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Toxicity Study of Aqueous Extract and Methylene Chloride Fraction of *Allanblackia monticola* (guttiferae)

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Abstract

The aqueous extract and methylene chloride fraction of *Allanblackia monticola* were screened for toxicity activity. Swiss mice administered single oral doses of 2, 4, 6, 8, 10 g/kg (aqueous extract) or doses of 4 to 16g/kg (methylene chloride fraction) and monitored for death and growth impairment for seven days (acute toxicity). In subacute toxicity, experimental mice, received daily doses of 300, 600 and 1200 g/kg for 45 consecutive days and the toxic effects of the fraction were assessed using biochemical parameters as well as the study of the histological section of vital body organs (liver, kidneys).
The acute toxicity evaluation of aqueous extract has permitted to show that lethal dose (DL50) of aqueous extract is 6.4g/kg. The DL50 of methylene chloride fraction is situated above 16.5g/kg. The sub-acute treatment of mice with the methylene chloride fraction for 6 weeks has not revealed significant difference on the rate of serum proteins, of serum creatinine and that of ALT. The rate of the AST differs significantly from male to female respectively at the doses of 600 and 1200 mg/kg of body weight. There were no significant differences between the controls and treated in the body weight, organ weight of male and female mice. The histology of the kidneys has not shown significantly difference as compared to controlled animals.

KEYS WORDS: TOXICITY, *ALLANBLACKIA MONTICOLA, AQUEOUS EXTRACT, METHYLENE CHLORIDE FRACTION*

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Introduction

Allanblackia monticola Staner L. C *(Guttiferae)* has been used traditionally in Cameroon and other countries as remedy against amoebic dysentery, diarrhoea, indigestion, pulmonary infections, skin diseases, headache and generalised pain [1]. Its phytochemical studies revealed the presence of secondary metabolites belonging to xanthones, triterpenoids, phytosterols and biflavonoids with antiplasmodial and antimicrobial activities [2,3]. Our previous pharmacological screening of *Allanblackia monticola* extracts has revealed anti-inflammatory and anti- nociceptive activities of its methylene chloride fraction [4]. The present studies was designed to determine the acute and subacute toxicity tests of the aqueous extract, methylene chloride fraction stem barks of *Allanblackia monticola* in mice.

Methods

Plant

The stem barks of *Allanblackia monticola* were collected in July 2005 at Bangante, West Region, Cameroon. The identification was done by M. Zapfack, Department of Vegetal Biology and Physiology, University of Yaoundé I. A voucher specimen documenting the collection is deposited at the National Herbarium of Cameroon under the reference 61168 HNC.

Air dried stem bark of *Allanblackia monticola* were reduced to fine powder (1000g) and macerated in methylene chloride /methanol (1/1) for 48 hours at room temperature. The filtrate was concentrated in vacuum with rotavapor at 55°C giving viscous residue (yield of 2.8%). 25 g of the viscous residue was fractionated by flash chromatography using CH_2Cl_2 and methanol as mobile phases to yield 8 g of methylene chloride fraction and 13 g of methanol residue.

Aqueous extract was obtained by decoction of 1kg of stem bark powder of *Allanblackia monticola*, After filtration, filtrate was dried in oven at 50°C during 24 hours. 24g of powder was obtained equally to the ratio of 2.4%.

Animals

Males and females mice weighing 20-30g were used for this study. Animals were housed in the animal house of the Faculty of Science of the University of Yaoundé I, in plastic cages, under standard light (from 6 a.m to 6 p.m) and temperature (22 °C). Animals were feed with standard food and water ad libitum. tap water being available ad libitum. Animals were fasted overnight before treatment.

Acute toxicity test

The test was carried out following the methods described by WHO, 2000a[5]. Mice were deprived of food for 12 hours prior to extract administration and allocated (n=10) into six extract treatments groups (0, 2, 4, 6, 8, 10 g/kg, aqueous extract) and five groups (0, 2 4, 8 et 16g/kg, methylene chloride fraction). Extract treated mice received product by intra gastric means while control animals were given the vehicle. The animals were observed continuously for 2 hours for many behavioural changes.

Sub-acute toxicity test

Following the methods described by WHO, 2000a[5], mice were deprived of food and water 12 hours before the experience. They were divided into 4 groups (n=10, males and females) corresponding treated with fraction of methylene chloride fraction at the doses of 0, 300, 600 and 1200 mg/kg. The animals were administrated the plant extract by intragastric route once daily for 43 consecutive days. Control group (0 mg/kg) received vehicle. During the study, food intake was measured daily, while body weights were recording weekly. By the end of 43 days, animals were sacrified by decapitation. Blood was collected without anticoagulants for the determination of biochemical parameters as, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein using commercial kit obtained from Randox.

Statistical analysis

All values were presented as means ± S.E.M. One way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical evaluation. P values less than 0.05 ($p < 0.05$) were considered significant.

Results

Acute Toxicity

Aqueous extract

Oral administration of a single dose of aqueous extract of *Allanblackia monticola* did not produce significant changes in behavior skin effect. Aggressiveness was decreased slightly there by dose of 4g/kg of body weight. Mobility also decreases at the doses of 2 to 6g/Kg of body weight. At the dose of 8g/kg of the body weight

mice became constant; the breast rhythm increased at the dose of 6g/kg.

No mortality of animal was observed at the dose of 2g/ kg. The percentage of mortality increase from 30% to 100% respectively, at the doses of 4g/kg and 10g/kg of body weight. The means rate of mortality 48 hours after single dose taking of the aqueous extract of *A. monticola* was 46%. The lethal dose 50 of aqueous extract of *A. monticola* was 6.4g/kg of body weight.

Methylene chloride fraction

Administration of methylene chloride fraction at the dose of 2 – 16 g/kg decreased mice mobility. The animals lost aggressiveness at the dose of 4g/kg. Compare to control animals, Tact sensibility decreased prior 2g/kg of body weight.

There was not mortality in mice at the doses between 0 to 4g/kg of body weight. Mortality means observed was 5%. Lethal dose 50 of methylene chloride fraction was more than 16g/kg of body weight.

Subacute toxicity of methylene chloride fraction of *Allanblackia monticola*

Effect on the mice body weight in sub acute treatment

The male body weight increase significantly during treatment compared to control group. However, body weight of male treated with methylene chloride fraction at the dose of 1200g/kg decreased significantly (96.81%) when compared to control group (111.49%). at the second week. (figure 1)

Effect of methylene chloride fraction on relative organs weighs.

Repetitive administration of methylene chloride fraction does not affect significantly relative organs weight like: kidney, lungs, heart, spleen and liver when compared to control group. (Figure 2 A and B).

Effect of methylene chloride fraction on the biochemical parameters.

Effect on total proteins rate

Meyhlene chloride fraction of *A monticola* did not decreased significantly the rate of serum proteins at all dose in female mice. This diminution was 29.42% and 25.68% at the dose of 1200mg/kg of body weight; respectively in male and female. There was no significant difference of proteins rate between male and female. (figure 3)

Effect on creatinine rate

Plant extract does not increase significantly creatinine rate in male and female mice during 6 weeks repetitive administration of the extract. (figure 4)

Effect on transaminases rate

Effect on alanine aminotransferase (ALT) rate

Six weeks treatment did not modify significantly the level of ALT in male and female compared to control group. However, this extract increases slightly ALT level at the dose of 600 mg/kg (32.93%) in male: at the same dose this rate decreased in female (3.17%) when compared to control group. (figure 5)

Effect on aspartate aminotransferase (AST) rate

Repetitive administration of *A monticola* during 6 weeks increase in dose dependent way the rate of serum AST from 33.6±1.17U/ml in control group to 59.48+4.25U/ml in female treated group at the dose of 1200 mg/kg, In male mice, serum AST rate increased from 32.45 ± 1.30 U/ml in control group to 45.65 ± 3.08 U/ml in animals treated with dose of 600 mg/kg. (figure 6)

Effect of methylene chloride fraction on histology of detoxications organs

Histopathology observations of the liver

In control group hepatic parenchyma is constituted with hepatocyte enclosed with sinusoid capillary. We can also see kupfer cells. Sinusoids capillaries run between hepatic cell stratums. Hepatocytes are disposing in order around centrolobular vein.

In animals treated with extract at the dose of 300mg/kg during six weeks, leukocyte near portal vein was observed. There was no structural modification in hepatic cell compared to control. Periportal inflammations, hepatocytes degenerescence, cellular vacuolization at the dose of 1200mg/kg were observed. (figure 7)

Histopathological studies of the kidney

Normal structure of kidney where glomerule is surrounded with Bowman capsule which bound urinary chamber well distinctive was observed. We can also observe proximal and distal tubules well established. Mice treated with methylene chloride during 6 weeks do not showed structural modifications. Glomerule and tubules are well distinguished. The dose of 1200mg/kg also showed normal structure. (figure 8)

Discussion

Study of acute toxicity of *A monticola* showed no mice mortality at least up to a dose of 2g/kg (aqueous extract) and 4g/kg (methylene chloride fraction). Methylene chloride fraction or aqueous extract administrated in single dose, are provoked toxicity signs causing mobility and respiratory alterations at the dose of 2g/kg of body weight. *A monticola* at dose of 4g/kg provoked decreased of aggressiveness. *A monticola* extract can contain sedative substances - like which act on nervous centers or on motility.

In animals treated with aqueous extract or with methylene chloride fraction with doses more than 2g/kg, feces aspect passed from granular to mole aspect. This modification suggested perturbation of gastro- intestinal tract due to the intestinal transit acceleration which can cause weak reabsorption of water and fast elimination of extract in feces.

Evaluation of acute toxicity showed that mortality appeared at a dose of 8g/kg (methylene chloride fraction) and 4g/kg (aqueous extract). Thus, tolerate maximal dose was 4g/kg for methylene chloride fraction and 2g/kg for aqueous extract. The percentage of mortality increased with the rise of the dose of aqueous extract showing a lethal dose 50 (LD_{50}) at 6.4g/kg while 16g/kg of methylene chloride fraction caused only 10% of mortality. Since all products that LD_{50} is upper than 5g/kg is consider like non toxic [7, 8, 9], these result suggested that *A. monticola* stem bark extract is less toxic.

Ours results also show that methylene chloride fraction is less toxic than aqueous extract; because aqueous extract showed mortality (30%) at dose of 4g/kg versus 10% for methylene chloride fraction at the dose of 8g/kg. This observation suggested that many toxic compound of *A. monticola* can be polar substances; also *Allanblackia monticola* stem bark contained less polar compounds which can attenuate toxicity of polar substances.

Sub chronic toxicity study showed that, rate of total serum proteins did not change significant during the treatment. However the decrease of proteins level from 25.68% (females) and of 29.42% (males) was observed at the dose of 1200mg/kg. Serum proteins are mainly synthesized in the liver, their decrease can be the consequence of an insufficiency of the anabolism and the defect of synthesis in relation with an achievement of the hepatic cells [10]. Transaminases (ALT, AST) and creatinine are, respectively, the indicators of the hepatic and renal function. The serum rate of the ALT increases in case of destruction of hepatic cells. The rate of the AST can also increase in case of destruction of hepatic cells where it is mainly synthesized [11]. The creatinine is generally produced by the body during the muscular contraction and is completely excreted at the level of the kidney. The methylene chloride fraction *A. monticola* provoked non significant increase serum creatinine level in female mice. This could explain the absence of histological change observed at the renal level. There is no effect on the rate of ALT. The rate of AST increased significantly in male and female mice respectively in the doses of 600 mg/kg and 1200 mg/kg explained an achievement of the hepatic cells.

Also, histological structure of liver cutting of mice treated with methylene chloride fraction at the dose of 1200 mg/kg showed, periportal inflammation, degeneration of hepatocytes and a cellular vacuolization.

In subacute treatment, methylene chloride fraction of *Allanblackia monticola* caused during fourth week of treatment, significant decrease of the body weight in females animals. This reduction of the body weight would be due probably to an action of the plant on the assimilation of food. The change of the weight is used as indicator of the opposite effect of products [12, 13, 14].

Conclusion

The present study confirms in part, the safety of *Allanblackia monticola* since aqueous extract and methylene chloride fraction of the stem bark did not cause any lethality at the lower doses and slight changes in the general behavior in acute and chronic toxicity.

Acknowledgments

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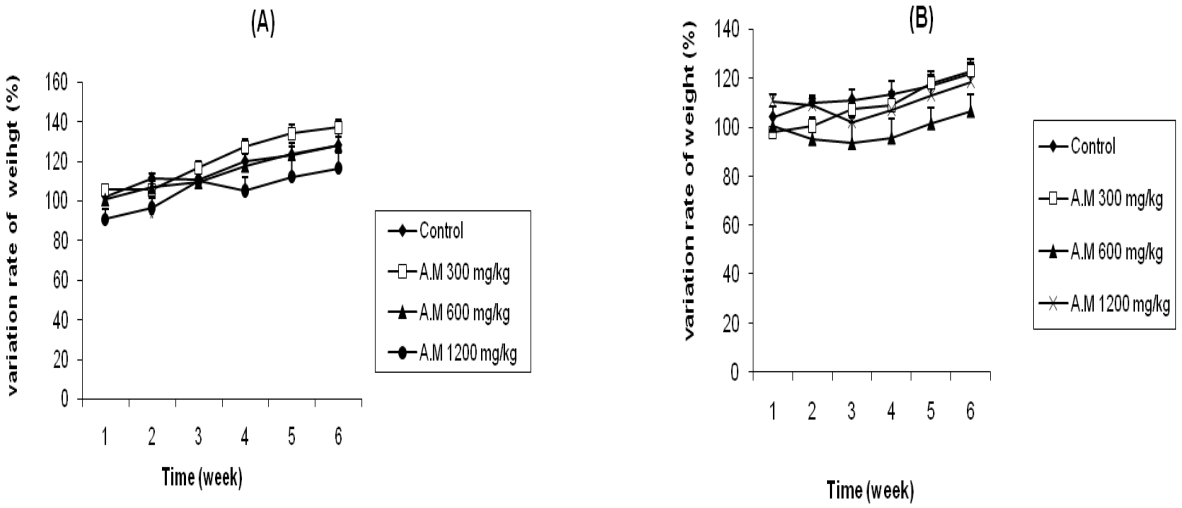


Figure 1: Variation of body weight of male (A) and female (B) mice during sub acute toxicity of methylene chloride fraction

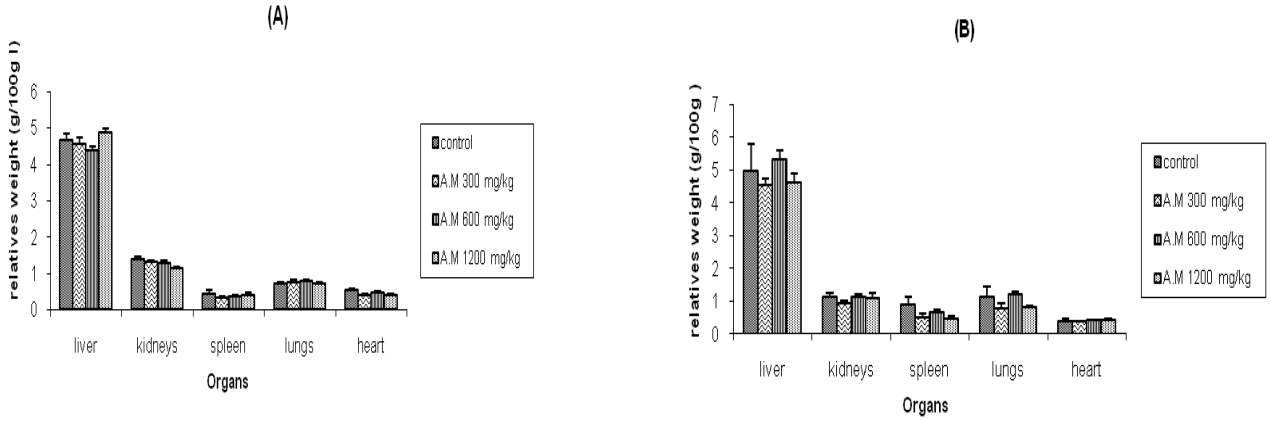


Figure 2: effect dose of methylene chloride fraction of *A. Monticola* on the relative organs weight in male (A) and female (B) mice during sub acute toxicity

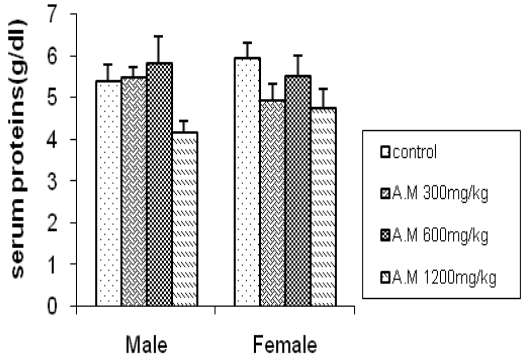


Figure 3: effect of methylene chloride fraction of *A. monticola* on total proteins in male and female mice.

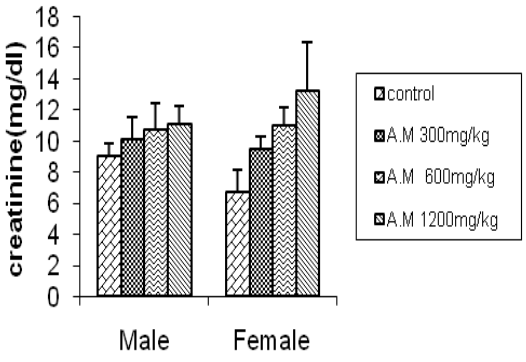
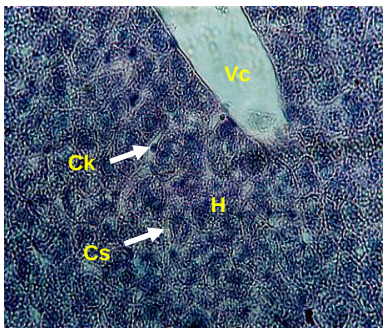
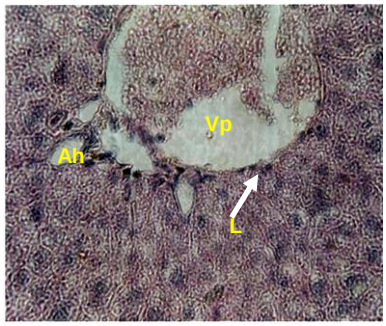


Figure 4: effect of methylene chloride fraction of *A. monticola* on creatinine rate during sub acute treatment in male and female



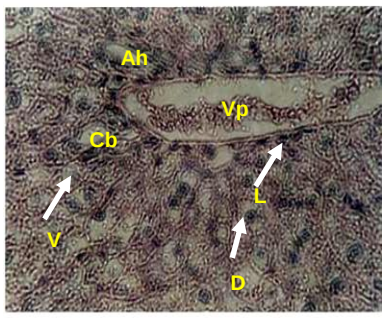
A: Cutting of mice liver control group (HE x 400).

Hepatic parenchyma is constitute with hepatic cells (H) surrounded with sinusoids capillary (CS) where you can see kupfer cells (Ck). Hepatocytes are disposing by row around centrolobular vein (Vc).



B : Cutting of mice liver treated with extract 300 mg/kg (HE x 400).

Presence of leukocytes (L) around portal vein (Vp) and hepatic artery (Ah).



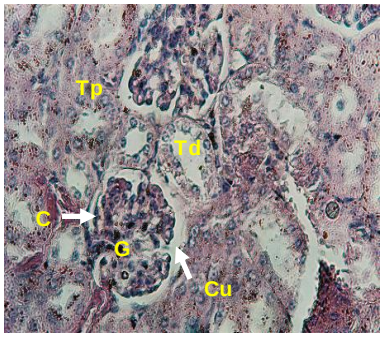
C : Cutting of mice liver treated with extract 1200 mg/kg (HE x 400).

Add to peri portal inflammation

(L) we observed hepatic cells degenerescence (D) and cell vacuolization (V).

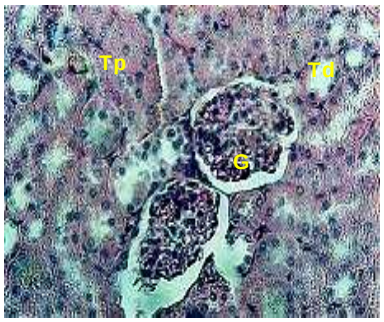
Cb : Canalicule biliaire.

Figure 7: photomicrographs of the liver control group (A) and those treated with methylene chloride fraction of *Allanblackia monticola* at the doses of 300 mg/kg (B) and 1200mg/kg (C) during 6 weeks.



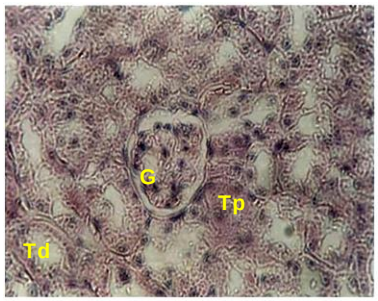
A : Cutting of mouse kidney control group (HE x 400).

The cutting presents normal architecture. glomerule (G) is surrounded with the capsule of bowman (C) which bounds urinary chamber very different (Cu). The by- passed tubes proximal (TP) and distal (Td) are established.



B : cutting of mouse kidney treated with extract, dose of 300 mg/kg (HE x 400).

The cutting presents no anomaly. Glomerule and tubules are very different and do not present visible structural modification.



C: Cutting of kidney of mouse treated with extract, dose of 1200 mg/kg (HE x 400).

At this dose, cutting is like that of mice control group.

Figure 8: photomicrographs of the mice kidney control group (A) and those treated with methylene chloride fraction of *Allanblackia monticola* at the doses of 300 mg/kg (B) and 1200mg/kg (C) during 6 weeks.