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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.

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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 *Mitragynainermis*, an allegatednephroprotective 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

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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 (Zarei*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, lèn*in*Fon*or*Nago, kabe* in *dendi* (Akouegninou*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 (Akouegninou*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 37^{th} 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 was started 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.

		Mitragynainermis
Tanins	catechic	+
	gallic	-
Anthraquinones		-
Alcaloides		+
Flavonoides		+
Mucilages		+
Saponosides		+
Terpens et sterols		+
Coumarines		-

Table 2:- Animals group.

	Nomber of animal			
	Control group	D1 (100mg/Kg BW)	D2 (1000mg/Kg BW)	D3 (2500mg/Kg BW)
D0	3	3	3	3
D+7	3	3	3	3
D+30	3	3	3	3

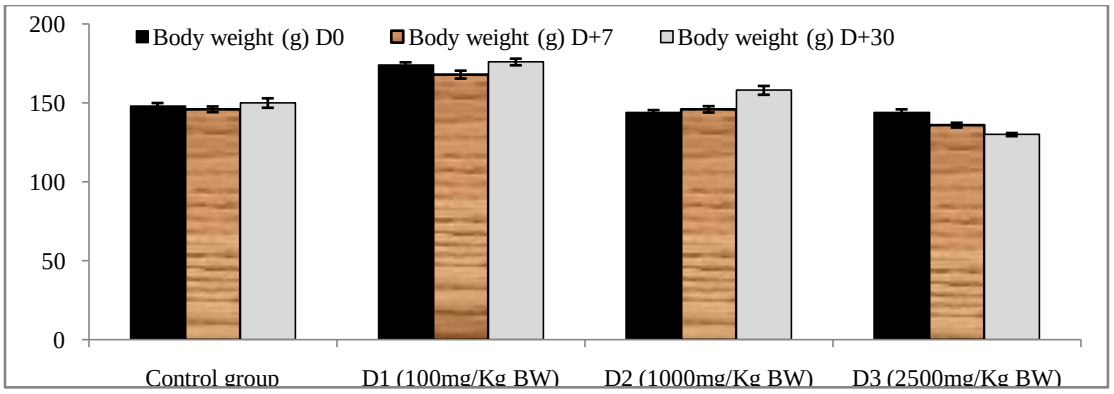
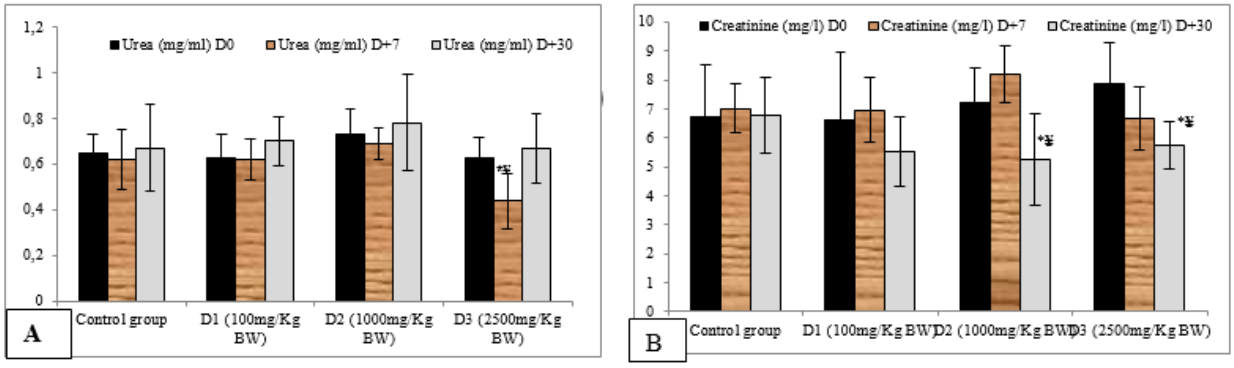
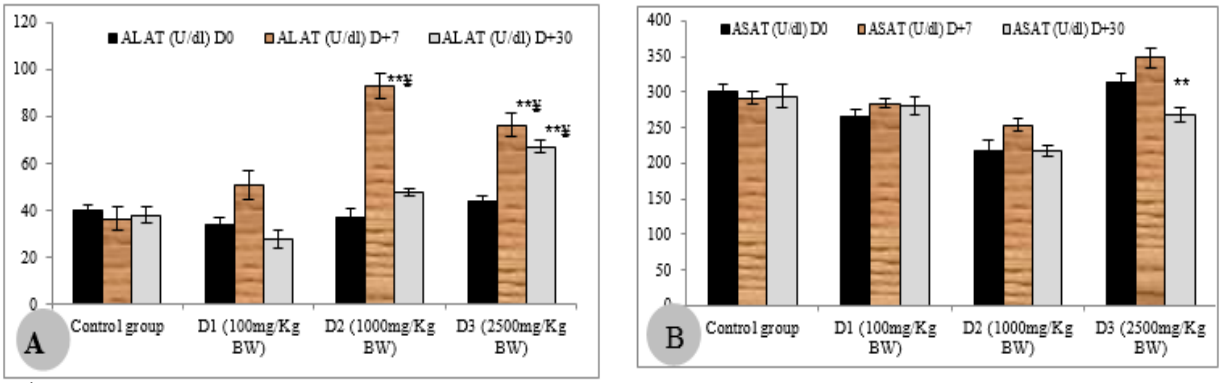


Figure 1:-Body weight of rats.



Significantly different ($p<0.05$) ***** : compared to control group ;**¥** :Compared to D0 in the same group

Figure 2:- Effect of extract on kidney function.



Significantly different ($p<0.05$) ****** : compared to control group ;**¥** :Compared to D0 in the same group

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 Konkon*et al.* (2008) and those of Wakirwa*et al.* (2013) in *Mitragynainermis* 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 *Mitragynainermis* leaves extracts (Konkon*et al.*, 2008 and Wakirwa*et 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. Konkon*et 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, Uthman*et al.*, 2013, Adoum*et al.*, 2012, Atinga*et al.*, 2015, and Konkon*et 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 (Saad*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 flirtation 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. Itslevels 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 *Mitragynainermis* 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:-

1. Adoum, O. A., Nenge, H.P., and Bashir C. (2012): The steroidal component and hypoglycaemic effect of stem bark extract of *Mitragynainermis*(wild) o. kundze (rubiaceae) in alloxan induced diabetic wistar rats. International Journal of Applied Biology and Pharmaceutical Technology, 3(2):169 – 174.
2. Akoegninou A., FloreAnalytique du Bénin. Cotonou, Wageningen, 1034 p., 2006.
3. Atinga V., Midala T.A.S., Timothy S.Y., Moh'd A. S. (2015): Evaluation of *Mitragynainermis* (wild) leaf extract as an anticonvulsant agent in pentylenetetrazole induced seizures in mice. Advances in Biomedicine and Pharmacy, 2 (5)
4. Guyton A. C., Hall J. E., (2012): Textbook of Medical physiology. 12thed, pp, 450-454, Philadelphia: W.B. Saunders.
5. Higgins C., (2016) Urea and the clinical value measuring blood urea concentration. http://acutecaretesting.org, July 2016.
6. Kolawole S. O., Kolawole T.O., etAkandji M. A., (2011): Effects of aqueous extract of Khayasenegalensis Stem Bark on biochemical and hematological parameters in Rats; J. of Pharm. And Toxic., 6 (6): 602-607.
7. Konkon N.G., Adejoungona A.L., Manda P., Simagn D., Nguiesan K.E and Kone B.D. (2008): Toxicological and Phytochemical Screening Study of M. inermis (Wils) O. Kuntze (Rub.). Anti diabetic Plant. J. Medicinal Plant Research, 2 (10): 279 – 284.
8. Koudoro Y. A., Dedome O. L. , Yehouenou B., Yovo M., AgbangnanDossa C P., et al., (2014): "Free radical scavenging and antibacterial potential of two plants extracts (Khayasenegalensis and Pseudocedrelakotschyi) used in veterinary pharmacopoeia in Benin", Elixir Appl. Chem., 76: 28720-28726 ,
9. Ndiaye M., Bassène E., Sow M. and Sembène M. (2008): Effetsbiocidés des extraitstotauxaqueuxd'*Anogeissusleiocarpus*(dc) wall (combrétacées) et de *Mitragynainermis* (willd.) O ktze (rubiacées) sur les chenilleslépidoptères du *Gossypiumhirsutum* l (malvacées). Journal des Sciences et Technologies, 6 (1): 29 – 35
10. Ouedraogo S., Ranlayo H. R., Ndiaye M., Kabore Z. I., Guissous, I.P., Bucher, B. and Andrianotsitohaina, R. (2004): Cardiovascular properties of aqueous extract from *M. inermis* (Wild). J. Ethnopharmacol., 93: 35 – 350.
11. Saad B, Azaizeh H, Abu-Hijleh G, Said S., (2006): Safety of traditional Arab herbal medicine. Evidence-based Complementary and Alternative Medicine, 3: 433– 439
12. Shafaei A., E. Farsi, B. M. K. Ahamed, M. J. A. Siddiqui, I. H. Attitalla, I. Zhari and M.Z. Asmawi, (2011): Evaluation of toxicological and standardization parameters and phytochemical investigation of Ficusdeltoidea leaves", Am J BiochemMol Biol., 1: 237-243,
13. Ssegawa P., J. M. Kasenene, (2007): Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. J. Ethnopharmacol., 113: 521–540.
14. Tijani A. A., Adekomi D. A., Ogedengbe O. O. and Adekeye A. O. (2012): Some of the effects of aqueous leaf extract of Malabar nightshade on the kidney and liver of albino wistar rats;European Journal of Experimental Biology, 2 (2):337-342
15. Timothy S.Y., Helga B.I., Bomai H.I. and Musa A.H., (2015): Acute and subchronic toxicity study of the aqueous and ethanolic extracts of Mitragynainermis bark in albino rats'', Inter. J. Pharmacol. Toxicology, 5: 24-32
16. Uthman G. S., Gana S. and Zakama S., (2013): Anticonvulsant screening of the ethanol leaf extract of Mitragynainermis (wild) in mice and chicks'', IJRPBS, 4: 1354-1357
17. Zarei A., Vaezi G. H., Malekirad A. A., Abdollahi M., (2015): Effects of ethanol extract of Salvia hydrangea on hepatic and renal functions of streptozotocin-induced diabetic rat. Avicenna J Phytomed, 5 (2): 138-147