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Safety study of the aqueous extract of the leaves of Lantana cumara L. (Verbenaceae), a plant used in Benin in the treatment of infections.

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Subject description : *In Benin, many medicinal plants were used by the populations for the treatment of infections. However, these herbal remedies should be used with great caution as they can be toxic.*
Objectives: *In this perspective, this work consisted in carrying out a study of the Acute Oral Toxicity and the Sub-Chronic Oral Toxicity (TOC) of aqueous extracts from the leaves of Lantana cumara L. (Verbenaceae), a plant widely used in Benin to treat skin infections.*
Method: *Wistar rats were given a single dose of 2000 mg / kg aqueous extract of Lantana cumara leaves by gavage for the Acute Oral Toxicity (TOA) test. For the sub-Chronic Oral Toxicity (TOC) test, rats were force-fed with the extract at a daily dose of 300 mg / kg of body weight per day for 28 days. The weight of the rats was taken and the blood samples collected on Day 0 and then respectively on day 14 for the TOA and Day 28 for the TOC. The renal balance was carried out by assaying serum creatinine, the hepatic balance by AST and ALT transaminases and the haematological balance by the blood count. The liver, kidneys and spleen were removed for histological analysis. The results were analyzed on the Student's test, the significance level set at 5%.*
Results: *The weight of the rats did not vary significantly on acute or subchronic oral toxicity tests suggesting an absence of physical disturbance to the rats. Serum creatinine did not change significantly, suggesting preservation of renal function. The same was the same for the AST and ALT transaminases, indicating an absence of hepatic cytolysis. Hematologically, the hemoglobin level and the number of blood platelets did not change significantly, suggesting that the extract did not create anemia and did not influence blood clotting. The hepatic, renal and splenic parenchyma did not show atypia.*
Conclusion: *The aqueous extract of the leaves of Lantana cumara (L.) did not reveal acute or subchronic toxicity and offers prospects for its use.*
Keywords: *Lantana cumara, oral toxicity.*

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I. INTRODUCTION

In recent years, there was a resurgence of interest in herbal medicine, in fact more and more people were using medicinal plants to treat themselves WHO (2007). It should be noted that the traditional use of any plant for therapeutic purposes did not guarantee its safety (Ukwuani et al., 2012). If the pharmacological effects of many plants was proven in various laboratories, their toxicity was generally unknown. Therefore, evaluating the toxicity of herbal preparations was important in determining the safety of these remedies (Atsamo et al. 2011).

The plant species Lantana camara (of the Verbenaceae family) was the most widespread species of the genus Lantana native to tropical and subtropical America. Lantana camara L. was a perennial shrub, it grew up to 2 to 3 meters and could reach about 2.5 meters in width (Lonare et al., 2012). Lantana camara was used in many parts of the world to treat a wide variety of disorders. In Central and South America, the leaves was made

into a poultice to treat sores, chickenpox, and measles. Lantana extract was used against fever, colds, rheumatism, asthma, high blood pressure. In Ghana, a whole plant infusion was used for bronchitis and powdered root in milk given to children with upset stomach (Saxena et al., 2012). In Asian countries, the leaves were used to treat cut wounds, rheumatism, ulcers, and parasitic diseases. Decoctions were used externally against leprosy and scabies (Verma, 2018). This plant was rich in secondary metabolites as alkaloids, phenolic acids, coumarins, tannins, terpenes and flavonoids… (Bahorun et al., 1996). Polyphenols, in particular, were endowed with multiple therapeutic virtues, they play a very important role, mainly in the fight against cancer, cardiovascular diseases, against bacteria and fungi, thus explaining their great use in the manufacture of drugs (Bruneton, 2009).

The aim of this work was to study the acute and subchronic toxicity of methanolic extracts from Lantana camara leaves in Wistar rats.

II. MATERIALS AND METHODS :

Animal material:

The animal material consisted of albino Wistar rats with an average body weight of 130 to 157 g, having free access to water and food and acclimatized to the conditions of breeding of the animal house of the laboratory of Molecular Pathophysiology and Toxicology of the Faculty of Sciences and Techniques (FAST) of the University of Abomey-Calavi UAC) in the Republic of Benin. The breeding took place in a well ventilated room, with a day-night rhythm of 12 hours. The animals were kept in wire mesh cages with feeders and drinkers. Their daily diet consisted of a mixture of foods in the form of croquettes and marketed by Veto Services (Benin). The enclosure was regularly cleaned to ensure optimal development of the animals and avoid infections.

Identification and preparation of plant material:

Identification

Lantana cumara leaves were collected in Abomey Calavi in Benin in December 2019. The samples collected were identified and authenticated at the National Herbarium of Benin (YH 512/ HNB). The University of Abomey Calavi. The leaves were dried at moderate temperatures (20-25 ° C), protected from moisture for four weeks. They were then ground into powder and stored in appropriate containers at room temperature.

Preparation of the aqueous extract

50 g of powder from the Lantana cumara leaves were boiled in 500 ml of distilled water in a 1000 ml flask for 30 minutes. After cooling, the mixture was filtered through a Bushner. This operation was repeated six times for a total mass of 300 g.

Acute oral toxicity tests :

Acute oral toxicity tests were performed in accordance with the recommendations of the Organization for Economic Co-operation and Development Guideline 423 for the Testing of Chemicals (OECD, 2002). The substance was tested in a sequential process in which three animals, including 8-12 week old female and non-pregnant multiparas were used at each stage. The absence or manifestation of substance-related mortality in a one-step dosed group would determine the next walk. The initial dose was chosen from the following four doses : 5, 50, 300 and 2000 mg / kg weight. We administered by gavage to the animals 2000 mg of Lantana cumara extract / kg of body weight. The animals were observed carefully for four (4) hours and then daily for 14 days. They were weighed and blood was taken by orbital puncture at the start of the experiment and then after 14 days.

Subchronic toxicity tests :

Five Wistar rats received Lantana cumara extract at 200 mg / kg body weight daily for 28 consecutive days by gavage (Biswas et al., 2010). They were weighed and blood was taken by orbital puncture at the start of the experiment and then after 28 days.

Blood tests :

Serum creatinine was assayed to explore renal function. AST and ALT transaminases were assayed for liver function. White blood cell count, hemoglobin level and blood platelet count were measured as haematological parameters.

Histology:

At the end of the experiment, the animals were dissected. The liver, kidneys and spleen were removed, fixed in Bouin's solution and embedded in paraffin. The specimen sections (5 m) were mounted on glass slides, dewaxed

and hydrated. For histological analysis, the sections were stained with hematoxylin and eosin (H&E), according to a standard protocol (Sènou et al, 2009). Photos were taken at 400X magnification.

Statistical analysis:

Means were compared using the Mann-Whitney test. The significance threshold was set at 5%.

Results:

The acute oral toxicity of the aqueous extract of the leaves of Lantana cumara was evaluated by measuring the weight of the rats as a physical parameter, AST and ALT transaminases as hepatic parameters, serum creatinine as a renal parameter, the number of white blood cells as an immune parameter, hemoglobin level to assess anemia and blood platelet count for hemostasis (Table 1).

***Table 1** : Acute oral toxicity*

Parameters	Means at D0	Means at D14	Pvalue	Difference
Rat weight (g)	136 ± 6	157 ± 4	0.2	no significant
Creatinine (mg /L)	10.5 ± 0.44	10.4 ± 0.41	0.9	no significant
Transaminase AST (U / L)	193 ± 6	121 ± 5	0.1	no significant
Transaminase ALT (U / L)	112 ± 10	115 ± 8	0.9	no significant
White Blood Cells (G/L)	11.2 ± 1.1	11.0 ± 1.1	0.9	no significant
Hemoglobin level (G/L)	15.4 ± 0.6	14.3 ± 0.5	0.7	no significant
Number of blood platelets (G/L)	673 ± 46	680 ± 51	0.9	no significant

The mean weight of the rats was 136 ± 6 g on day 0 and 157 ± 4 g on day 14, indicating that during the 14 days of the study, the extract promoted weight growth. However, the increase was not statistically significant. The mean creatinine level was 10.5 ± 0.44 g / L. There was no significant change on day 14 (10.4 ± 0.41), suggesting no deterioration in renal function. The AST and ALT transaminases were 193 ± 6 IU / L and 112 ± 10 IU / L, respectively, on day 0. On day 14, the AST transaminase was reduced (121 ± 5 IU / L) and the ALT transaminase. (115 ± 8 IU / L) experienced a slight increase on day 14. However, these variations were not statistically significant, suggesting protection of liver function. The mean number of blood leukocytes was 11.2 ± 1.1 G / L. It did not change significantly on day 14, suggesting no disturbance in immune function. The hemoglobin level was 15.4 ± 0.6 g / dL on day 0. There was no significant change on day 14 (14.3 ± 0.5 g / dL), indicating that the rats were not anemic by the treatment. The D0 blood platelet count was 673 ± 46 G / L. There was a slight increase to 680 ± 51 G / L on D14. This variation was not statistically significant, suggesting the absence of disturbance of blood coagulation phenomena.

Since no toxic effects were observed during the acute toxicity study, an additional study was conducted to assess the sub-chronic toxicity of the aqueous Lantana cumara L. extracts during a 28 day experiment in the Wistar rat. Subchronic oral toxicity was assessed by the same parameters previously measured for acute oral toxicity, namely rat weight as physical parameter, AST and ALT transaminases as liver parameters, serum creatinine as renal parameter, number of blood cells. White blood cells as an immune parameter, the hemoglobin level as an erythrocyte parameter and the number of blood platelets for blood clotting phenomena (Table 2).

***Table 2 :** Subchronic oral toxicity*

Parameters	Means at D0	Means at D14	Pvalue	Difference
Rat weight (g)	156 ± 5	182 ± 3	0.1	no significant
Creatinine (mg /L)	10.9 ± 2	8.53 ± 0.9	0.2	no significant
Transaminase AST (IU / L)	147 ± 7	144 ± 6	0.9	no significant
Transaminase ALT (IU / L)	101 ± 2	80.7 ± 2	0.1	no significant
White Blood Cells (G/L)	11.1 ± 1.0	9.0 ± 0.99	0.4	no significant
Hemoglobin level (G/L)	14.8 ± 0.1	14.9 ± 0.1	0.9	no significant
Number of blood platelets (G/L)	587 ± 40	612 ± 32	0.9	no significant

The mean weight of the rats was 156 ± 5 g on day 0. There was an increase on day 28, which was however not statistically significant, suggesting the absence of physical damage in the rats. The mean serum creatinine level was 10.9 ± 2 mg / L on D0. It did not significantly change on day 28, suggesting no disturbance in renal function. ASAT transaminase was 147 ± 79 U / L on day 0. There was no significant change on day 28 (144 ± 6 U / L). However, the ALAT transaminase which was on D0 at 101 ± 2 U / L experienced a non-significant drop on D28 with a value of 80.7 ± 2 U / L. The absence of an increase in AST and ALT transaminases suggested protection of liver function. The mean blood leukocyte count was 11.1 ± 1.0 G / L on day 0. It did not change significantly on day 28, suggesting no disturbance in immune function. The hemoglobin level was 14.8 ± 0.1 g / dl on day 0. There was no significant change on day 28 (14.9 ± 0.1 g / dl), which indicated that the extract did not not caused anemia in rats. The number of blood platelets on D0 was 587 ± 40 G

/ L. We note an increase in this rate to 612 ± 32 G / L. However, the increase was not statistically significant, suggesting the absence of disturbance in coagulation phenomena.

Analysis of physical, hepatic, renal, immune, erythrocyte and coagulation parameters showed that the aqueous extract of leaves of Lantana cumara was not toxic at the acute and sub-chronic condition. So, we continued our investigations with a histological study to explore possible morphological alterations of the liver, kidneys and spleen.

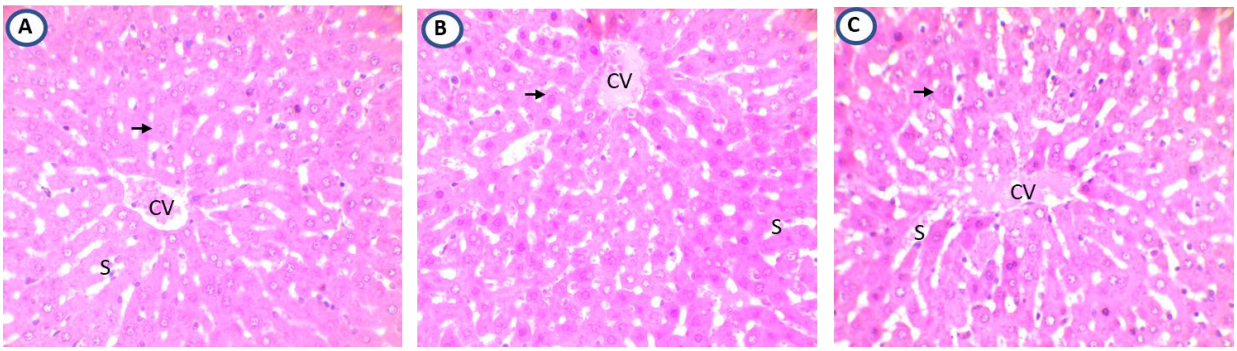

Figure 1 : Liver histology in acute and subchronic oral toxicity tests of aqueous extract of Lantana cumara leaves (magnification 400X).

In acute (Figure 1B) and subchronic (Figure 1C) oral toxicity tests, the liver of rats force-fed with aqueous extract of Lantana cumara showed no visible atypia. Normal-looking hepatocytes (arrows) were neatly arranged in radial cords around the central vein (CV). Venous sinusoids (S) were clearly visible as observed in control rats (Figure 1A).

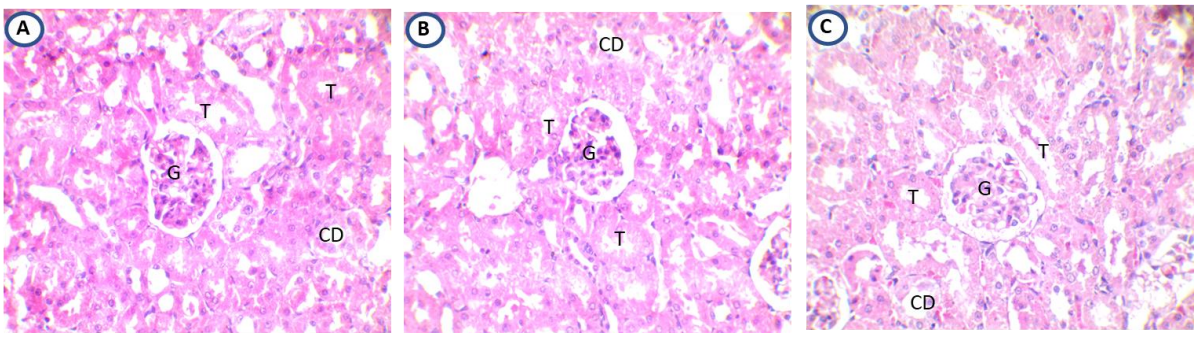

Figure 2 : Renal histology in acute and subchronic oral toxicity tests of aqueous extract of Lantana cumara leaves (magnification 400X).

In the acute (FIG. 2B) and subchronic (FIG. 2C) oral toxicity tests, the renal parenchyma of the rats fed with the aqueous extract of Lantana cumara retained its typical appearance observed in the control rats (FIG. 2A). The glomeruli (G), the proximal and distal tubes (T) as well as the collecting ducts (CD) showed no visible atypia.

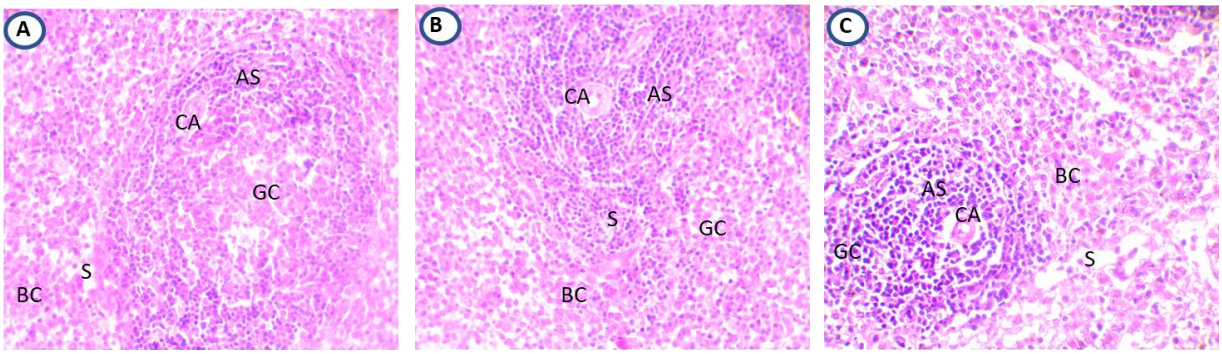

Figure 3 : Histology of the spleen in acute and subchronic oral toxicity tests of the aqueous extract of Lantana cumara leaves (magnification 400X).

In the acute (Figure 3B) and subchronic (Figure 3C) oral toxicity test, the splenic architecture of rats force-fed with aqueous extract of Lantana cumara was not modified and was normal as in the controls (Figure 3A). The central arteries (CA), peri-arteriolar sleeves (AS) and germinal centers of the white pulp appeared typical. The was the same for the venous sinusoids (S) and the Billroth cords (BC) of the red pulp which retained the typical architecture.

III. DISCUSSION :

Although medicinal plants showed many biological activities, little was known about the toxic potential of their bioactive substances (Rosidah et al., 2009). In this work we studied the safety of the aqueous extract of the leaves of Lantana cumara orally in acute and sub-chronic condition as recommended by OECD (2002). The parameters analyzed were physical (rat weight), hepatic, renal, immune, erythrocyte, thrombocyte and organ histology.

The aqueous extract of Lantana cumara leaves did not modify the behavior and weight of rats in acute or subchronic oral toxicity tests. It was suggested that oral and subchronic administration of the aqueous extract of Lantana cumara did not alterd the normal growth of rats.

Subchronic administration of Lantana cumara aqueous extract at a dose of 200 mg / kg resulted in an insignificant decrease in ALT enzyme levels in treated rats. These observations may suggest that the aqueous extract of Lantana cumara would have hepatoprotective effects. These observations corroborate with those of Luka et al. (2014) who explained in a similar study that a decrease in the liver enzymes AST, ALT and Alkaline Phosphatase could indicate a hepatoprotective effect of the crude ethanolic extract of the roots of Diospyros mespiliformis hochst (ebenaceae). This result was confirmed by the liver histology which remained normal.

Renal balance showed a non-significant decrease in creatinine on the sub-chronic toxicity test, suggesting that the aqueous extract of Lantana cumara leaves was not nephrotoxic. The observation was confirmed by histology which showed a renal parenchyma with glomeruli, proximal and distal tubules as well as collecting ducts apparently normal.

The immune function assessed by the white blood cell count was not affected in the acute or subchronic oral toxicity tests. The histology of the spleen, a peripheral immune organ, was not altered, confirming the plant's non-toxic character. According to Mukinda, JT & Syce, JA (2007), the analysis of blood parameters was relevant because it gave information on the hematopoietic-tick function (evaluation of cells of the myeloid lineage), on the appearance of allergies (studies of white blood cells) and on intravasucular effects such as hemolysis. The haematological balance did not show any significant difference between the treated rats and the control rats. These results were consistent with the work of NEA (2021) in Ivory Coast on Lantana camara and Lantana rhodesiensis, indicating that the toxicity of L. camara on humans, specifically on children, was not significant. Furthermore, according to Ghisalberti (2000), the green fruits of L. camara were toxic to humans and livestock. This could be explained by the change of plant organ or by the difference in environment and socio-cultural traits.

IV. CONCLUSION

Oral administration of the aqueous extract of Lantana cumara leaves did not show acute toxicity in wistar rats at the doses studied. Subchronic toxicity study in rats also did not show any toxicity of the aqueous extract of Lantana cumara leaves at administered doses. Histological study of the organs of the liver, kidneys and spleen did not show any signs of toxicity from the aqueous extract of the leaves of Lantana cumara. However, it would be wise to study chronic toxicity to assess the effect of the extract in the longer term.

Reconnaissance :

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