INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

PRELIMINARY ASSESSMENT OF TOXICITY OF *CROTON MACROSTACHYUS* STEM BARK (EUPHORBIACEAE) EXTRACTS

Mbiantcha M[1*], Ngueleface TB[1], Ndontsa BL[2], Tane P[2] and Kamanyi A[1].

[1]Laboratory of Animal Physiology and Phytopharmacology, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.

[2]Laboratory of Natural Products, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.

ABSTRACT

The present investigation was carried out to evaluate the safety of aqueous and methylene chloride/methanol extracts of Croton *macrostachyus* stem bark by determining its potential toxicity. For the cytotoxicity study, the aqueous and organic extracts were administered to larvae of *Artemia Sp*., the number of death is determined after 6 hours and 24 hours; while for the acute study, the extracts were administered to mice. In the sub-chronic study, the extracts were administered orally for 28 days. In the cytotoxicity study, aqueous and organic extracts showed LC_{50} (lethal concentration 50) values of 569 and 425 µg/ml, respectively. In acute toxicity study, aqueous extract did not provoke death until the dose 16 g/kg; whereas the organic extract caused general behaviours, adverse effects and mortality. Mortality increased with increasing doses, with LD_{50} (lethal dose 50) of 10.2 and 9.4 g/kg *b.w*. respectively for male and female mice. In the sub-chronic study in rats, we observes a significant variation of ALT (alanine aminotransferase) and proteins serum levels; AST (aspartate aminotransferase) hepatic level. In view of the dose of *C. macrostachyus* consumed in traditional medicine, there is a wide argin of safety for the therapeutic use of the extract of *Croton macrostachyus* stem bark.

Keywords: *Croton macrostachyus*; Cytotoxicity; Acute and chronic toxicity.

INTRODUCTION

The use of plants for therapeutic purposes is getting increasingly popular as they are believed to be beneficial and free of side effect[1]. However, the rationale for the use of medicinal plants has twelled largely on long-term clinical experience with little or no scientific data on their efficacy and safety[2]. Medicinal plants have their use as drugs based simply on a traditional folk use that has been perpetuated along several generations[3]. With the upsurge in the use of plants medicines, a through scientific investigation of these plants is imperative based on the need to validate their folkloric use[4].

Croton macrostachyus (Hochst) pipe cleaner ex and Galinier (Euphorbiaceae) is a large what with cylindrical trunk. The stem is more or less pyramidal in sharp with widespread branches. The stem is gray clear, smooth and fissure with age. Leaves are almost as heart-shaped large that long, they have 10 to 15 cm of length, they are flexible, green or brunette according to the season and present some prominent ribs. Flowers are regrouped in inflorescence on stems of about 25 cm of long. They are visible but their life span is very short. They are colour creamy and slightly fragrant yellow. Fruits are regrouped along an axis. This plant is wide spread in Africa and in America; it is present in the hottest parts of the tropical forests of mountain. Outside of forests, species are distributed extensively in the more humid sectors[5, 6].

Croton macrostachyus is very common plant in Cameroonian traditional medicine. Its roots and fruits are used for constipation, diabetes and as purgative. Leaves are used to treat cough. Roots are used for malaria, venereal illnesses and like antidiabetic[7]. Our recent work showed that the stem barks of this plant have analgesic and anti-inflammatory activities[8].

Taking into account all these traditional uses of this plant, we become interested to the toxicity profile of this plant which had not been previously evaluated in order to bridge the gap in knowledge about it toxicological effects and to propose this extract *Croton macrostachyus* as a new drug for therapy of inflammation and pain. Hence, the cytotoxicity, acute and sub-chronic toxicity profile of the extract was studied on *Artemia Sp.*, mice and Wistar rats oral dosing for 4 weeks, since sub-chronic toxicity data are required to predict the safety associated to the use of medical product[9].

The use of *Artemia*, which permits to determines the LC_{50} value of the active compounds and extracts in saline medium in µg/mL[10], has been used in research on medicinal plants carried out in different countries in order to evaluate toxicity, gastro-protective action, and other biological actions, which in some cases have been related to pharmacological studies carried out for different chemical compounds[11, 12], as a screening method mainly for products of plant origin. In this study the effectiveness of the bioassay of *Artemia Sp.* for predicting the toxicity of plant extracts was evaluated by comparison with LD_{50} value-results obtained from oral acute toxicity tests in mice.

MATERIAL AND METHODS

Plant Material and extraction

The stem barks of *Croton macrostachyus* (Euphorbiaceae) were collected in the town of Bangangte (west region, Cameroon) in October 2006. The plant was identified by M. Ghogue Jean Paul of the National Herbarium, Yaounde-Cameroon where the specimen is preserved.

Preparation of extract

Aqueous extract

One thousand grams of the powder were boiled in five litters of distilled water for 20 minutes. The decoction was allowed to cool for 30 minutes at room temperature and then filtered. The filtrate was evaporated to dryness in an air oven at 40 °C, to give 25 g of the aqueous extract corresponding to an extraction yield of 2.5 %. This extract was dissolved in distilled water upon administration.

Methylene chloride/methanol extract

Six kilograms of the powder were macerated in 10 L of a mixture of CH_2Cl_2/CH_3OH (1:1 v/v) for 2 days at room temperature, filtered and concentrated at 45°C and 65 °C successively, using a rotative evaporator. This process produced 160 g of organic extract, which correspond to 2.67 % yield. This extract was dissolved in a solution made up of 2.5 % of dimethylsulfoxide (DMSO) and 2.5 % of tween 80 prior to oral administration.

Animals

Adult mice, *Mus musculus* weighing 20-30 g and adult *Wistar* rats weighing 150-200g, of both sexes and of 3 months old 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).

Cytotoxicity

Eggs of *Artemia sp.* are hatched in the sea water filtered and sterilized. Larvae are paid out and distributed test tubes. 10 larvae are placed whole in a test tube of 10 ml containing 5 ml of water of sea filtered and sterilized.

The extract is added in tubes in order to make bathe larvae in solutions of concentration 30 - 100 - 300 or 1000 µg/ml. The water of sea filtered and sterilized or solution of 2.5 % DMSO and 2.5 % tween 80 is introduced in control tubes. The surviving larvae will be counted 6 h and 24 h after administration of the extract. Each dose will be repeated 5 times[13, 14].

$$LC_{50} = LC_{100} - \frac{\Sigma(Z \times d)}{n} \quad with,$$

Z: the half sum o f animals having succumbed in 2 groups corresponding to successive doses.
n: the number o f animals by group
d: differences between 2 successive doses.

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 (2, 4, 8 and 16 g/kg *b.w.*) and six different concentrations of the organic extract (2, 4, 6, 8, 10 and 12 g/kg *b.w.*), and administered orally. The other groups were taken as a control and given vehicle (distilled water or solution of 2.5 % DMSO and 2.5 % tween 80). Animals were starved for 12 h prior to administration and were monitored continuously for 3 h after administration 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[15]:

$$LD_{50} = LD_{100} - \frac{\Sigma(Z \, X \, d)}{n} \quad where,$$

Z: the half sum o f animals having succumbed in 2 groups corresponding to successive doses.
n: the number o f animals by group
d: differences between 2 successive doses.

The graphic determination of the $LD_{50}$is carried out starting from the curve of variation of the death according to the dose, traced on semi-logarithmic paper.

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 and second groups were given vehicle of the extract (distilled water or solution of 2.5 % DMSO and 2.5 % tween 80) and taken as control. The remaining eight groups were given orally 0.3, 0.6 and 1.2 g/kg *b.w.* of aqueous and organic extracts of *C. macrostachyus* daily for 4 weeks.

Weekly body weight

The body weight of each rat was assessed during the acclimatization period, once before the beginning of administration, once every 7 days during the administration period and once on the day of sacrifice. The relative body weight of each animal was then calculated as follows:

$$Relative\ body\ weight = \frac{Absolute\ body\ weight\ of\ one\ time\ interval\ (g)}{Body\ weight\ of\ rat\ at\ the\ beginning\ of\ administration} \times 100$$

Feed and water consumption

The amounts of feed and water consumed were measured daily from the quantity of feed and water supplied and the amount remaining after 24 h.

Mortality and clinical signs

During the 4 weeks administration 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 urine of animals was taken and preserved at - 20 °C then animals was anesthetized by injection of thiopental. Blood has been collected by cardiac puncture, in plastic test tubes and allowed to stand for complete clotting. 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:

$$\textit{Relative organ weight} = \frac{\textit{Absolute organ weight (g)}}{\textit{Body weight of rat on day of sacrifice (g)}} \textit{X 100}$$

Serum and homogenate biochemistry

Serum sample were analyzed for the determination of total protein and creatinin respectively according to the methods described by Gornall[16] and Slot[17]. Enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed according to Reitman and Frankel[18].

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:

$$\textit{Renal clearance} = \frac{V\ X\ U}{P} \quad \textit{where}$$

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

Statistical analysis

The values were expressed as mean ± standard error of the mean (S.E.M). The statistical analysis of data was carried out by analysis of variance (ANOVA) using 5 % level of significance. The statistical package used was GraphPad InStat. A one-way ANOVA enable us to see the significant differences between the values. The Tukey test was used to identify these differences.

RESULTS

Cytotoxicity

The study of the toxicity of the aqueous and organic extracts of *C. macrostachyus* on larvae of *Artemia sp.* reveal that, after 24 hours of incubation, the aqueous extract at the concentrations of 300 and 1000 µg/ml provokes 24 and 100 % of mortality respectively; while the organic extract, at the same concentrations provokes 50 and

100% mortality. The LC_{50} determined by calculation are 0,569 and 0,425 mg/ml for the aqueous and organic extract, respectively (table 1).

Acute toxicity

Behavioural observation

The animals did not showed behavioural changes after the administration of the aqueous extract up to 8 g/kg. At the dose of 16 g/kg we observed a modification in the mobility, aggressiveness, sensitivity to noise and touch. All these parameters come back to normal at the end of 24 h.

The administration of the organic extract provokes a modification of the behaviours from the dose 6 g/kg. At the dose of 16 g/kg all animals are completely immobile, insensible to pain and noise. Faeces become liquid from the dose 4 g/kg.

Mortality patterns

The oral administration of a unique dose of the aqueous extract of 2, 4, 8 and 16 g/kg didn't provoke any death during the 7 days of experimentation.

All male mice treated with dose 2, 4, 6 and 8 g/kg of the organic extract remained alive during the 7 days of observation. But 40 % of mortality occurred in the 10 g/kg. Dose dependant mortalities of 20 and 60 % occurred in the 8 and 10 g/kg females groups, respectively. The plot of this progressive increase in mortality with respect to the dose of the extract showed that the oral LD_{50} values of the organic extract were about 10.2 and 9.4 g/kg *b.w.* respectively for male and female mice (figure 1).

Consumption and body weight trends

The aqueous extract causes no significant variation of the body weight of the animals. In addition, the body weight of the animals decreases with the dose of 4 g/kg and increases with the dose of 16 g/kg compared to the control group. The organic extract, administered at doses of 4 to 10 g/kg, cause a significant decrease in body weight of the animals on the 2^{nd} day compared with the control group. In addition, starting from the 3^{rd} day, no significant variation of the body weight was observed (figure 2).

The aqueous extract provokes an increase of the food consumption from the doses of 8 and 16 g/kg, this increase is significant at animals treated at doses of 10 g/kg of body weight at days 4, 5 and 7. The administration of the organic extract provokes a decrease of the food consumption in relation to the animals of the control group (figure 3).

The water consumption decreases significantly on the first day of treatment and increase significantly on the 2^{nd}, 6^{th} and 7^{th} day of treatment with the aqueous extract. The increase of the water consumption is only observed at strong doses of the organic extract (figure 4).

Sub-chronic toxicity

Clinical signs and mortality patterns

Animals resumed normal behaviour and activity immediately after each administration of the aqueous and organic extracts. During the 28 days of treatment, no death has been recorded for animals treated at the different doses.

Weekly body weight, feed and water consumption patterns

There were variable changes in the body weight of rats from day 0 to day 28 in all the groups. Body weight of treated and control rats increase throughout the duration of treatment. We observe an increase of the body weight with the dose in animals treated with the aqueous extract and a reduction of the body weight in animals treated with the organic extract (figure 5).

Food consumption patterns during 28 days treatment period (figure 6) revealed that, at the lower dose (0.3 g/kg) the aqueous extract provokes a significant decrease, while at strong doses the food consumption increases. Otherwise, the organic extract provokes an increase of the food consumption at all doses of extract; this increase is significant on the 2^{nd} and 4^{th} week at the dose of 0.6 g/kg.

The water consumption doesn't vary significantly after administration of the aqueous extract during 28 days of treatment. The organic extract provokes a significant decrease in the water

consumption on the first week of treatment, then a non significant increase during the last 3 weeks (figure 7).

Relative organ weight

There were no significant changes in the relative weights of the heart, kidneys, lungs and spleen of the treated rats in relation to control group. However, the doses 0.3 and 1.2 g/kg of aqueous extract produced a significant increase in the relative weight of the liver. The organic extract provoke a significant increase ($p<0.001$) of the mass of the liver at the all animals treated (figure 8).

Serum, hepatic and urine biochemical findings

Our data show a significant increase in serum concentration of ALT at high-dose of *C. macrostachyus* treatment tested in this study (0.6 and 1.2 g/kg). No significant changes were observed in AST serum level. The total protein level significant reduced in animals treated with the aqueous and organic extracts. Otherwise, the aqueous extract provokes a significant increase in the creatinin level at the dose of 0.6 g/kg (figures 9, 10 and 11).

No significant changes were observed in ALT, AST and protein hepatic level with the aqueous extract. The administration of the organic extract in animals during 4 weeks doesn't provoke any significant variation of the hepatic values of ALT and total protein. On the other hand, we observe a significant reduction of the hepatic value of AST at all doses compared to control group (figures 9 and 10).

The organic extract does not cause any significant variation of the urinary creatinin; nevertheless, we observe a reduction in urinary creatinin. In addition, the serum creatinin increases in all the animals treated with the two extracts; this increase is significant at the dose of 0.6 g/kg of aqueous extract. The clearance of creatinin decreases in the animals treated with the doses 0.6 and 1.2 g/kg of aqueous extract and this clearance increases in a dose dependent manner with the organic extract (figure 11).

DISCUSSION

The evaluation of the toxic action of plant extracts is indispensable in order to consider a treatment safe; it enables the definition of the intrinsic toxicity of the plant and the effects of acute overdose[19].

The *Artemia sp.* lethality assay is considered a useful tool for preliminary assessment of toxicity. In addition, the method is rapid, simple, reproducible and economical. A wide variety of biologically active chemical compounds, in particular cytotoxic agents, are toxic to *Artemia sp.*; the death of this organism when exposed to varying concentrations of these compounds forms the basis of a toxicity test. Bioactive compounds are nearly always toxic at high concentrations and, as toxicology can be described as pharmacology at higher doses, this premise has been applied to the screening of medicinal plant extracts in the *Artemia* toxicity test[20]. Indeed, Meyer[13]; Solis[21]; Hartl and Humpf[22] showed that larvae of *Artemia* constitute a good biological material for the assessment of cytotoxicity. The results of *Artemia* testing are summarized in Table 1. The results revealed that the aqueous and organic extracts of *C. macrostachyus* exhibited weak toxicity, with the LC_{50} values the 569 and 425 µg/ml for the aqueous and organic extracts, respectively. In toxicity evaluation of plant extracts by *Artemia* bioassay, an LC_{50} value lower than 1000 µg/ml is considered bioactive[13]. In our study all the extracts have LC_{50} values < 1000 µg/ml; therefore, they can have biological activity. Pharmacological properties of these plants have been demonstrated in preclinical and clinical studies, including those of *Croton macrostachyus* to relieve constipation, cough, diabetes and as purgative. This test is a quick, simple, practical, and low-cost method (it does not require aseptic techniques) and allows a great number of samples to be tested and processed adequately[23]. In general, the *Artemia salina* test is useful for the screening of plant extracts in order to predict their toxicity.

The aqueous extract of *C. macrostachyus* didn't provoke any death until the dose 16 g/kg during 7 days of observation; what could be explained by the fact that the aqueous extract of this plant doesn't contain any toxic compounds. Otherwise, exposure of mice to single *p.o.* doses of *C.*

macrostachyus produced a number of treatment-related effects, particularly in the group with the highest dose. What provokes the oral LD_{50} values of the organic extract the 10.2 and 9.4 g/kg *b.w.* respectively for male and female mices. Fortunately, ethno-medicinal doses commonly used have been estimated to be less than this. Following numerous legislations aiming at regulating the use in the trade of toxins, and supposing values of LD_{50} lower to 5 g/kg correspond to the highly poisonous substances and values of LD_{50} superior to 5 g/kg to the weakly toxic substances[24, 25], results show that the extract of *C. macrostachyus* possesses a very weak toxicity.

At the doses of 16 g/kg for the aqueous extract and 6 g/kg for the organic extract, we observe a depressive state of mice, resulting in a reduction in locomotion, sensitivity to pain, sensitivity to noise and aggressiveness. These behavioural changes would be in principle, except in particular cases, in report is with a reach of certain elements of the nervous system[26], or with a reduction of the sensory or motor conduction consecutive to a depressive action of the extract on the central nervous system whose picture clinic is super imposable the one observed in certain cases of medicinal poisoning[25], or then of an inhibition in the production of algogenics substances (prostaglandin, histamines...) that are regulators of the perception of the pinch[27], or to the action of extract on nociceptors, or to the inhibition at the central level of the transmission of the painful message[28].

The aqueous extract causes an increase in the body weight at the dose of 16 g/kg; a significant increase in food and water consumption. The increase in food consumption would be due to an action of the extract on the receptors of the hunger which would stimulate the appetite with consequently an increase of the body weight. In addition, in the animals having received the organic extract, the body weight, food consumption decrease and water consumption increased. The reduction of food consumption could be due to the loss of appetite (anorexia), which related to an inhibition of the feeling of hunger by action on the receptor of the hunger would be located in the stomach or then at an irritation of the lining of the gastro-intestinal tract, without interference with the absorption or the metabolism of the food of reserves. The reduction in food consumption would cause the reduction in the body weight observed in the animals

It has been pointed out that there is no real correlation between acute dose LD_{50} and prediction of adverse effects of chronic daily dosing[29]. In addition, the LD_{50} in animals does not predict the human lethal dose of a drug or the symptomatology of acute poisoning after overdose[30]. Nevertheless, the acute dose study provides a guideline for selecting doses for the chronic low-dose study, which may be more clinically relevant.

Animal behavioural changes were not altered during the sub-chronic dose study with the aqueous and organic extracts. The body weight of the animals treated during 4 weeks with the aqueous extract is weak, whereas the organic extract induces a significant reduction in the body weight. On the other hand, the extract causes an increase in food consumption and water consumption. These results show that the repeated administration of the various extracts would stimulate the receptors of the hunger, which would explain the increase of food consumption. However all these extracts caused a reduction in the serum and hepatic protein in all the treated animals. It is known that all variations in body weight is not always related to the variation of food consumption[31], this leads us to think that the reduction in serum proteins would prevent the mobilization of proteins in tissue[32], what would be at the origin of the dropping cell multiplication and the reduction in the body weight observed in the treated animals

The drop of the hepatic and serum protein could be explained by a bad metabolism of proteins. Thus, the extracts would cause insufficiency in the metabolism of the hepatocytes. Under normal conditions, the synthesis of serum proteins by the liver depends on the concentration of blood amino acids[33]. In the event of reduction in plasmatic proteins without process able to supply blood with amino acid, the blood proteins fall. The fall of the blood protein

could be also explained by the anomalies observed (vascular Congestion and exudates) on the histological cuts of the treated animals; these anomalies caused the reduction in the hepatic synthesis of proteins, since the synthesis of plasmatic proteins takes place in the liver[34].

The weight of the animal spleen doesn't present any significant variation, what could reveal the possibility of the absence of immunotoxic effect of the extract for it's true that the spleen plays a major role in the immunological mechanisms. A light reduction of the relative weight of the heart would mean a possible toxic effect of this organ induce by the extract.

The relative weight of the liver increases in all the treated animals. Moreover, the extracts cause vascular congestions and inflammatory exudates which would be at the origin of the inflammatory infiltrations and liquid, which could explain the increase in the weight of the liver. The function of the liver in detoxification would also be disturbed by the extracts, because the activity of enzymes ALT and AST decreases in the liver and increases significantly in blood. The increase of the serum enzymes ALT and AST would be correlated to the morphological damage which appears at the level of the liver[35]. The ALT and AST are markers of the syndrome of cytolysis or lesion of the hepatocytes[36]. The ALT being localised mainly in the hepatocytes, is regarded as the principal marker of the damage of the hepatocyte that the AST and can in this direction provide information on the sudden degree of the damage by the liver[37].

The extract involves a dose-dependent decrease of the relative weight of the kidneys. The extracts do not cause significant variation of the serum creatinin and urinary creatinin. Indeed, the values of reference of creatinin are included between 0.39-2.29 mg/dl[38]. Values of the serum creatinin obtained in this study, 0.74 mg/dl at controls group and 1.12 mg/dl in the animals treated with the high doses of *C. macrostachyus* extracts, are contained in the interval of reference. Consequently, the increase in the serum creatinin observed is not significant at the medical consideration. This is comforted by the fact that the renal clearance of creatinin does not show significant variation in all the treated animals. Thus, the presence of the antioxydant compounds such as the flavonoïds in the various extracts[39] probably protected the kidneys against a possible nephrotoxic effect. Because the *C. macrostachyus* extracts are pcontained in phenolic compounds, particularly the flavonoïds which are antioxydant compounds. This assumption is all the more probable as Djemna[40] showed the antioxydant effects of the aqueous and methanolic extracts of the stem barks of *C. macrostachyus*.

In conclusion, a number of significant physical, clinical and pathological changes were associated with the *p.o.* administration of *C. macrostachyus* to mices and *Wistar* rats, either acutely (up to 2 g/kg) or sub-chronically (up to 1.2 g/kg). The fact that no substantial toxic effect occurred in animals that were administered *C. macrostachyus* at a dose 0.6 g/kg *b.w.* suggest that the margin of safety of the extract is high at administration used clinically. The preliminary study seems to be conclusive but further investigations are necessary notably "*in vitro*" evaluation of cytogenotoxicity on cell lines registered in the guidelines and "*in vivo*" alcaline comet assay on at least five organ (like blood, liver, kidney, brain, lung).

Table 1: Mortality obtained of *Artemia salina* and estimate of LC_{50} values

Extracts	Concentration (µg/ml)	Mortality	LC_{50} values (µg/ml)	Limits 95 % Confidence (µg/ml)	Slope
Aqueous extract	30	0	569	0.00-1.62	6.29
	100	0			
	300	24			
	1000	100			
CH_2Cl_2/CH_3OH extract	30	0	425	294.3-305.9	9.056
	100	0			
	300	50			
	1000	100			

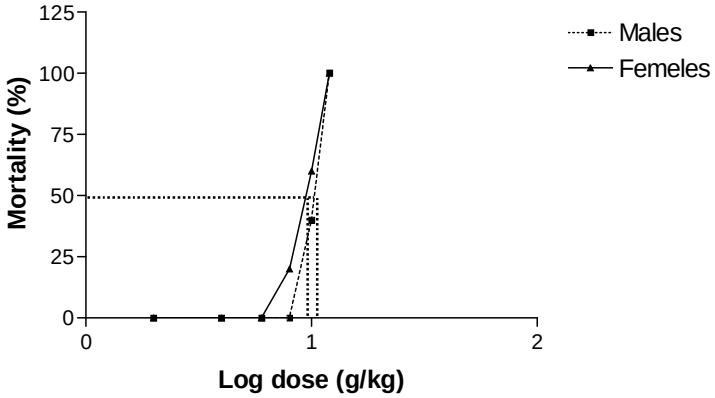


Fig. 1: Overall mortality (%) observed in mice having received a single oral dose of the CH_2Cl_2/CH_3OH extract of *C. macrostachyus* to 7 days

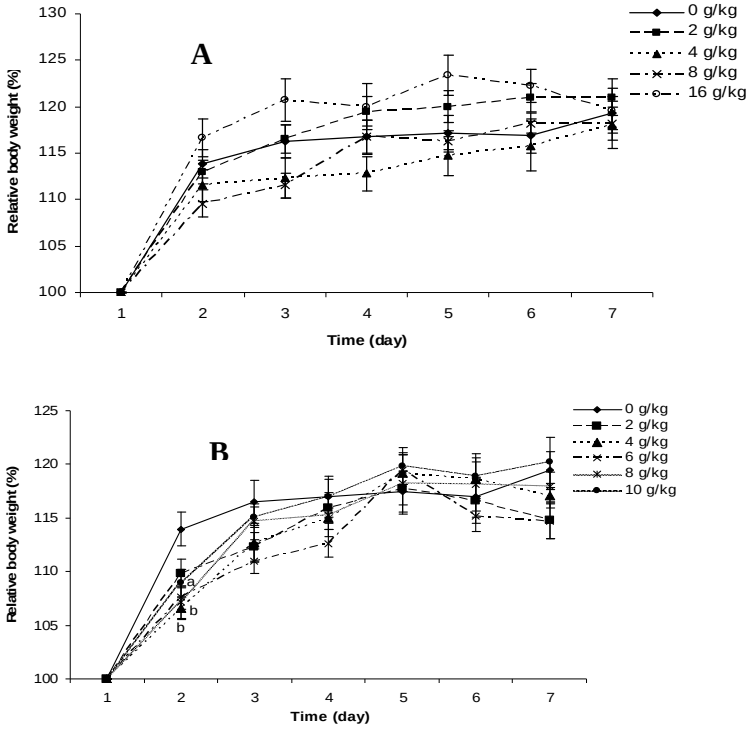


Fig. 2: Body weight trend for mice dosed once with aqueous (A) and CH_2Cl_2/CH_3OH (B) extracts of *C. macrostachyus*. Each data point represents the mean ± S.E.M. (n = 10). [a]p < 0.05; [b]p < 0.01 compared with the control group (0 g/kg).

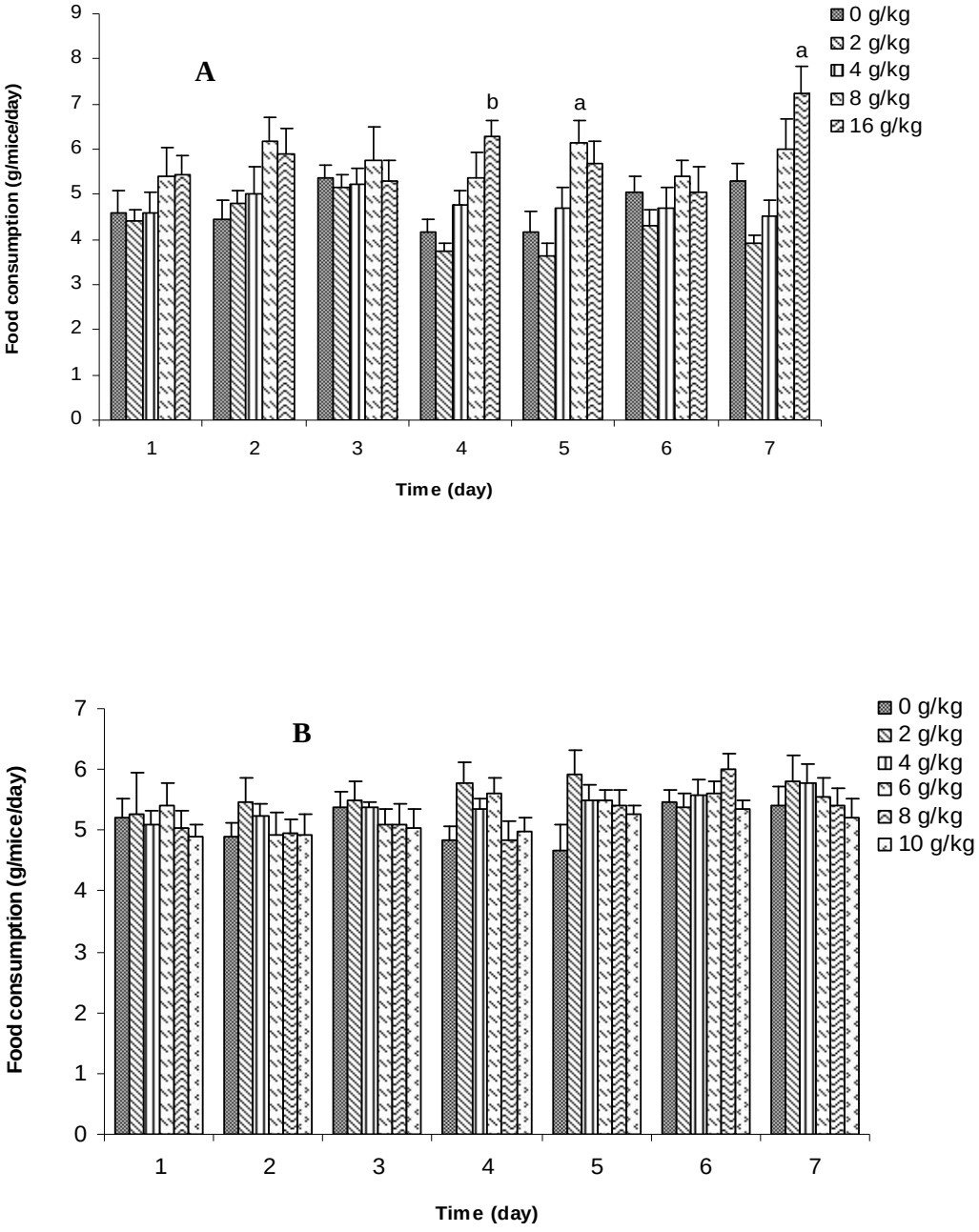


Fig. 3: Food consumption trend of mice dosed once with aqueous (A) and CH_2Cl_2/CH_3OH extracts of *C. macrostachyus*. Each data column represents the mean ± S.E.M. (n = 10). Values for consumption are based on total intake and average body weight of the preceding time interval. [a]p < 0.05; [b]p < 0.01 compared with the control group (0 g/kg).

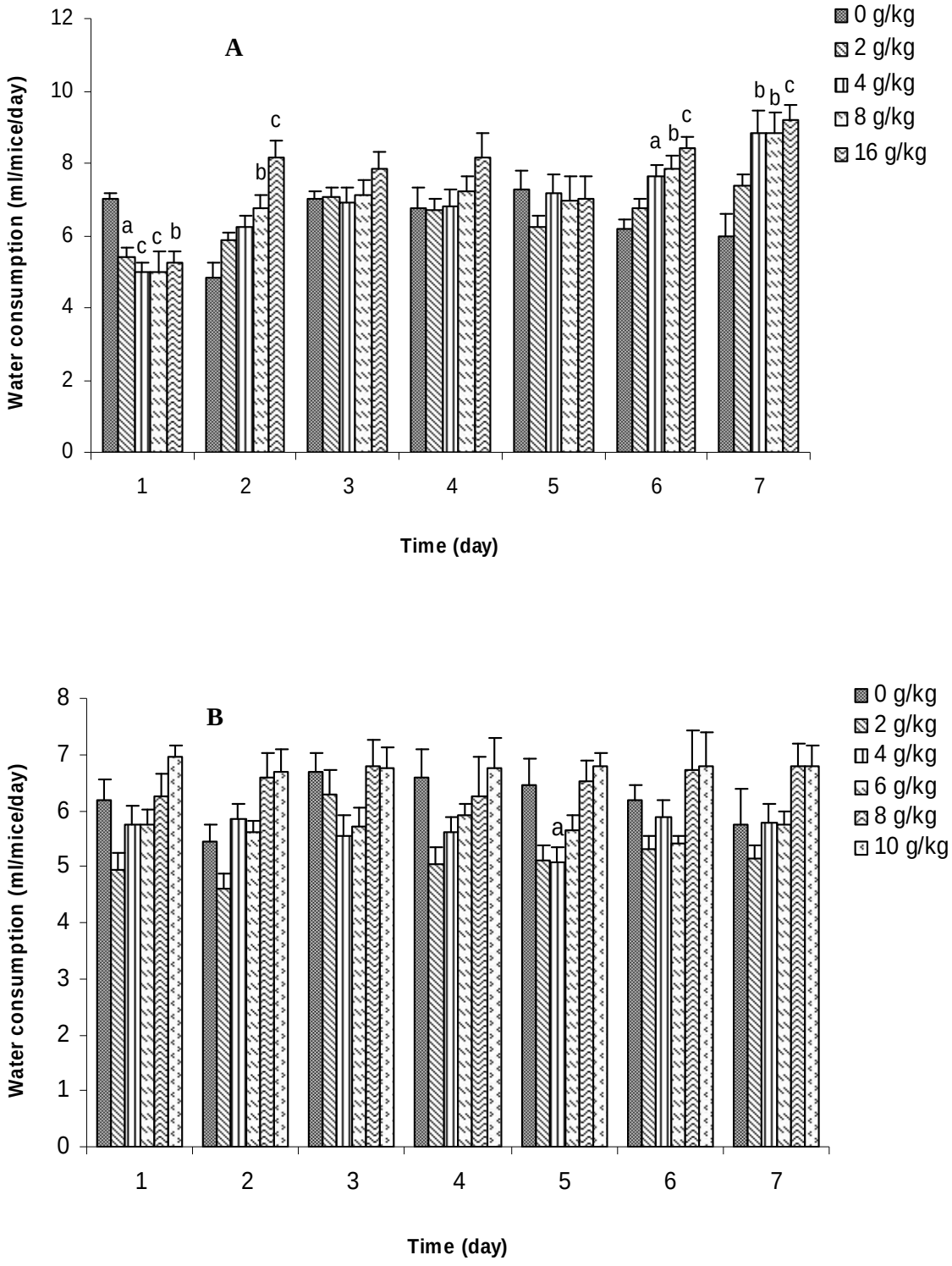


Fig. 4: Water consumption trend of mice dosed once with aqueous (A) and CH_2Cl_2/CH_3OH extracts of *C. macrostachyus*. Each data column represents the mean ± S.E.M. (n = 10). Values for consumption are based on total intake and average body weight of the preceding time interval. $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0,001$ compared with the control group (0 g/kg)

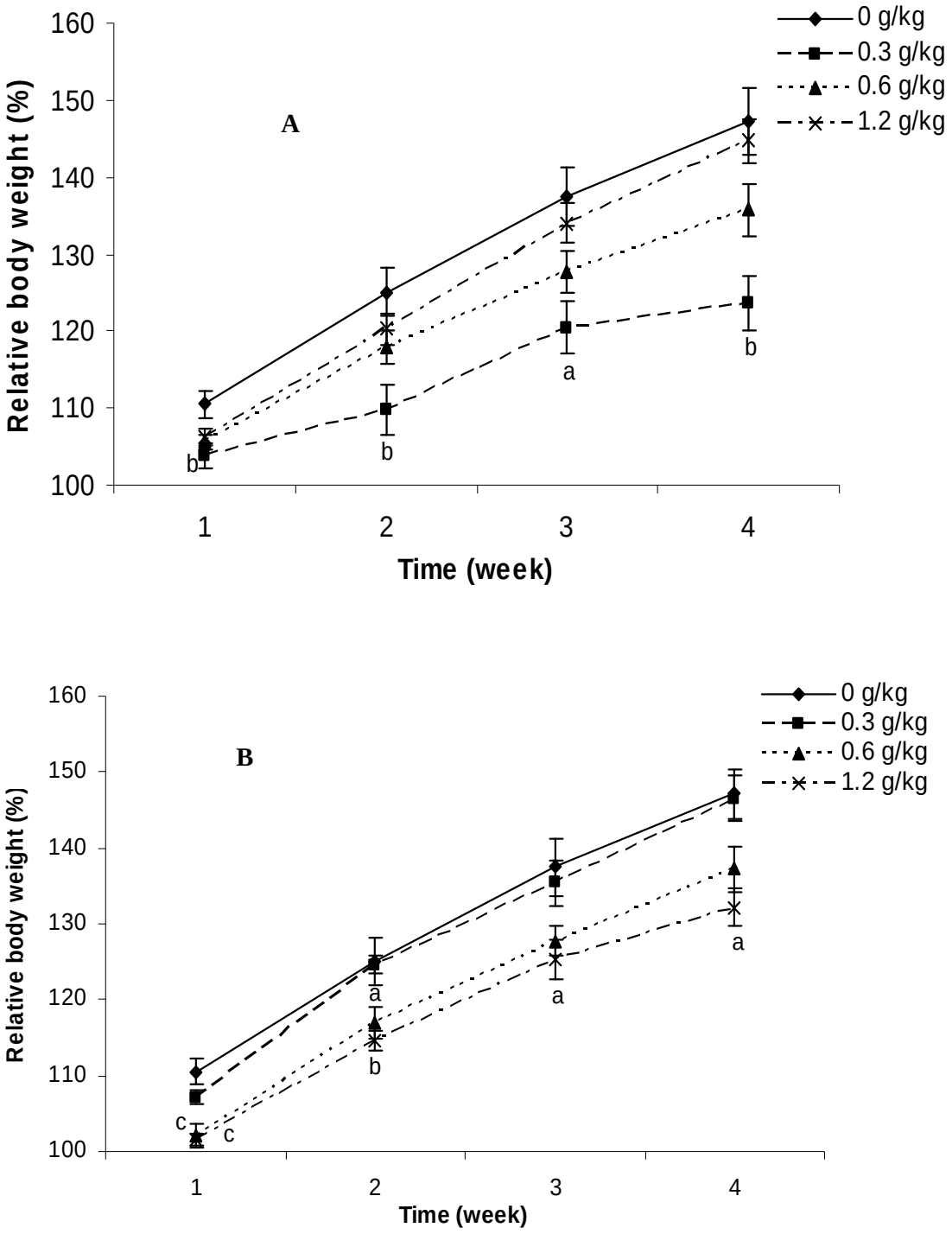


Fig. 5: Relative body weight pattern in Wistar rats fed with aqueous (A) and CH_2Cl_2/CH_3OH (B) extracts of *C. macrostachyus*. Each data point represents the mean ± S.E.M. (n = 10). $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$ compared with the control group (0 g/kg)

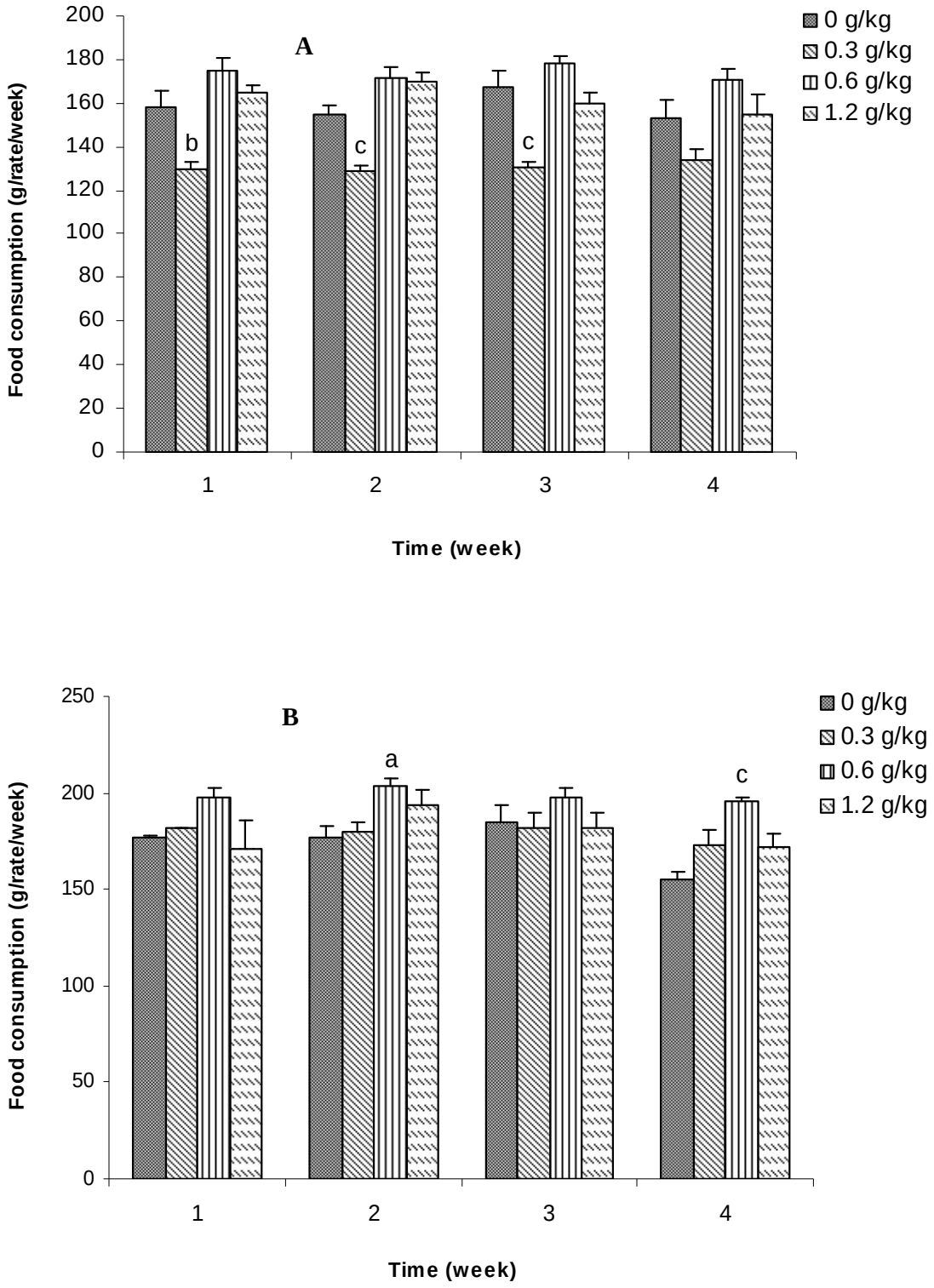


Fig. 6: Food consumption trends of rats fed with aqueous (A) and CH_2Cl_2/CH_3OH (B) extracts of *C. macrostachyus*. Each data point represents the mean ± S.E.M. (n = 10), $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$ compared with the control group (0 g/kg)

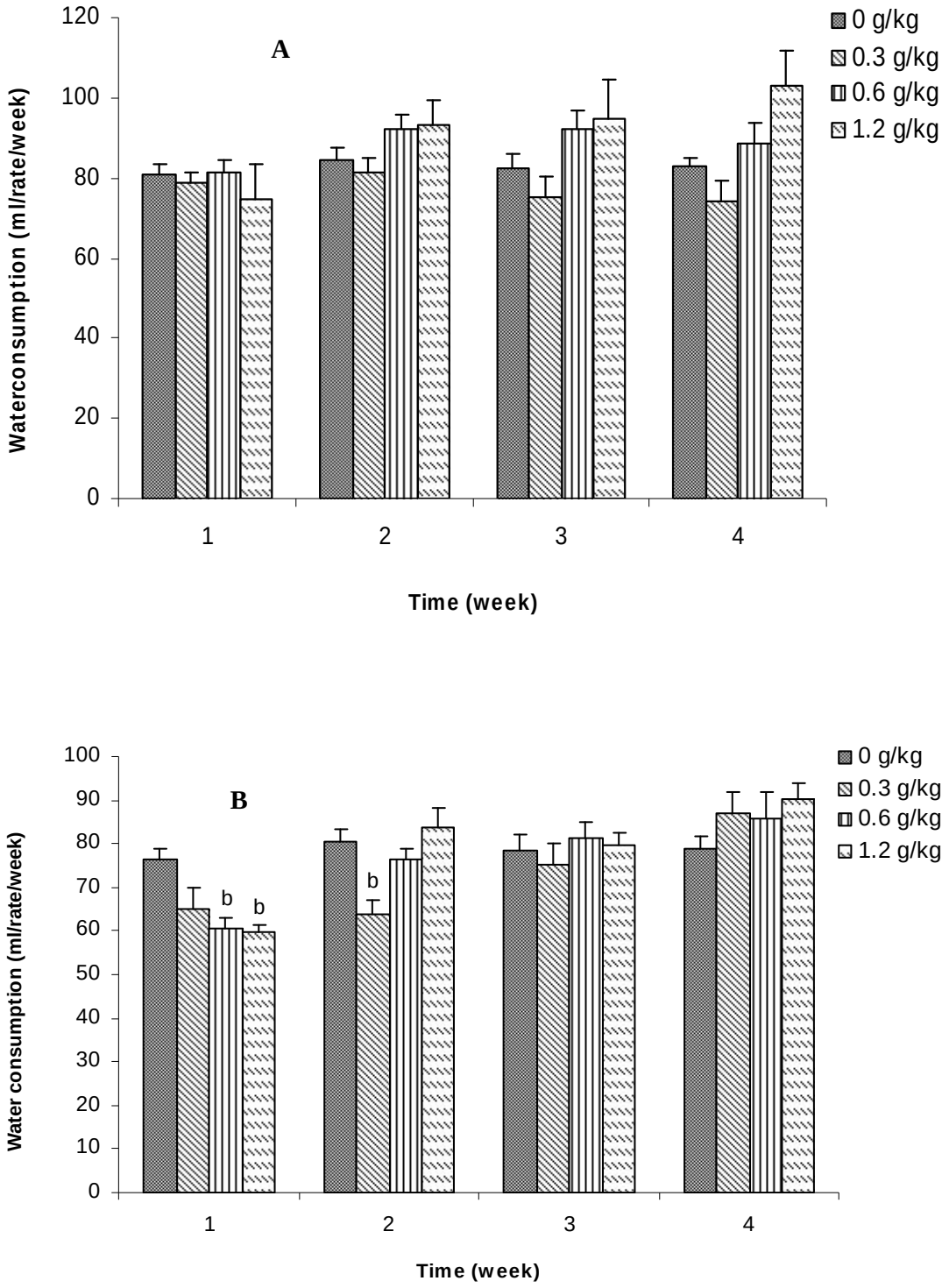


Fig. 7: Water consumption trends of mice fed with aqueous (A) and CH_2Cl_2/CH_3OH (B) extracts of *C. macrostachyus*. Each data point represents the mean ± S.E.M. (n = 10), $^{a}p < 0.05$; $^{b}p < 0.01$ compared with the control group (0 g/kg)

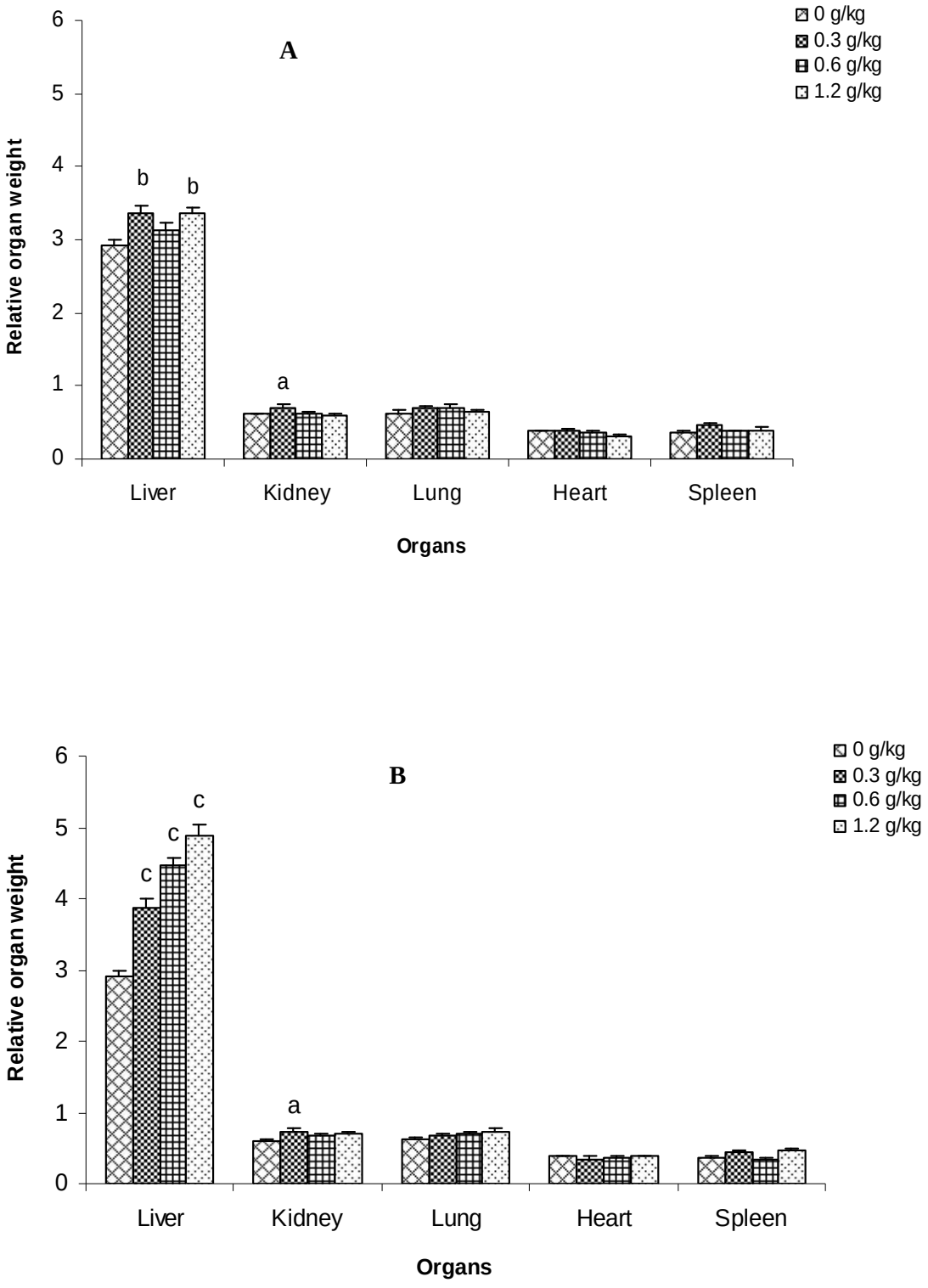


Fig. 8: Effect of aqueous (A) and CH_2Cl_2/CH_3OH (B) extracts of *C. macrostachyus* on the relative organ weights Wistar rats after 4 weeks oral dosing. Each data column represents the mean ± S.E.M. (n = 10). $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$ compared with the control group (0 g/kg)

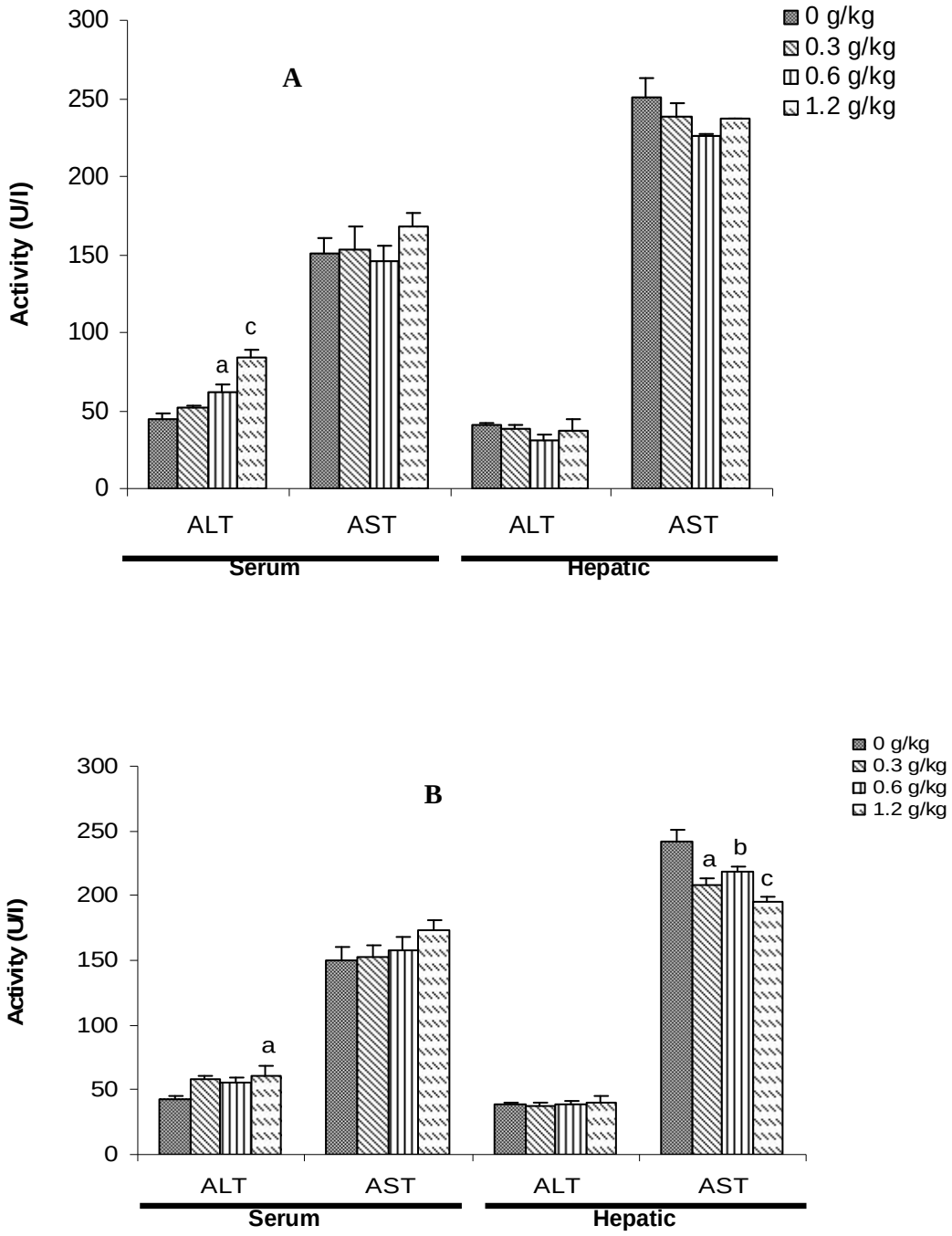


Fig. 9: Variation of the serum and hepatic enzymes ALT (alanine aminotransferase) and AST (aspartat aminotransferase) in the rat treated with the aqueous (A) and CH_2Cl_2/CH_3OH (B) extract of *C macrostachyus* in sub-chronic toxicity. Each point represents the mean of 10 values ±ESM. $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$; significant difference compared to the control group (0 g/kg)

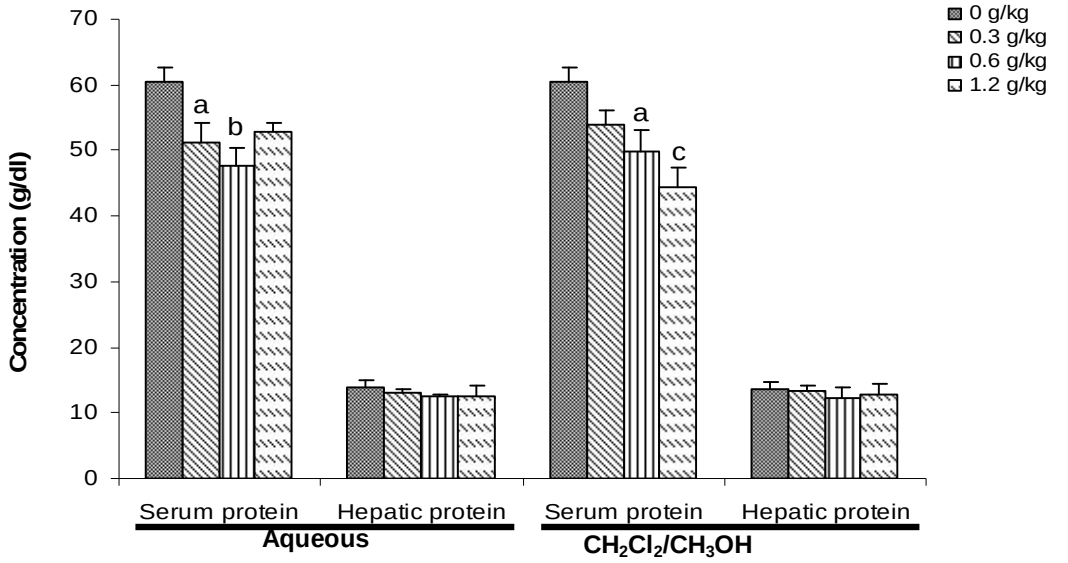
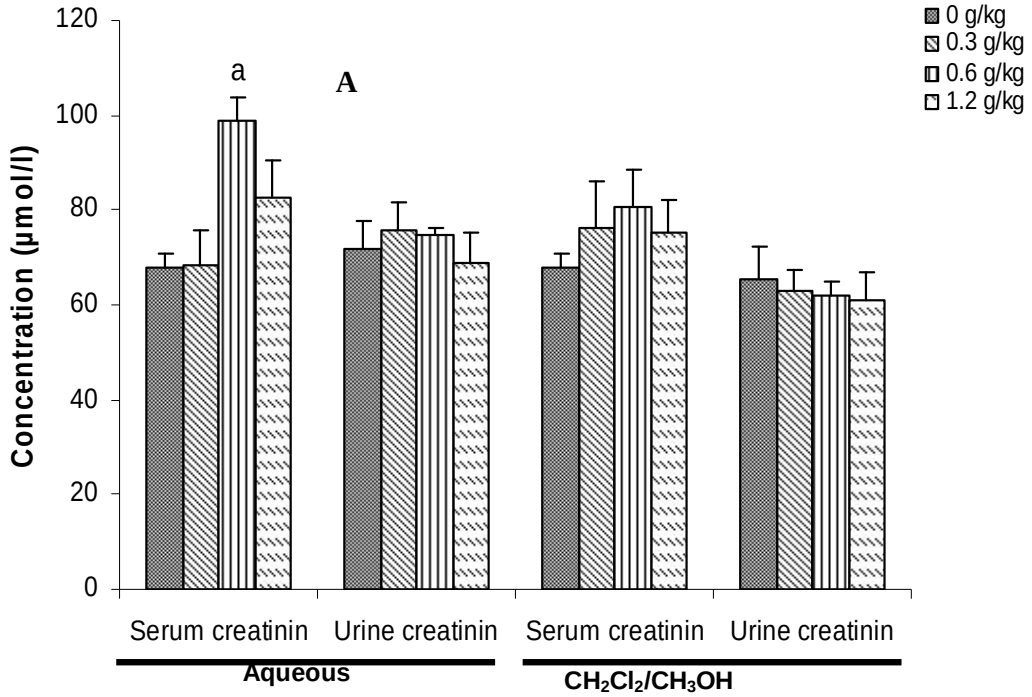


Fig. 10: Variation of the serum and hepatic proteins in the rat treated with the aqueous and CH_2Cl_2/CH_3OH extract of *C macrostachyus* in sub-chronic toxicity. Each point represents the mean of 10 values ±ESM. $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$; significant difference compared to the control group (0 g/kg)



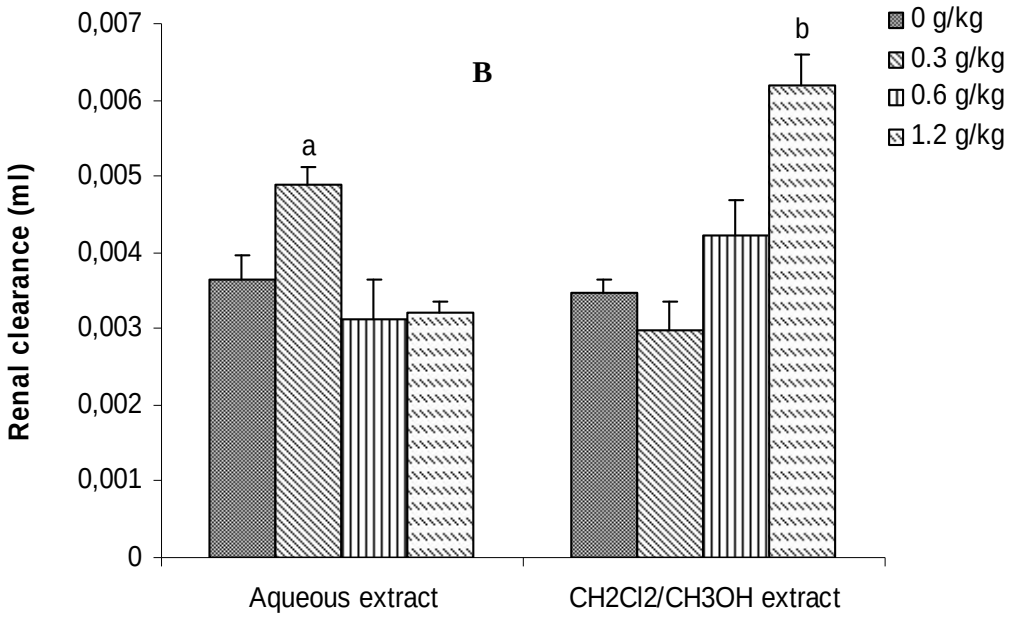


Fig. 11: Variation of the creatinin (A) and the renal clearance (B) in the rat treated with the aqueous and CH_2Cl_2/CH_3OH extract of *C macrostachyus* in sub-chronic toxicity. Each point represents the mean of 10 values ± ESM. $^{a}p < 0.05$; $^{b}p < 0.01$significant difference compared to the control group (0 g/kg)

REFERENCES

1. Leonardo DCL., Franco A., Gustavo ATI., Luciano MA., Lius EMES., Gabriele PDS., Isabela DMA., José ENN., Israel E. and Karla K. Toxicological evaluation by in vitro and in vivo assays of an aqueous extract prepared from Echinodorus macrophyllus leaves. Toxicology Letters. 2000; 116: 89-198.
2. Zhu M., Lew KT. and Leung P. Protective effects of plants formula on ethanol-induced gastric lesions in rats. Phytotherapy Research. 2002; 16: 276-280.
3. Kouitcheu MLB., Penlap BV., Kouam J., Essame O. and Etoa FX. Toxicological evaluation of ethyl acetate extract of Cylicodiscus gabunensis stem bark (Mimosaceae). Journal of Ethnopharmacology. 2007; 111: 598-606.
4. Sofowora EA. Medicinal Plants and Traditional Medicine in Africa, 1st ed. Sprectrum Books Ltd., Ibadan-Nigeria. 1989.
5. Adjanohoun EJ., Adjakidjè V., Ahyi MRA., Akeassi L., Akoegninou A., d'Almeida J., Apovo F., Boukef K., Chadare M., Dramane K., Eyme J., Gassita JN., Gbaguidi N., Goudoté E., Guinko S., Houngnon P., Issa Lo, Keita A., Kiniffo HV., Koné-Bamba D., Musampa Nseyya A., Saadou M., Sodogandji Th., de Souza S., Tchabi A., Zinsou Dossa C. and Zohoun Th. Médecine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques et floristiques en République du Bénin. Agence de Coopération Culturelle et Technique, ISBN 92-9028. 1989 ; 152.9.

6. Stuart KL. and Graham L. Alkaloids biosynthesis in Croton flavens.Phytochemistry. 1973 ; 12: 1967-1972.
7. Mbuya LP., Msanga HP., Ruffo CK., Birnie A. and Tengnäs B. The useful trees, shrubs for Tanzania. Identification, propagation and management for agricultural and pastoral communities. Technical handbook. 1994; No. 6:540.
8. Kamanyi A., Mbiantcha M., Nguelefack TB., Watcho P., Ndontsa BL. and Tane P. Anti-Nociceptive and Anti-Inflammatory Activities of Extracts from the Stem Bark of Croton macrostachyus (Euphorbiaceae) in Mice and Rats. Journal of Complementary and Integrative Medicine. 2009; Vol. 6 , Iss. 1, Art. 20.
9. Mc Namara B. Concepts in health evaluation of commercial and industrial chemical. In: Mehlman, M.A., Shapiro, R.E., Blumental, H. (Eds.). New Concepts in Safety Evaluation. Hemisphere. Washington DC. 1976.
10. Massele AY. and Nshimo CM. Brine shrimp bioassay for biological activity of medicinal plants in traditional medicines in Tanzania. East Afr. Med. J. 1995; 72(10): 661–663.
11. Mathews RS. Artemia salina as a test organism for measuring superoxide-mediated toxicity. Free Radia Biol. Med. 1995; 18(5): 919–922.
12. Fumaral F. and Garchitorena M. Artemia salina Recolección, descapsulación y desarrollo. Revista Aquamar. 1996; Año 4 Nº 3: 22–24.
13. Meyer BN., Ferrigni NR., Putnam JE., Jacobsen LB., Nichols DE. and Mc Laughlin JL. Brine shrimp: a convenient general bioassay for active plant constitauents. Planta Medica. 1982; 45: 31-34.
14. Ojala T., Vuorela P., Kiviranta J., Vuorela H. and Hiltunen R. A bioassay using Artemia salina for detecting phototoxicity of plant coumarins. Planta Medica. 1999; 65: 715-718.
15. Behrens B. and Karber G. Mathematic for Naturalists and Agriculturalist. PWN, Warszawa. 1983; 212.
16. Gornal AG., Bardwill GS. and David MM. Determination of serum proteins by the means of Buiret reactions. Journal of. Biology and. Chemistry. 1949; 177:751-766.
17. Slot C. Plasma creatinine determination: a new and specific Jaffe reaction method. Scand. J. Clin. Invest. 1965; 17: 381.
18. Reitman M. and Frankel S. A calorimetric method for the determination of serum glutamate oxalate and glutamate pyruvate transaminase. Am. J. Clin. Pathol.1957; 28: 56-63.
19. Cáceres A. Plantas de Uso Medicinal en Guatemala. 1er ed. Editorial Universitaria: San Carlos de Guatemala. 1996; 61, 67, 167, 236, 283, 325, 328.
20. Campos MD., Cruz IR., Alba MA., Corral GC., Angeles-Lopez GE., Navarrete A. and Mata R. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. Journal of Ethnopharmacology. 2007; 110: 334 –342.
21. Solis PN., Wright CW., Anderson M., Gupta MP. and Phillipson JD. A microwell cytotoxicitiy assay using Artemia salina (brine shrimp). Planta Medica 1993; 26: 250–252.
22. Hartl M. and Humpf H. Toxicity assessment of fumonising using the brine shrimp (Artemia salina) bioassay. Food chem. toxicol. 200; 38: 1097-1102.
23. Ohno Y., Kaneko T., Inove T., Morikawa T., Yoshida A., Fujii A., Masuda M., Ohno T., Hayashi M., Mumma J., Akiyama J., Hagaki H., Ohkoshi K., Okumura H., Kakishima H., Kasai Y., Kurishita A., Kujima H., Saijo K., Sakamoto K., Sugiura S., Sunouchi M., Takano K., Tatsumi H., Tani N., Chiba K., Nakamura T., Matsukawa K. and Matsushige C. Interlaboratory validation of alternative methods to the eye

irritation test for the safety evaluation of cosmetic ingredients: an overview of the plan and the results. Animal Alternatives, Welfare and Ethics. 1997; 27: 1155–8.

24. Delongeas J.L, Burnel D., Netter P., Grignon M., Mur J.M., Royer R.J., rignon G. Toxicité et pharmacocinétique de l'oxychlorure de Zirconium chez la souris et le chat. J. pharmacol. 1983 ; 14 : 437.
25. Diezi J. Principe de base et répercussion clinique. Dans Pharmacologie : Des concepts fondamentaux aux applications thérapeutiques, Schoderet M., (Eds) Frison Roche, Paris, Slatkine, Genève. 1992 ; (2ème ed) : 33-35.
26. Olivier B. and Berver. Medicinal plants in tropical West Africa. New-York, Cambridge University press. 1986; 15-269.
27. Lehninger AL. Principes de Biochimie. Ed. Flammarion. Paris. 1985; 272-274.
28. Nguelefack TB., Fotio AL., Watcho P., Wansi SL., Dimo T., Kamanyi A. Analgesic Properties of the Aqueous and Ethanol Extract of the Leaves of Kalanchoe crenata, Phytotherapy Research. 2004; 18: 385-388.
29. Zbinden G. Experimental and clinical aspect of drug toxicity. Advances in Pharmacology. 1963; 2: 1–112.
30. Zbinden G. and Flury-Roversi M. Significance of the LD50 test for the toxicological evaluation of chemical substances. Archives of Toxicology. 1981; 47: 77 –99.
31. Case MT., Sibinski LJ. And Steffen GL. (1984) Chronic oral toxicity and oncogenicity studie of flecainide, an antirythmic, in rats and mice. Toxicology. Applipharmacology. 1984; 73 (2): 32-242.
32. Dohm GL. Protein metabolism during endurance exercice. Med. Proc.1985 ; 44: p348.
33. Ntchapda F. Quelques aspects de la toxicité et des propriétés cardiovasculaires des extraits de feuilles de Celtis durzndii Engler (Ulmacées) chez le rat. Thèse de Doctorat/Ph.D. Université de Yaoundé I. Yaoundé, Cameroun. 2006.
34. Kathleen DP. and Jaunes TP. Mosby's diagnostic and laboratory test reference, Mosby year book, St Louis USA. 1992; 843.
35. Brautbar N. and Williams II J. Industrial solvents and solvent and liver toxicity: rick assessment, rick factors and mechanisms: review. International Journal of Hygiene and Environmental Health. 2002; 205 : 479-491.
36. Brissot P., Deugnier Y., Messner M., Dalamaire D. and Lescoat G. «La biologie hépatique en 1988». Gastro-enterologie. 1998; 17 :467-473.
37. Al Mammary M., Al-Habori M., Al-Aghbari AM. and Baker MM Investigation into the toxicological effects of Catha edulis leaves: a short-term study in animals. Phytotherapie. Research. 2002; 16:127-32.
38. Matsuda H., Tanaka A. and Itakura A. Immunology and hematology. In: Krinke, G.J. (Ed.), The Laboratory Rat. Academic Press, London. 2000; 437–446.
39. Mbiantcha M. Propriétés Analgésique, Anti-inflammatoire et Profil Toxicologique des Extraits de Croton macrostachyus (Hochst) pipe and Galinier et de Dioscorea bulbifera L. var sativa. Thèse de Doctorat/Ph.D. Université de Dschang. Dschanf, Cameroun. 2010.
40. Djemna AF. Evaluation des Effets Hypotensifs et Antioxydants des Extraits Aqueux et Méthanolique des Ecorces de Croton macrostachyus(Euphorbiacée) chez le rat. Thèse de Master of Science. Université de Dschang. Dschang, Cameroun. 2009.