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Research Article

ANALGESIC PROPERTIES AND TOXICOLOGICAL PROFIL OF AQUEOUS EXTRACT OF THE STEM BARK OF *ANTHOCLEISTA VOGELII* PLANCH (LOGANIACEAE)

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ABSTRACT

The aim of this work was to evaluate the analgesics properties and toxicological profile of aqueous extract of the stem barks of *Anthocleista vogelii*. The analgesics tests were carried out on pain induced by acetic acid and formalin; while the toxicological study related to the acute and sub-chronic toxicity. The extract at 125, 250 and 500 mg/kg, reduced the number of abdominal constrictions induced by acetic acid with 40.42, 65.62 and 68.75 % respectively. In the formalin test, extract provokes 56.48, 59.49 and 89.93 % of inhibition respectively with the same doses; while the second phase is marked by an activity higher of the extract, with 94.16, 97.47 and 100 % of inhibition. In acute toxicity study, the extract was administered orally at doses 0, 4, 8, 16 and 20 g/kg. The animals were observed for any toxic symptoms up to 7 days. The extracts don't provoke a death until the dose 20 g/kg. The food consumption and the water increases significantly in all animals treated. In a sub-chronic toxicity study, the extract was tested at the dose of 0, 250, 500 and 1000 mg/kg *p.o.* once daily for 28 days. The body weight and water consumption decreases whereas food consumption increases. The relative organ weight not varies significantly. The serum and hepatic levels of ALT increases significantly, whereas the levels of ASAT, proteins and creatinin (serum and urinary) levels do not vary significantly in all the treated animals compared with the animals of the group controls.

Keywords: *Anthocleista vogelii*, acid acetic, formalin, acute and sub-chronic toxicity.

INTRODUCTION

The pain is an abnormal and painful impression received by a part alive and perceived by the brain. It materializes by the anxiety, the cries, diminish them, the tears, mental anguish and a risk of slip towards the depression at the time of its passage towards chronicity[1]. These demonstrations can be both intense and fast (acute pain), or slow and progressive (chronic pain)[2,1]. The assumption of responsibility implies mechanical, surgical and medicamentous treatments (opioïdes, antipyretic and anti-inflammatory drugs not stéroïdiens).

Anthocleista vogelii Planch. is a tree of the Loganiaceae family which commonly grows around river edges and banks or in marshy areas of the tropical humid forest of West Africa [3] with great concentration in Cameroon and Gabon[4]. This plant is 6 to 20 meters high, usually with buttrous roots, with branches having spikes, which also have sessile leaves and short petals. The stem bark of this plant is used in African traditional medicine for the treatment of gastro-intestinal disorders, to cure fever, stomach ache and as purgative while the combination of the stem bark and the leaves is used as anti-inflammatory and anti-diabetic agents and also in the treatment of wounds[4].

In Cameroon, the stem bark is reported to be used to treat abdominal pains[5]. Recent findings in our laboratory indicated the antiulcer properties of the xanthone 1-hydroxy-3,7,8-trimethoxyxanthone isolated from the methanol stem bark extract of *Anthocleista vogelii*[6]. We are reporting in the present study, the analgesic properties and toxicological profile of aqueous extract of the stem bark of *Anthocleista vogelii* Planch (Loganiaceae).

MATERIALS AND METHOD

Animals

Adult mice, *Mus musculus* weighing on average 26 ± 3.15 g and adult Wistar rats weighing on average 170 ± 4.59 g, of both sexes were used for these studies. These animals were raised in the animal house of the Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang, Cameroon, under standard natural conditions and had free access to water and food. All experimental procedures used in the present study followed the "Principles of Laboratory Animal Care" from NIH publication Nos. 85-23 and were approved by the ethic committee of the Cameroon Ministry of Scientific Research and Technology which has adopted the guidelines established by the European Union on Animal Care and Experimentation (CEE Council 86/609).

Plant material and extraction

The extracts of plant were prepared starting from the stem barks of *Anthocleista vogelii collected* in Mars 2009 in Bandjoun (west region). Mr. Paul Mesili, now a retired Botanist of the Cameroon herbarium, Yaoundé**,** carried out the authentication of the plant material. A voucher specimen coded BUD 0636 was deposited at the Botany Department, University of Dschang for future reference. The collected fresh stem bark was air dried and ground into fine powder in a high speed grinding mill.

One kilogram of fine powder were macerated in 3000 ml of distilled water during 72 hours and filtrated. The filtrate was evaporated to dryness in an air oven at 40 °C to give 98.6 g of the aqueous extract corresponding to an extraction yield of 9.86 %. This extract was dissolved in distilled water upon administration.

Pharmacological activities

Nociceptive activity

Acetic acid-induced abdominal writhing test

This was performed according to Ngueleface *et al*.[7] Mice (six per group) were injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg. The extract (125, 250 and 500 mg/kg, p.o.), efferalgan/codeine (50 mg/kg, p.o.) and distilled water (p.o.) were administered 1 hour prior to treatment with acetic acid. The writhings induced by the acid, consisting of abdominal constrictions and hind limbs stretchings, were counted for 30 min after acetic acid injection. The percentage analgesic activity was calculated as follows:

$$Percentage\ analgesic\ activity = \frac{N - NI}{N} \quad X \quad 100$$

Where *N* is the average number of stretchings of control per group. *N*I is the average number of stretchings of test per group.

Formalin-induced pain.

The procedure described by Gaertner *et al*.[8] was used. Pain was induced by injecting 20 µl of 2.5% formalin (40% formaldehyde) in distilled water in the subplantar of the right hindpaw. Rats (six per group) were given extract (125, 250 and 500 mg/kg, p.o.), efferalgan/codeine (50 mg/kg, p.o.), and distilled water (p.o.) 1 hour prior to injecting formalin. These rats were individually placed in a transparent Plexiglass cage (25 cm × 15 cm × 15 cm) observation chamber. The amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0 to 5 min (first phase) and 15–30 min

(second phase) were counted after injection of formalin. These phases represented neurogenic and inflammatory pain responses, respectively[9]. The percentage of analgesic activity (%I) at each phase was calculated using the following formula:

$$\%I = \frac{C - T}{C} X\ 100$$

Toxicity study

Acute toxicity

In order to study any possible toxic effect or changes in normal behaviour, groups of 10 mice (5 males and 5 females) were used in this experiment. The acute toxicity of the plant was studied by preparing four different concentrations of the aqueous extract (4, 8, 16 and 20 g/kg *b.w.*) and administered orally. The other group were taken as a control and given vehicle (distilled water). Animals were kept without food for 12 h prior to dosing and were monitored continuously for 3 h after dosing for any sign of toxicity. The symptoms, mobility, aggressiveness, sensitivity to the pain, sensitivity to the noise, the broadcast of stools and the mortality were checked. Animals were kept under observation for 7 days and were monitored daily for changes in body weight, food and water consumption and for any sign of toxicity.

The LD_{50} values were determined according to the formula of Behrens *et al.*[10]:

$$LD_{50} = LD_{100} - \frac{\sum (Z \times d)}{n} \quad \text{with,}$$

Z: the half sum of animals having succumbed in 2 groups corresponding to doses that follow themselves.
n: the number of animals by group
d: differences between 2 doses that follow themselves.

Sub-chronic toxicity

The rats were divided into groups of 10 animals each. They were kept under the same conditions as described above. The first groups was given vehicle of the extract (distilled water) and taken as control. The remaining three groups were given orally 250, 500 and 1000 mg/kg b.w. of aqueous extracts of *Anthocleista vogelii* daily for 4 weeks.

Weekly body weight

The body weight of each rat was assessed during the acclimatization period, once before the beginning of dosing, once every 7 days during the dosing period and once on the day of sacrifice.

The relative body weight of each animal was then calculated as follows:

$$\text{Relative body weight} = \frac{\text{absolute body weight of one time interval (g)}}{\text{body weight of rat on commencement of dosing day (g)}} X\ 100$$

water supplied and the amount remaining after 24 h.

Mortality and clinical signs

During the 4 weeks dosing period, all animals were observed daily for clinical signs and mortality patterns once before dosing, during dosing and up to 3 h after dosing.

Preparation of serum samples

24 hours after the administration of the 28th day, the urines of animals have been appropriated and have been preserved to -20 °C then animals have been anesthetized by injection of thiopental. Blood was collected by cardiac puncture and part of blood was put in tubes containing of heparin for blood numeration and the other part was put in tubes not containing an anticoagulant. The clotted blood samples were centrifuged at 4900 rpm for 15 min and serum samples were aspirated off and frozen.

Relative organ weight and preparation of homogenate samples

After taking the blood, the abdominal cavity of each animal was opened and organs namely the heart, lung, spleen, liver and kidneys were quickly removed, cleaned with ice-cold saline, weighed and stored at –20 °C.

A part of the liver tissue was thawed and homogenized 20 times (w/v) by homogenizer in plug phosphates (KH_2PO_4, $NaHPO_4$, pH 7.4). The homogenates were centrifuged at 6000 rpm for 30 min to obtain the supernatant.

The relative organ weight of each animal was then calculated as follows:

$$\text{Relative organ weight} = \frac{\text{absolute organ weight (g)}}{\text{body weight of rat on day of sacrifice (g)}} \text{ X } 100$$

Serum and homogenate biochemistry

Serum sample were analyzed for the determination of total protein and creatinin respectively according to the methods described by Gornall *et al*.[11] and Slot[12]. Enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed according to Reitman and Frankel[13].

The homogenates of the liver tissues were analyzed for the determination of the total protein hepatic concentration, the enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), while using the same methods that previously.

The urine was analyzed for the determination of creatinin concentration using the method cited above. The renal clearance of creatinin was given for each dose of extract by using the following formula:

$$\text{Renal clearance} = \frac{V \times U}{P} \quad \text{with,}$$

V= Urinary volume by minute
U= Concentration of the substance in the urine
P= Concentration of the substance in plasma

Statistical analysis

The measured parameters were expressed as means ± standard error of mean. Statistical analysis was performed using ANOVA and Tukey as post hoc test.

RESULTS

Nociceptive activity

Acetic acid-induced abdominal writhing test

The intraperitoneale injection of acetic acid in the mice induced a pain which materializes by abdominal contractions. The administration of aqueous extract of the stem barks of *Anthocleista vogelii* at the doses of 125, 250 and 500 mg/kg reduced significantly ($p< 0.001$) the number of abdominal contractions compared with the control group (figure 1). The extract at the doses of 125, 250 and 500 mg/kg reduced the number of abdominal contractions with 40.42, 65.62 and 68.75 % of inhibition respectively. Paracetamol-codeine used as reference product at the dose of 50 mg/ml significantly reduces ($p<0.001$) the number of contractions with 84.27%.

Formalin test

Figure 2 shows the effects of aqueous extract of *Anthocleista vogelii* on the pain induced by the formalin. It comes out from this figure that the animals of the control group licked the leg during 144.00 seconds during the first five minutes which followed the injection of formalin and during 105.67 seconds of the 15th to the 30th minute. The administration of extract at the doses of 125, 250 and 500 mg/kg inhibited significantly ($p<0.001$) and dose dependent manner the two phases on the pain induced by the formalin. At the first phase, the extract (125, 250 and 500 mg/kg) inhibit the pain with 56.48, 59.49 and 89.93 % and paracetamol-codeine (50 mg/kg) significantly reduce ($p<0.01$) this pain with 78.94 %. At the second phase, the activity of the extract increases considerably, it reaches 94.16, 97.47 and 100 % respectively at the doses of 125, 250 and 500 mg/kg. Whereas the activity of the paracetamol-codeine is only 72.32 %.

Toxicity study

Acute toxicity

No case of death was recorded until the dose of 20 g/kg of body weight at the end 7 days of observation after administration single of the extract. With the exception of the saddles which remained granulous independently of the dose used, all the behavioural parameters (mobility, sensitivity to the noise and the touch and aggressiveness) know a dose-dependent reduction. All these behavioural parameters are cancelled starting from the dose of 8 g/kg except for the sensitivity to the pain which is cancelled with the dose of 12 g/kg. Administration of the single dose of the extract does not significantly influence the relative weight of the animals compared to the animals of the group controls (figure 3). The food consumption increases significantly in all the animals treated compared to the animals of the control group (figure 4), whereas the water consumption significantly remains low in the animals treated with the various doses of extract (figure 5).

Sub-chronic toxicity

The daily administration of the extract during 28 days provoke a decrease of the body weight of the treated animals, this reduction is significant in the animals treated with the dose of 500 mg/kg (figure 6). Food consumption undergoes a significant increase with the dose of 1000 mg/kg at the 3rd week of treatment (figure 7); while the water consumption decreases significantly to the 4th week in the animals treated with the strong doses of extract (figure 8).

The relative weight of some organs of the animals treated after 28 days is represented by figure 9. This reveals that the aqueous extract of the stem barks of *A. Vogelii* does not cause significant variation of the relative organ weight of the liver, kidneys, heart, lungs and spleen. The levels of serum and hepatic ALT increase in dose dependent manner in all the animals treated with the extract. This increase is significant with the dose of 1000 mg/kg. In addition, the levels of serum and hepatic AST do not shown significant variation in all the treated animals (Figure 10). On the other hand levels of serum and hepatic proteins not varying significantly in all the treated animals (figure 11); just like creatinin level (serum and urinary) and even the clearance of creatinin (figure 12).

DISCUSSION

The aim of this study was to evaluate the analgesics and antioxydant properties of aqueous extract of the stem barks of *A. vogelii*. The results obtained showed that the administration by oral way of the extract significantly inhibits the pains induced by the acetic acid and the formalin on the one hand; on the other hand this extract did not show significant effect on the parameters of the oxidative stress.

The results obtained show that the extract decreases the noxious answers of the chemical stimuli in the abdominal contractions induced by injection of the acetic acid. The intraperitoneale injection of acid acetic produces pains by activation of the chemo-sensitive nociceptors[14] or by irritation of visceral surface, which leads to the release of histamine, serotonin, bradykinines and the prostaglandins[15,16].

Thus, the antinociceptive activity of the agonists opioids, the partial agonists opioids, the no steroids anti-inflammatory drugs can be determined by the test with the acetic acid. The inhibition of the pain induced by the acetic acid can thus be due to the action of the extract on the chemo-sensitive nociceptors, on the nociceptors located on visceral surfaces, the release of the algogenes substances or the transmission of the nervous message[14]. On this type of pain, the peripheral and central analgesics have inhibiting effects. Extracted having had an inhibiting effect on this type of pain, this suggests that extract would act like peripheral and/or central analgesics. In order to elucidate the mechanism of action of the extract, this was tested on the two phases of the pain induced by the formalin. The under-plantar injection of formalin causes a pain which appears in two phases: the first phase or neurogenic phase during which there are activation of the fibers C and release of the substance P, the second phase or inflammatory phase during which there is release of serotonin, histamine, the bradykinin and of the prostaglandins[17,18,8]. According to stai *et al*.[14], the peripheral analgesics such as the aspirin and paracetamol inhibit only the second phase while the central analgesics (narcotic) inhibit the two phases. The aqueous extract of the stem barks of *Anthocleista vogelii* have an outstanding activity during the second phase. What lets think that the extract would carry on their activity at the peripheral level. However, the extract also presented an activity at the first phase, which means that their action at the central level is not the least. Their dominating activity during the second phase suggests that they have an anti-inflammatory drug activity.

As for the toxicological study, it revealed a relatively low toxicity of the stem barks of *Anthocleista vogelii*. In acute toxicity, the extract did not cause any death at the end of 7 days of experimentation. According to Delongeas *et al*.[19] and Schorderet[20], all product whose DL_{50} are higher than 5 g/kg is regarded as nonpoison. The aqueous extract of stem barks *of A. vogelii* could be regarded as nonpoisons, but within sight of the behavioural modifications that they involve such as a fall of mobility, sensitivity to the noise, sensitivity to the pain and the aggressiveness but which return to the normal at the end of 24 hours, they can rather be regarded as being relatively toxic. It is known that the drugs which prevent the perception of the pain (analgesics) inhibit the conversion of the arachidonic acid by inhibition of the synthesis of prostaglandins[21]. Our results let think that the extract would act by direct action at the medullary level into depressing the transmission of the noxious messages and indirectly on the level of the cerebral trunk by stimulation of the control inhibiting going down[22,23]. The body evolution tightens with the general fall except with the dose of 16 g/kg which it, presents a positive evolution as of the 4th day of treatment, while food consumption increase significantly throughout the experimentation, which means that the extract would stimulate the appetite while probably acting on the receivers of the hunger located in the stomach.

In sub chronic treatment, the body weight of the animals treated with the aqueous extract of the stem barks of *A. vogelii* during four weeks remained weak compared to the controls group, whereas food consumption increase throughout the experiment. This confirms the results obtained in acute treatment and can be justified by the fact that the extract would cause bad food assimilation. The liver and kidney constitute the principal organs of detoxification and so represent the first targets of all substances in the body. Thus, these two organs are very often affected in the event of toxicity. In this study, we evaluated impacts of extract by determine the levels of ALT, AST, proteins and creatinin. The relative weight of the liver does not vary significantly of all the treated animals. But on the other hand the weight of the liver undergoes a light fall in the animals treated compared to the animals of the control group. This fall would be due to the destruction of several hepatic cells. Because the results show that the level of serum ALT increases significantly in the animals treated with the strong dose of extract. The evaluation of the serum level of enzymes ALT and AST is correlated with the morphological damage on the level of the

liver[24]. Indeed, ALT and AST are the markers of the syndrome of cytolysis or destruction of the hepatocyte cells[25], they are good function words of the liver[26,27]. They are levels increase in blood at the time of the destruction of the hepatic cells, and this in all hepatic pathologies (infectious or toxic). This attack of the liver would be due to the oxidative activity of several compounds hepatotoxic contained in the extract, since the oxidative activity of many substances is the principal cause of the intoxication of the liver[28]. Although the extract provokes a bad food assimilation and destruction of certain hepatic cells, it does not affect in a considerable way the hepatic function (proteinic synthesis and detoxification). Because the results show an increase in the protein level (serum and hepatic) and hepatic level of enzymes ALAT and ASAT.

Creatinin is formed in the organization by nonenzymatic dehydration of the creatin synthesized by the liver and stored on the level of the muscles. Its elimination is entirely renal and its concentration in blood varies physiologically with the muscular mass[29]. The extract did not modify the weight of the kidneys, or significantly the serum and urinary levels of creatinin. Nevertheless, we notes a rise of the serum level of the creatinin and a fall of his urinary level, which suggests that the extract would lower the renal function (function of filtration) by decreasing the elimination of creatinin at the renal level. The renal clearance corresponds to the volume of plasma completely purified by the kidney, for this substance, by unit of time. The renal elimination of a substance can correspond to three phenomena which are at the origin of the renal clearance to knowing: glomerular filtration, tubular secretion and the tubular reabsorption. All the factors likely to modify renal physiology, the urinary pH and fixing with plasmatic proteins lead to a modification of the renal clearance. It is the case of the age (immature renal function at the new-born baby, faded at the old person), of the renal insufficiency, the cardiac insufficiency and the hepatic attacks. It is to be noted that in spite of an increase in the clearance according to the dose, this one remains low compared with the controls group, which means that the aqueous extract of stem barks of *A.vogelii* would reduce glomerular filtration.

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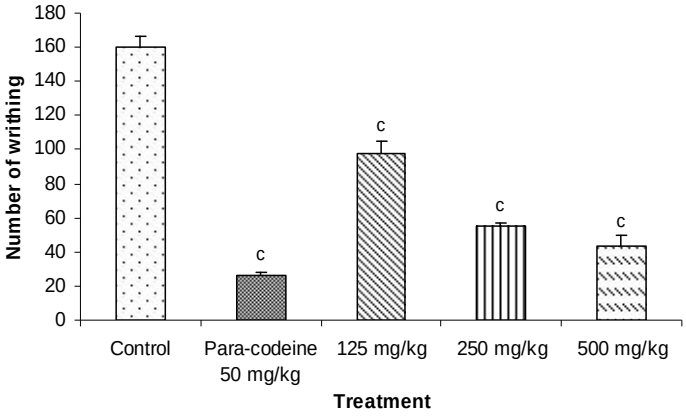

Fig. 1: Effects of aqueous extract of the stem bark of *A. vogelii* on acetic acid-induced pain in mice. Each bar represents the mean ± SEM of 6 animals; $^{c}p<.001$ statistically significant compared to control

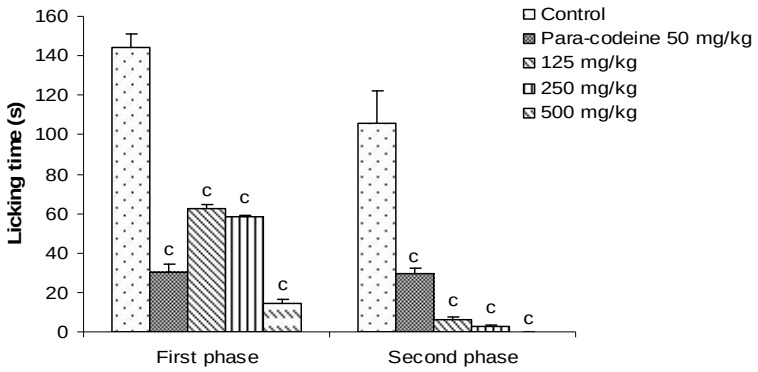

Fig. 2: Effects of aqueous extracts of the stem bark of *A. vogelii* on formalin-induced pain in mice. Each bar represents the mean ± SEM of 6 animals; $^{c}p<.001$ statistically significant compared to control

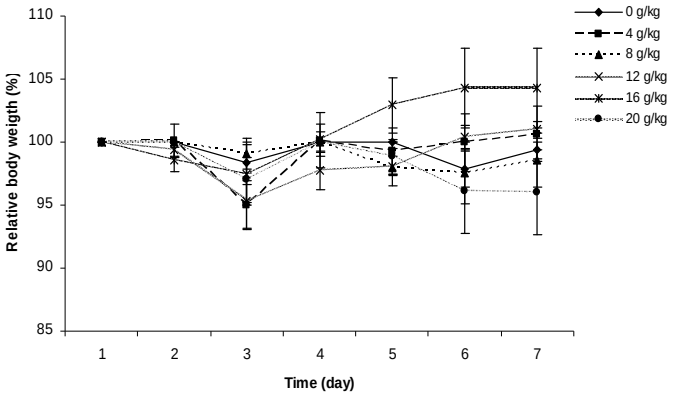

Fig. 3: Body weight trend for mice dosed once with aqueous extracts of the stem bark of *A. vogelii*. Each data point represents the mean ± S.E.M. (n = 10)

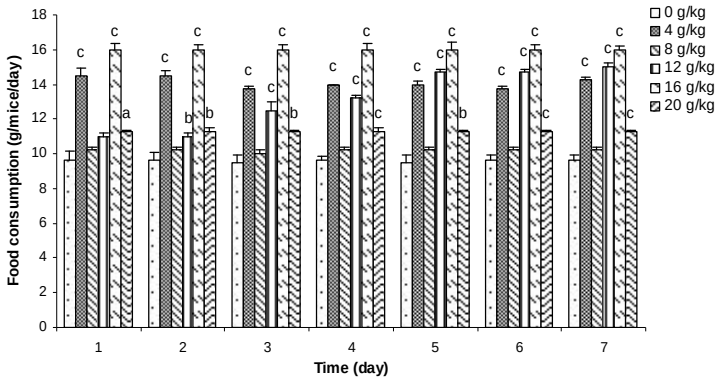

Fig. 4: Food consumption trend of mice dosed once with aqueous extract of the stem bark of *A. vogelii*. Each data column represents the mean ± S.E.M. (n = 10). Values for consumption are based on total intake and average body weight of the preceding time interval. $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$ compared with the control group (0 g/kg)

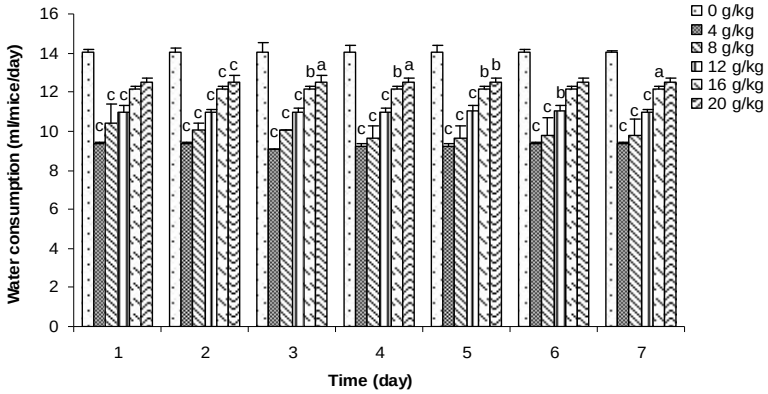

Fig. 5: Water consumption trend of mice dosed once with aqueous extract of the stem bark of *A. vogelii*. Each data column represents the mean ± S.E.M. (n = 10). Values for consumption are based on total intake and average body weight of the preceding time interval. $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0,001$ compared with the control group (0 g/kg)

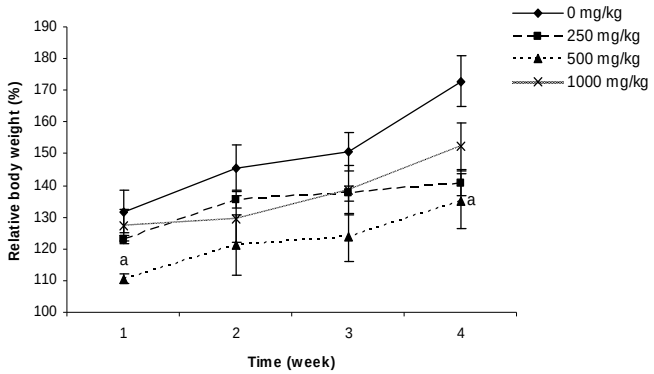

Fig. 6: Relative body weight pattern in Wistar rats fed with aqueous extract of the stem bark of *A. vogelii*. Each data point represents the mean ± S.E.M. (n = 10). $^{a}p < 0.05$ compared with the control group (0 g/kg)

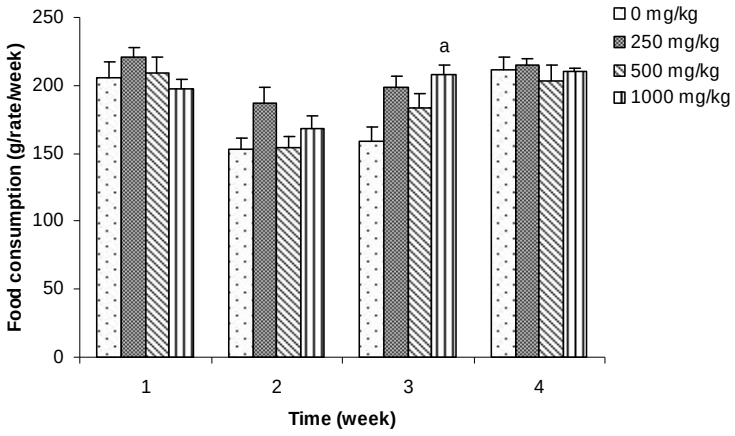

Fig. 7: Food consumption trends of rats fed with aqueous extract of the stem bark of *A. vogelii*. Each data point represents the mean ± S.E.M. (n = 10), [a]p < 0.05 compared with the control group (0 g/kg)

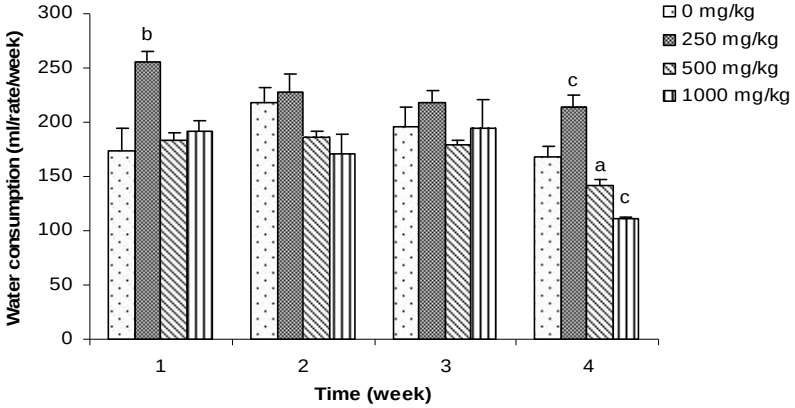

Fig. 8: Water consumption trends of mice fed with aqueous extract of the stem bark of *A. vogelii*. Each data point represents the mean ± S.E.M. (n = 10), [a]p < 0.05; [b]p < 0.01; [c]p < 0.001 compared with the control group (0 g/kg)

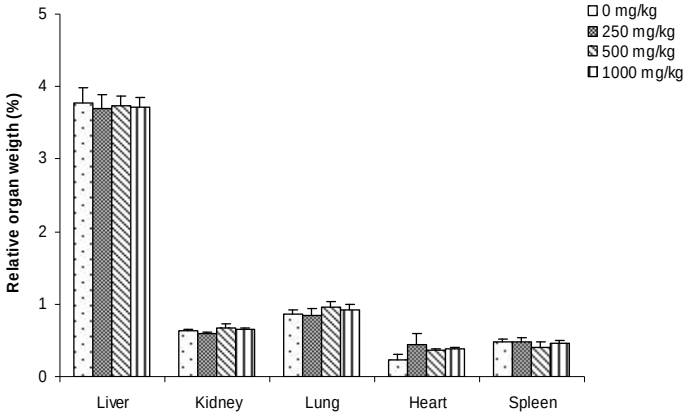

Fig. 9: Effect of aqueous extract of the stem bark of *A. vogelii* on the relative organ weights Wistar rats after 4 weeks oral dosing. Each data column represents the mean ± S.E.M. (n = 10)

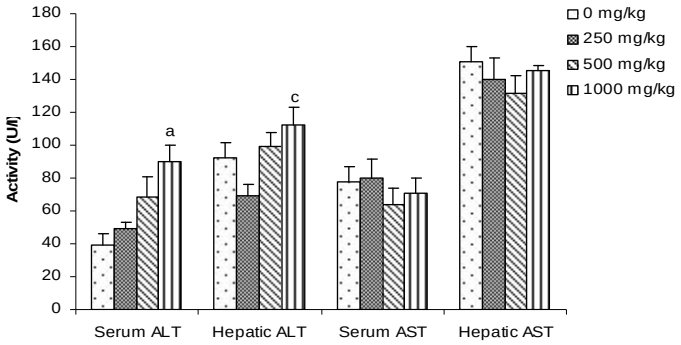

Fig. 10: Variation of the serum and hepatic enzymes ALT (alanine aminotransferase) and AST (aspartat aminotransferase) in the rat treated with the aqueous extract of the stem bark of *A. vogelii* in sub-chronic toxicity. Each point represents the mean of 10 values ±ESM. $^{a}p < 0,05$; $^{b}p < 0,01$; $^{c}p < 0,001$; significant difference compared to the control group (0 g/kg).

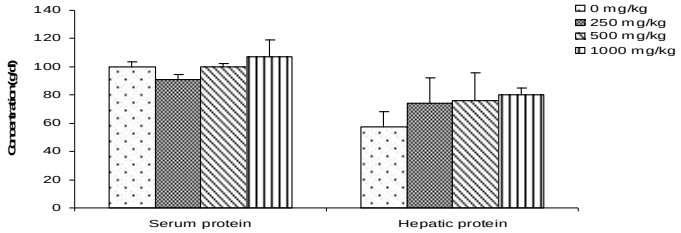

Fig. 11: Variation of the serum and hepatic proteins in the rat treated with the aqueous extract of the stem bark of *A. vogelii* in sub-chronic toxicity. Each point represents the mean of 10 values ±ESM

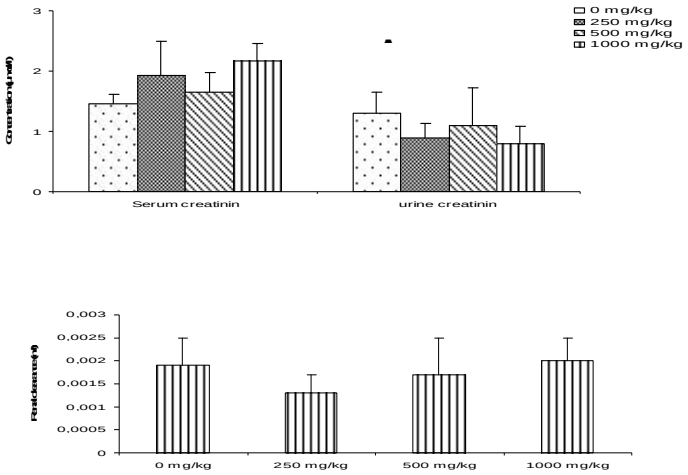

Fig. 12: Variation of the creatinin (A) and the renal clearance (B) in the rat treated with the aqueous extract of the stem bark of *A. vogelii* in sub-chronic toxicity. Each point represents the mean of 10 values ±ESM