Starting day (Fives days before first gavage), D+ 7 means the day after the last gavage, D+ 30 means 30 days after the last force feeding.
We recorded no particular signs of toxicity on animal during 24 hours following first drug administration. RecordedSigns are piloerection, convulsions, lethargy, diarrhea and sleep. The alive animals number was also recorded. From the following table 2, no death was recorded during the experimental period.

Change in body weight of rats administered with the aqueous extract of M. inermis are shown in figure 1.
As per figure 1, there was no significant variation of animals body weight during the experiment (P> 0.05) in all groups.

**Table 1:** Phytochemical groupements in M. inermis stem back.

<table>
<thead>
<tr>
<th>Mitragynainermis</th>
<th>Tanins</th>
<th>Catechic</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gallic</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcaloides</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucilages</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponosides</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terpenes et sterols</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coumarines</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Animals group.

<table>
<thead>
<tr>
<th>Number of animal</th>
<th>Control group</th>
<th>D1 (100mg/Kg BW)</th>
<th>D2 (1000mg/Kg BW)</th>
<th>D3 (2500mg/Kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>D+7</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>D+30</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
Significantly different (p<0.05) *: compared to control group ¥: Compared to D0 in the same group

**Figure 1:** Body weight of rats.

**Figure 2:** Effect of extract on kidney function.

**Figure 3:** Effect of extract on Liver function.

**Effect of the drug administration on biochemical parameters:**

**Effect on kidney function:**

Drug administration to rats at 100 mg/Kg BW and 1000 mg/Kg BW did not show any effect on the urea level in blood during the first 7 days and also after 30 days of observation. But at 2500 mg/Kg BW, the extract reduced significantly the urea level in blood (Fig. 2 A). At 1000 mg/Kg BW, the extract significantly reduced the blood creatinine level that had been increased by the seven-day administration (Fig. 2 B). The 2500 mg/Kg (BW) dose also reduced blood creatinine level after 30 days from the drug administration stop day.
At 100 mg/Kg (BW), the extract did not change the ALAT level in blood, but at 1000 mg/Kg (BW) and at 2500 mg/Kg (BW), the extract increased ALAT level significantly after seven consecutive days of drug administration (Fig.3 A). After 30 days, the normalization of ALAT level was noticed in group D2, which was significantly higher in comparison to the starting level and the control group (Fig.3 A). Blood level in ASAT was affected by seven-day administration of the extract at 2500 mg/Kg (BW) but normalized after 30 days of observation (Fig.3 A). Other doses (D1 and D2) didn’t affect ASAT levels. 100 mg/Kg (BW) and 1000 mg/Kg (BW) doses did not induce significant changes, although there was a small variation after seven-day drug administration (Fig.3 A).

Discussion:-

In the current study, the phytochemicals found presented results similar to those of Konkonet et al. (2008) and those of Wakirwaet al. (2013) in *Mitragyna inermis* leaves extracts (infusion and decoction); however, contrary to the above mentioned studies saponosides was also present. Similarly to the current study, Ndiaye et al. (2008) also reported finding saponosides besides other phytochemicals such as flavonoids, alkaloids and tannins. The main differences in the findings could be attributed to the difference in plant parts or solvent systems used in different studies. Indeed, these two research groups used *Mitragyna inermis* leaves extracts (Konkonet et al., 2008 and Wakirwaet al., 2013). Ndiaye et al. (2008) used both leaves and stem back whilst the present study used only stem back extract for the phytochemical screening. This difference can also be attributed to climate and soil conditions of the harvesting areas (Olaleye, 2007).

The present study showed no particular signs such piloerection, convulsions, lethargy, diarrhoea and sleep. Moreover, after 37 days experimental period, no death within animals was recorded. Such results suggested that the crude aqueous extract of *M. inermis* do not possess acute toxicity up to 2500mg/kg of body weight in rats. Several research groups came to the same conclusion. Konkonet al. (2008) using *M. inermis* leaves extracts estimated LD50 over 5000 mg/kg in mice and concluded that leaves freeze-dried aqueous extract of *M. inermis* was less toxic. Timothy et al. (2015) estimated the LD50 of the ethanolic extract of *M. inermis* stem bark at 1587.45 mg/kg for intraperitoneal route and at 4298.84 mg/kg for oral route. Atinga et al. (2015) also concluded that methanol leaf extract *M. inermis* in mice is relatively less toxic after finding intraperitoneal LD50 to be more than 2000mg/kg of body weight. Furthermore, in the current study, the observation of less toxicity was reinforced by other research groups’ conclusions using different solvents (water, methanol); in different animal (mice, rats) and different route (oral and intraperitoneal) (Timothy et al., 2015, Uthmanet al., 2013, Adoumet al., 2012, Atinga et al., 2015, and Konkonet al., 2008).

The experimentation period did not affect animal’s body weight suggesting that the crude aqueous extract of *M. inermis* doesn’t affect animal appetite. Because kidney and liver toxicity has been reported following the use of phytotherapeutic products (Saadet et al., 2006), biochemical parameters evaluation was important. In this study, it was determined the effect of a regular administration during one week of aqueous extract of two medicinal plants acclimated in Benin on rat serum creatinine, urea, ASAT and ALAT levels as bio-markers of kidney and liver activities. Since plasma urea concentration reflects the balance between urea production in the liver and urea elimination by the kidneys, its increase in plasma can be caused by increased urea production, decreased urea elimination, or a combination of the two. Urea is the dominant urinary osmole in most mammals and may be concentrated a 100-fold above its plasma level in humans and even more in rodents. The present study results showed a significant decrease in blood urea at 2500 mg/kg of body weight. This finding suggested that extracts could increase urea elimination by kidney acting on glomerular filtration rate (Higgins, 2016). Creatinine, which is a waste product in the blood that came from muscle activity and it, is normally removed from the blood by kidneys. Its levels rising when kidney function slows down. Generally, increased urea levels are associated with nephritis, renal ischemia and urinary tract obstruction (Guyton, 2012). Similarly, to urea, creatinine level is reduced significantly by drug administration at 1000 mg/kg and 2500 mg/kg of body weight. That observation supposed a nephroprotective effect of extract.

Transaminases are enzymes having significant metabolic activity within cells and their increase suggested a cellular lesion particularly in the liver, heart, kidney or muscular (Sanogo, 2008). Seven-day administration of the extract increased both ALAT and ASAT levels at 1000 mg/kg of body weight (ALAT) and at 2500 mg/kg of body weight (ALAT and ASAT). Interestingly, the levels of those parameters decreased and normalized after 30 days from drug administration date suggesting that the first seven days of drug intake can cause damage to either liver, heart, kidney or muscular cells.
Conclusion:-
The phytochemical screening in crude aqueous extracts from stem bark of *Mitragyna inermis* revealed different phytochemical compound such as tannins, alkaloids, flavonoids, terpenes and sterols but saponosides was absent in leave *M. inermis*. The solvent and plant part used have been shown to be the main causes of the difference. Toxicological study allowed confirming the less toxicity of crude aqueous extracts from stem bark of *M. inermis* using oral route in rat wistar. Biochemical essay also revealed and confirmed less toxicity, suggesting the beneficial effect of the extract up to 2500 mg/kg of body weight for the liver; but the safety duration of the extract intake must be determined for the further pharmacological study of crude aqueous extract from stem bark of *M. inermis*. However, seven-day drug administration affect ALAT and ASAT levels negatively at 2500 mg/kg of body weight.

References:-