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Research Article

Acute and Sub-Acute Toxicity of *Sida veronicifolia* Aqueous Extract in Female Wistar Rats

M D Yemele[1], P B Telefo[1*], H S I Mapon[1], C Nangue[3], C S P Fodouop[1,2], N S Njina[1], J T Mbemya[1], L L Lienou[1], S R Tagne[1,2], C S Goka[1], F Ngoula[4], F Nguemo[5], P F Moundipa[6]

[1]*University of Dschang, Faculty of Science, Department of Biochemistry, P.O.BOX 67, Dschang, Cameroon*
[2]*University of Ngaoundere, Department of Biomedical Sciences, P.O. Box 454, Ngaoundere, Cameroon*
[3]*Pathological Analysis Department of the Central Hospital of Yaounde, Cameroon*
[4]*University of Dschang, Faculty of Agronomy and Agricultural Sciences, Department of Animal Production, Cameroon*
[5]*University of Cologne, Institute of Neurophysiology, Germany*
[6]*University of Yaounde I, Faculty of Science, Department of Biochemistry, P.O.BOX 812, Yaounde, Cameroon*

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ABSTRACT

Ethnopharmacological relevance: *Sida veronicifolia* Lamb (Malvaceae) is commonly used in traditional medicine of Cameroon for the treatment of pregnancy and childbirth discomfort; however, no toxicological studies have been reported on the plant. Aim of the study: The present study was carried out to evaluate the acute and sub-acute toxicity of the aqueous extract of this plant has been evaluated in female rats. Materials and methods: In the acute toxicity test four female Wistar rats were treated with a single dose of the AESV (5000 mg/kg) administered by oral gavage and observed for 14 days in order to identify signs of toxicity or death. In subchronic toxicity study, rats were divided into 4 groups of 5 animals each. Animals of group 2 to 4 received, by daily gavage three doses 60, 240 and 960 mg/kg of the AESV for 28 days. Body weight and food consumption were measured every four days and at the end of treatment were analysed hematological, biochemical and histopathological parameters. Results: Evaluation of acute toxicity indicated no apparent clinical change in the animals; the LD50 is higher than 5000 mg/kg. In the sub-acute toxicity study, the AESV did not affect the general behavior. Moreover, no significant difference was observed on the body weights and blood, urinary and organs biochemical parameters. However, food consumption significantly decreased at the dose 960 mg/kg; vascular congestion of hepatic portal vein, few hepatic fibrosis at the doses 240 and 960 mg/kg and few renal necrosis at the dose 960 mg/kg were registered on liver and kidney sections. Also, a significant decrease of lymphocytes percentage associate to an increase of those of granulocytes was observed in all treated groups comparatively to control. Conclusion: The results of the present study showed evidence of the non-toxic effect of *S. veronicifolia* at 60 mg/kg, the dose commonly used in traditional treatments of pregnancy complaints. However, in sub-acute treatment, higher doses could provoke histopathological damages in the liver and kidneys which could in part reversible. Thus the extract should be used with caution.

Keywords: Pregnancy complaints, *Sida veronicifolia*, aqueous extract, acute and sub-acute toxicity, histological section.

INTRODUCTION

The use of plant in traditional medicine in Cameroon is as old as the history of African and Cameroonian people[1]. Medicinal plants play a very significant role in health care needs of rural populations in Africa and other developing countries, especially in treatment of infectious diseases and all related reproductive system discomfort. At the international level, the issue of medicinal plants is of great concern in many heath care systems. A survey done by WHO reported that approximately 80% of the global population still depend on traditional plant-based medicines for their basic health care needs[2,3]. The administration of these natural products or synthetic drugs to a biological system may induce different types of interactions and a series of dose-related responses. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous[4]. Many studies have reported the toxic effects of herbal medicines[5-8].

S. veronicifolia Lam is a plant of Malvaceae family which presents long unicellular acicular trichomes and small 5-armed stellate hairs[9]. It is used in folkloric medicine as abortifacient, pain-killer, ecbolic, land conservator, analgesic during labour, shorter of postpartum bleeding as well as in the treatment of pregnancy ailments, dysmenorrhoea, male infertility (poor erection), gonorrhea, malnutrition and debility[10-13]. In association with other plants *S. veronicifolia* is used for falling hair and premature graying. Studies have proved the muscarine-like active principle of the water soluble fraction from the alcoholic extract of *S. veronicifolia*[14] and the presence of antioxidant active compound in S. *veronicifolia* extract[11]. Although many belonging *Sida* plants *sp*, especially *S. Acuta*; *S. rhombifolia*[12], *S. cordifolia*[15,16] and *S.*

**Author for Correspondence: bphelix@yahoo.co.uk*

veronicifolia[11] have been proven to treat diseases of the reproductive system, and their effectiveness in traditional treatment of pregnancy discomfort, no much is known about their dose-related toxicity at the histological as well as biochemical level. Moreover, an ethnobotanical survey done in Menoua-division (west-cameroon) revealed that *S. veronicifolia* is one of the most used plants for the treatment of pregnancy ailments and is used at the unlimited doses for longer period (3 to 6 months) of administration[13]. In the view of determining, some of the possible side effects which may be associated with the use of *S. veronicifolia* as antidote for pregnancy discomfort, this study was therefore designed for evaluating the acute and sub-acute toxicity effects of the aqueous extract of this plant on liver and kidney functions in female rats.

MATERIAL AND METHODOLOGY

Plant material

S. veronicifolia was collected in its vegetative state in June 2014 in Bamendou village, (Menoua Division, West Region of Cameroon). The taxonomic identification of the plant was done by the Cameroon's National Herbarium (CNH) under the voucher specimen number. 29010/SRF/CAM.

Animals

Female Wistar albino rats, weighing between 150 and 180 g and of 10 to 12 weeks' old were used. They were acclimatized during 10 days, in the Animal House of the Department of Biochemistry (University of Dschang, Cameroon), under standard animal house conditions and allowed free access to food and water for the same period.

Chemicals

INMESCO (Germany) Kits for aspartate aminotransferase, alanine aminotransferase, alkalin phosphatase, total and direct bilirubin, total cholesterol, HDL-cholesterol, triglyceride, creatinine, and urea were obtained from commercial firm.

Acute toxicity study

The doses adjustement method of OCDE[17] for acute toxicity studies was used for the estimation of the lethal dose (LD_{50}) of the plant using female rats. The highest dose recommended by this method (5000 mg/kg) was used because of previous results obtained from cytotoxic study on the aqueous extract of *S. veronicifolia* (AESV) which shown that AESV is not toxic[18]. Six acclimatized female Wistar albino rats were used in this study. Animal 1 and 2 were used control and received only distilled water (1 ml per 100 g of body weight), while animal 3 to 6 were test group and were treated orally with a single dose of AESV (5000 mg/kg). The different groups of rats were housed in a separate cage. All animals were fasted for 18 hrs prior to the administration of the plant extract or distilled water. They were continuously and hourly observed during the first day after treatment to detect any signs of toxicity such as: changes in autonomic or behavioral responses (locomotion, aggressiveness), spontaneous activity (reaction to tail pinch and to noise), social interactions, aspect of mucosa and feces, eye coloration and corneal reflex, appearance of hair, trembling, salivation and mortality. When animals are gathered together, it is an indicator of communication (i.e. gathering); they are said to be in activity when they are roaming in the cage; they are say to be reactive when any attempt to touch them, they react by biting; normal reaction to noise is when the rats are unsettled on hearing a noise; the cries of rats when pinched on their tail is an indicator of normal reaction to pinch; the tail is normal when it is flexible (i.e. no rigid); rigid tail is a sign of anger[19,20]. After the first day following the treatment, animals were supplied with food and water ad libitum, and were further closely observed once daily for 13 days in order to identify signs of toxicity or death. The body weight of each rat was measured every 2 days throughout the observation period. At the end of this period, all survivors were killed to examine macroscopic alterations in their vital organs.

Sub-acute toxicity study

Twenty female rats were randomly distributed into four (04) groups of five animals each. Animals of groups 2 to 4 (test groups) were treated for 28 days with different doses of AESV [60 mg/kg (traditional healer dose), 240 mg/kg and 960 mg/kg respectively)] while, animals of group 1 received 1 ml/100 g body weight per day of distilled water (control) for the same period. During treatment, animals were daily weighed, treated with the corresponding dose of extract (test groups) or distilled water (control) before being allowed to food and water (tap water) *ad libitum*. At the end of the treatment period, prior to sacrifice, animals were subjected to a 12 hours' food fasting at the end of which their urine was collected and stored at -20°C for the dosage of proteins, creatinine and urea. Then animals were anesthetized by inhalation of chloroform vapors, dissected and their blood collected by cardiac puncture into sterilized dry test tubes or test tubes containing EDTA. Blood containing EDTA was used for complete blood count while the other was left for 2 hours in refrigerator at 4°c before being centrifuged at 2 500 rpm for 15 minutes. The sera obtained were used for the determination of the effect on toxicity biochemical makers. The liver, lungs, spleen, heart and kidneys of each animal were removed, weighed and their protein as well as hepatic transaminases levels were determined.

Complete blood count

The complete blood count was performed using an automated hematology analyzer. Hematological evaluations included red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), blood platelet count (PLT), white blood cell count (WBC), lymphocytes count and granulocytes count.

Preparation of Homogenates and Biochemical Analysis

The homogenates of various organs were obtained by grinding a fixed weight of the organ in 3 ml of phosphate Buffer (pH 7.4; 0.1 M). After centrifugation at 3000 rpm for 15 min, the supernatant was taken and preserved at -20°C. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline Phosphatase (ALP) activity assays as well as Creatinine and bilirubin levels were evaluated. The lipidic profile [total cholesterol (TC), High Density Lipoprotein (HDL) and triglycerides (TG)] were performed spectrophotometrically using INMESCO

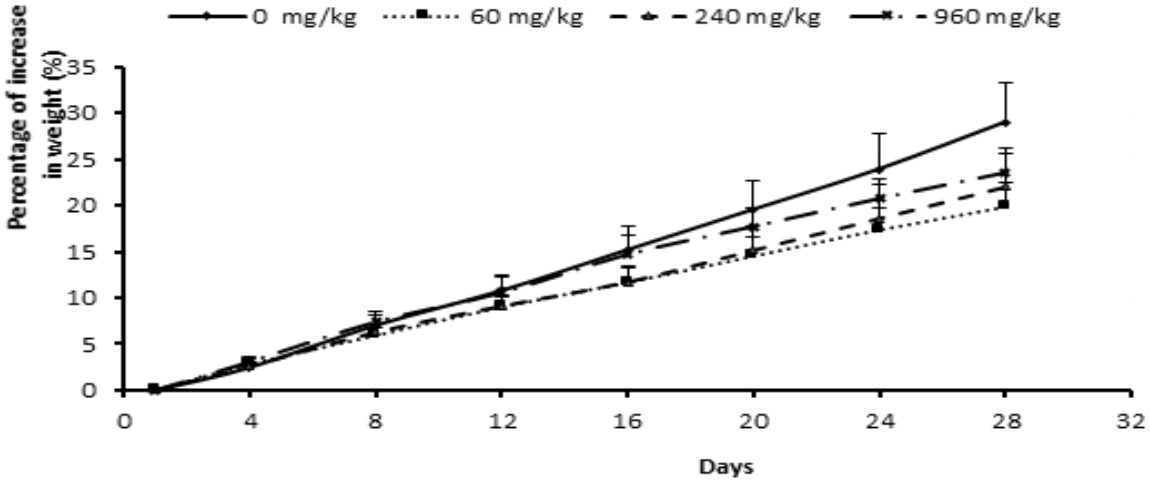

Figure 1: Evolvement of the body weight gain of the animals during the administration period. Each curve represents the mean ± s.e.m. of the values for 5 animals. The values presented are the means of the percentages values of the body weight of each animal relatively to the starting weight.

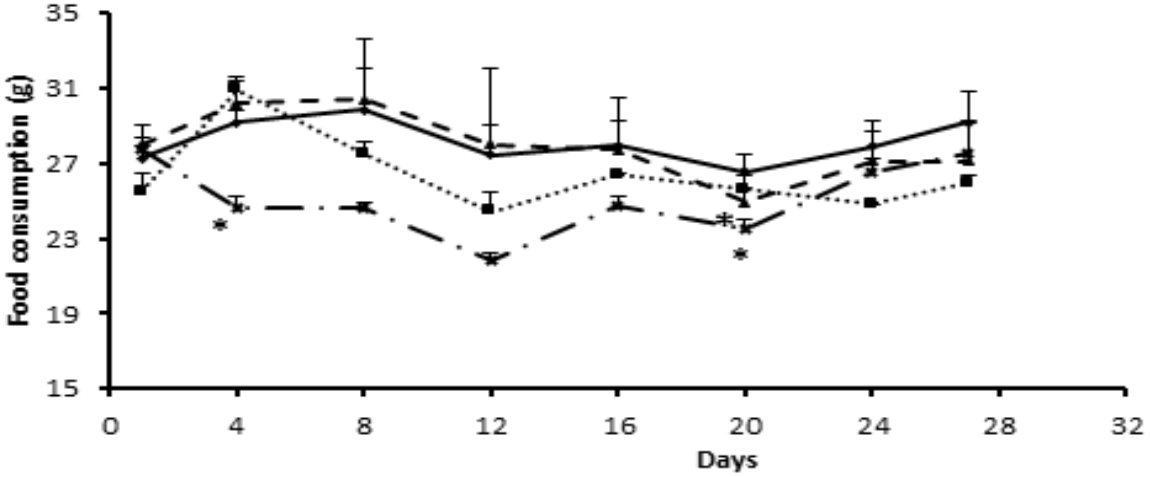

Figure 2: Evolvement of the food consumption of the animals during treatment period. *The dose is significantly different at P 0.05 from the control at the corresponding value. Each curve represents the mean ± s.e.m of the values for 5 animals.

(Germany) kits in accordance with the procedures described by Schumann and Klauke[21]. Urinary proteins were measured by the Bradford method[22] while total serum and tissues (liver and kidney) protein levels were measured by the Biuret method[23]. Ions (Na^+ and K^+) concentrations were measure by Flame photometry. Low Density Lipoprotein (LDL) level and Arterioclerosis Index (AI) were calculated as described respectively by Roeschlau[24] and Ibrahim *et al.*[25] using the following formulas

$$LDL = TC - \frac{TG}{5} - HDL$$

$$\mathrm{IA} = (\mathrm{CT} - \mathrm{HDL})/\mathrm{HDL}$$

Histological Cut

Tissue cross sections were done on liver and kidneys fixed in 10% formol as described by Vanhulle *et al.*[26]. After sacrificing the animals, small pieces of liver were fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissue was embedded in paraffin wax and sectioned into five micrometres thick with the rotary microtome, then stained with hematoxylin and eosin. Then the sections were examined with light microscope and photographed using a microscopic camera.

Statistical analysis

All measured variables were expressed as the Mean ± standard error on the mean (SEM). Statistical analyses were performed with SPSS software. The statistical differences between the values were shown by ANOVA (Analysis of Variance) test. Comparison of means were done using the Fisher LSD test and the significance of the differences was established at the 5% level ($p<0.05$)[27].

RESULTS

Acute toxicity

Overall, the study of acute toxicity revealed no adverse change in the behavior of female rat at 5000 mg/kg as compared to the control and no mortality was registered. On the other hand, there was no significant change in the body weight, as a toxicity indicator and the macrocospic anatomopathological studies did not show any alteration in the analyzed organs (Table 1). Therefore, the LD50 of the extract is over 5000 mg/kg.

Sub-chronic toxicity

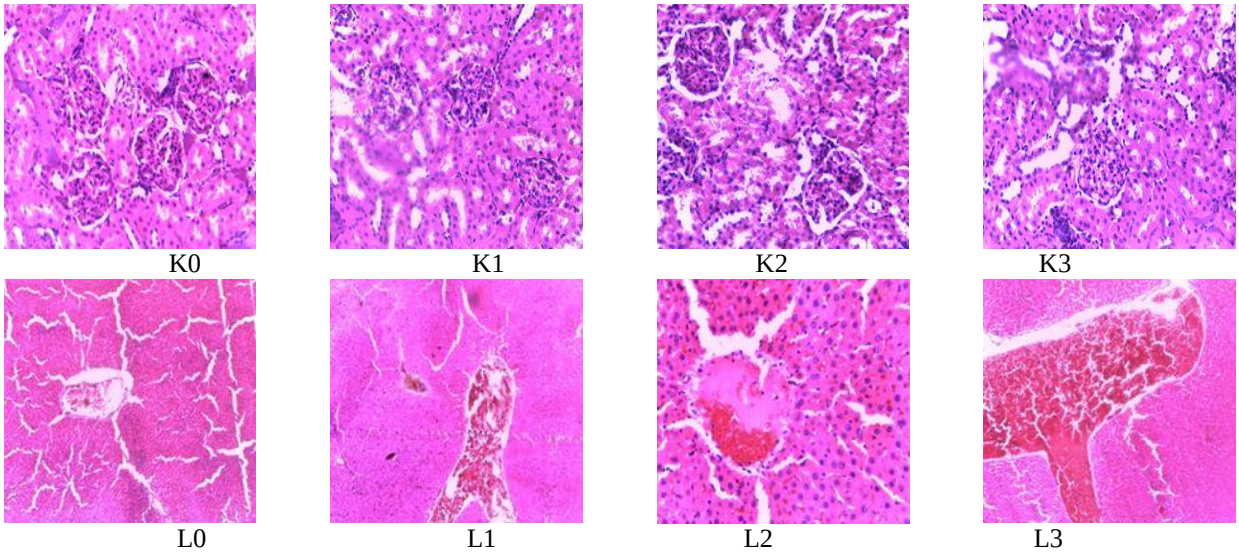

Figure 3: Effect of treatment on liver and kidney histology; K0: kidney of control group; K1: kidney of rat treated at 60 mg/kg; K2: kidney of rat treated at 240mg/kg; K3: kidney of rat treated at 960 mg/kg; L0: liver of control group; L1: liver of rat treated at 60 mg/kg; L2: liver of rat treated at 240mg/kg; L3: liver of rat treated at 960 mg/kg

Table 1: Acute toxicity study of aqueous extract of Sida véronicifolia (AESV) in female rates.

Rat No	Dose received (mg/kg of body weight)	Weight of animals (g)		Signs of Toxicity	Onset of Toxicity	Duration of study
		Before Test	After Test			
1	0	155	168	No sign of Toxicity	Nil	14days
2	0	170	181	No sign of Toxicity	Nil	14days
3	5 000	172	177	No sign of Toxicity	Nil	14days
4	5 000	151	159	No sign of Toxicity	Nil	14days
5	5 000	164	176	No sign of Toxicity	Nil	14days
6	5000	175	191	No sign of Toxicity	Nil	14days

Effect of aqueous extract of S. veronicifolia (AESV) administration on increase of body weight and food consummation.

In the sub-chronic toxicity study, the extract at all doses caused no significant changes in the body weight of the rats (Figure 1) and significant decrease in food intake comparatively to the control on days 4 and 20 of treatment at the higher dose 960 mg/kg (Figure 2).

Effect of aqueous extract of S. veronicifolia administration on hematological parameters, organs relative weights and on histopathology of liver and kidney

Subchronic oral treatments with *S. veronicifolia* at doses of 60, 240 and 960 mg/kg caused no significant changes on the following hematological parameters: red blood cells (RBC), white bood cells (WBC), haemoglobin, hematocrit and MCV. However, an increase in platelets count was observed in all treated groups and it was significant ($P<0.05$) at the dose of 60 mg/kg. Lymphocyte levels significantly dropped ($P<0.05$ and $P<0.01$) in all experimental groups as compared to the control (Table 3). Also, no significant changes were observed in all visceral relative weights excepted in the kidneys of rats treated at the dose 240 mg/kg ($P<0.05$) compared to animals in the control group (Table 2). Morphological analyses of vital organs showed no apparent alterations in treated animals compared to the control group while histology of liver and kidney revealed the presence in the liver of slight periportal fibrosis and vascular congestions at all doses. These vascular congestions were severe with the highest dose (Figure 3).

Effect of aqueous extract of S. veronicifolia administration on biochemical parameters

Subchronic oral administration of different doses of AESV did not lead to a significant effect on ALT, AST, ALP, ratio ALT/ALP, bilirubin, triglycerides, creatinine, index of arteriosclerosis, liver proteins, and on serum levels of K^+ and Na^+ at all doses comparatively to the control group (Table 4). At the doses 240 and 960 mg/kg, there were significant ($p<0.05$) increases in serum and hepatic AST level respectively comparatively to the control group. A significant increase ($p<0.05$) of the urinary urea levels was also noticed in animals treated with AESV at the dose 60 mg/kg as compared to the control group. There were decreases on urinary K^+ levels and on blood urea levels in all treated groups relatively to control with a significance ($p<0.05$) at the dose of 960 mg/kg. Significant ($P<0.05$) decreases were also observed in HDL cholesterol and kidneys protein levels respectively at the doses 240 and 960 mg/kg comparatively to the control (Table 4).

DISCUSSION

Although herbal medicinal products are widely considered to be of lower risk compared with synthetic drugs, they are not completely free from the possibility of toxicity or other

Table 2: Effects of sub-chronic oral administration of the aqueous extract of S. veronicifolia on relative weights of various organs.

	organes	Doses (mg/kg)			
		0	60	240	960
Relative weight (g/100g of BW)	Liver	3.89±0.23	3.86±0.13	3.68±0.30	3.9±0.17
	Kidneys	0.78±0.02	0.75±0.03	0.68±0.04*	0.78±0.03
	Spleen	0.33±0.04	0.35±0.06	0.24±0.06	0.30±0.03
	Heart	0.39±0.01	0.35±0.04	0.36±0.01	0.38±0.01
	Lung	0.76±0.04	0.74±0.05	0.67±0.04	0.70±0.04
	Ovaries $x10^{-1}$	0.46±0.03	0.44±0.9	0.55±0.03	0.52±0.06
	Womb	0.27±0.06	0.28±0.06	0.33±0.05	0.23±0.03

*Values significantly different at ($p<0.05$) from those of the control group (ANOVA and Fisher LSD). Each value represents the mean ± s.e.m of the values for 5 animals; BW, Body weight

adverse effects[28]. Thus, toxicological evaluation of plants diagnosed by certain biochemical markers like ALT, AST,

Table 3: Effects of sub-chronic oral administration of the aqueous extract of S. veronicifolia on hematologic parameters of rats.

Parameters	Doses (mg/kg)			
	0	60	240	960
WBC (x 10^3/µl)	7.26±0.68	6.86±1.03	5.98±1.09	5.92±0.42
RBC (x 10^6/µl)	6.19±1.26	6.31±0.23	5.98±0.43	6.52±0.42
Haemoglobin (g/dl)	14.14±0.93	12.42±1.47	13.30±1.20	14.88±0.87
Hematocrit (%)	32.70±5.67	34.67±1.19	32.30±2.20	34.92±2.64
Platelets (x 10^3/µl)	219.76±52.86	447.00±43.73*	353.60±35.85	354.00±95.86
Lymphocytes (%)	84.54±2.14	65.68±2.62**	67.58±4.51*	71.38±3.16*
Granulocytes (%)	9.62±1.52	26.70±2.23***	24.92±3.87**	22.64±2.34**
MCV (fl)	57.84±6.13	55.20±1.63	54.24±1.42	53.56±1.75

*or ** or ***Values significantly different at ($p<0.05$) or at ($p<0.01$) or at ($p<0.001$) from those of the control group (ANOVA and Fisher LSD). Each value represents the mean ± s.e.m. of the values for 5 animals; RBC, Red blood cells. WBC, white blood cells; MCV, mean corpuscular volume.

derived products, including extracts forms an essential part of scientific validation of medicinal plants[29]. Acute toxicity tests give clue to the range of doses that could be toxic to the animal and the estimation of the therapeutic index of drugs and xenobiotics. Based on DL_{50} which is over 5000mg/kg, and no significant change observed in the animal's body weight, behavior and organs after macrocospic anatomopathological studies, AESV can then be classified as orally nontoxic substances according to the scale of Hodge and Sterner[30] or to many laws regulating the use and trade of drugs. Subchronic toxicity evaluation of substances or plant extracts by repeated daily dosing for a period of 28 to 30 days is a well-established method, and it is a legal requirement in several countries. During the toxicity studies, the body weight changes serve as a sensitive indication of the general health status of animals while the food intake is used as indicator of their metabolism[31]. Then, the weight gains and food intake observed in all animal groups suggest that, at the oral doses administered AESV did not interfere with the general health status and the normal metabolism of animals since that of treated animals was not significantly different from control group. However, the decrease of food intake observed on days 4 and 20 at the dose 960 mg/kg may be due to the anorexic effect of our plant extract, which is sticky. Exposure to natural products can principally induce changes or histological damage in the liver (which is the target organ of metabolism) and /or the kidney (which is the main organ of elimination)[32]. Liver injury can be ALP and bilirubin. Hepatocellular or cytolytic injury involves predominantly a serum aminotransferase levels elevation, usually preceding increases in total bilirubin level and modest increases in alkaline phosphatase level[33,34]. Cholestatic injury is characterized by predominantly initial alkaline phosphatase level elevations that precede or are relatively more prominent than increases in the levels of serum aminotransferases[33,34]. Generally, mixed types of injuries, involving both hepatocellular and cholestatic mechanisms, occurs[35]. Also the ratio ALT/ALP plays an important role in deciding the type of liver damage by hepatotoxins. The ratio is greater than or equal to five during hepatocellular damage while the ratio is less than or equal to two during cholestatic liver damage. During mixed type of liver damage, the ratio ranges between two and five[36]. In our study, except the significant increase in AST level, there was a non-significant change in all other parameters (ALT, ALP, bilirubin, liver weight and proteins level) and the ratio ALT/ALP is less than 1 in all treated groups. All these findings indicate that the extract had no deleterious effect on liver function. However, the liver histology presented slight periportal fibrosis and vascular congestions at all doses but, these histological modifications are of less importance when not linked with modifications in biochemical parameters as in this study; macroscopic and histopathological observations and investigations of additional clinical biochemistry parameters allows the confirmation of hepatotoxicity Creatinine, urea and

Table 4: Effects of sub-chronic oral administration of the aqueous extract of S. veronicifolia on biochemical parameters of rats.

Parameters	Doses (mg/kg)			
	0	60	240	960
Serum				
Protein level (mg/ml)	45.28±2.92	49.38±2.39	48.66±2.62	52.00±2.64
ALT (UI/L)	14.19±2.13	12.56±1.98	15.12±1.50	15.59±1.45
AST (UI/L)	66.89±9.04	86.67±8.67	92.72±5.55*	69.91±7.86
ALP (UI/L)	22.42±3.37	19.85±3.13	23.89±2.38	24.63±3.30
ALT/ALP	0.63	0.63	0.63	0.63
Total Bilirubin (µmol/l)	2.53±0.37	1.83±0.53	2.85±0.50	1.92±0.31
Direct Bilirubin (µmol/l)	1.08±0.21	0.64±0.10	1.11±0.12	1.23±0.27
Indirect Bilirubin (µmol/l)	1.44±0.55	1.19±0.59	1.74±0.51	0.78±0.31
Creatinine (mg/dl)	0.31±0.07	0.46±0.05	0.51±0.09	0.49±0.17
Urea (mg/dl)	54.29±3.64	52.86±1.75	47.14±2.86	46.43±1.60*
Sodium (mg/dl)	49.42±0.24	49.42±0.24	49.42±0.24	49.42±0.24
Potassium (mg/dl)	3.45±0.55	3.45±0.55	3.45±0.55	3.45±0.55
Total cholesterol (mg/dl)	69.85±3.45	67.23±2.90	60.34±9.81	56.21±10.21
HDL (mg/dl)	17.01±1.03	14.46±1.38	11.12±2.20*	14.98±1.62
LDL (mg/dl)	52.84±2.90	52.77±2.11	49.22±11.07	41.23±8.85
Triglycerides (mg/dl)	76.55±4.81	74.16±11.82	103.90±14.08	79.70±7.32
AI	3.14±0.20	3.77±0.34	5.54±1.73	2.65±0.38
Urine				
Protein level (mg/ml)	0.32±0.03	0.38±0.02	0.50±0.07**	0.30±0.03
Creatinine (mg/dl)	12.26±0.75	15.57±1.17	16.06±2.50	11.03±3.04
Urea (g/l)	69.74±3.48	101.00±12.91*	86.57±8.22	68.54±8.07
Sodium (mg/dl)	13.17±0.65	13.17±1.13	14.63±0,00	11.71±0.93
Potassium (mg/dl)	84.30±4.23	78.81±8.26	87.97±2.59	59.56±1.36*
Liver				
Protein levels (mg/g)	80.96±3.48	83.55±10.60	98.66±12.46	88.25±16.66
ALT (UI/L)	1.86±0.34	2.56±0.35	2.33±0.32	2.09±0.51
AST (UI/L)	35,34±2,64	46,53±14,73	38,19±6,91	86,67±9,64*
Kidneys				
Protein level (mg/g)	8.90±0.60	8.96±0.66	7.82±0.79	6.85±0.33*

*or ** Values significantly different at ($p<0.05$) or at ($p<0.01$) from those of the control group (ANOVA and Fisher LSD). Each value represents the mean ± s.e.m. of the values for 5 animals; AI, Arteriosclerosis index

electrolytes are common parameters often measured to assess the state of the kidneys[37]. Administration of different doses of AESV did not lead to a significant effect on creatinine levels when compared to the control. The creatinine is known as an effective indicator of renal function and especially of the glomerular filtration rate. Kidney malfunction causes a rise above the normal threshold of serum creatinine and decreased urinary levels[38]. Thus, this results recorded suggest that AESV did not affect the renal function. Also the K^+ and Na^+ serum levels were not affected by the administration of different doses of AESV while they were decreases on urinary K^+ levels and on blood urea levels in all treated rats. Moreover, an increase of the urinary urea levels in animals treated with AESV at the 60 mg/kg doses was noticed and no adverse morphological and histological effects were observed on the kidneys of the experimental rats. These observations proved that extract has no deleterious effect on the kidneys but may improve their function. In effect, dysfunction of the Kidney leads to the increases in serum levels of urea and electrolytes and decreases in their urinary levels[37]. Measurement of hematologic parameters can be used to determine the extent of the deleterious effect of foreign substances including plant extracts on the constituents of the blood[39]. The various hematological parameters studied namely the Middle corpuscular volume (MCV), hemoglobin (HBS) and hematocrit (HCT) levels, related to the total population of red blood cells are indicators for evaluating the toxic potential of medicinal plant extracts in biological systems[40]. These tests are relevant to the assessment of the toxicity of a substance, since changes in the haematological system of an animal have greater predictive value for human toxicity[41]. So the non-significant effect of the administration of different doses of AESV on red blood cells (RBC) could mean that the balance between the rate of production (erythropoiesis) and destruction of blood cells has not been adversely affected. Haemoglobin, hematocrit and MCV did not significantly change during this study, showing that neither the formation of RBC's hemoglobin nor their morphology and osmotic fragility has been changed[42]. However, the significant ($P<0.05$ and $P<0.01$) decrease in lymphocytes (the main effector cells of the immune system) level observed may suggest inhibition of the immune system[43]. Fortunately, that decrease was compensated by the increase ($P<0.05$, $P<0.01$ and $P<0.001$) in granulocytes

levels with all AESV treated groups as compared to the control. In addition, no significant change was observed in White Blood Cells (WBC); it suggests that AESV has little effect on the immunological parameters of the treated rats. The quantification of the main carriers of individual lipoprotein cholesterol (Total cholesterol.) or Low Density Lipoproteins (LDL) and High Density Lipoprotein (HDL), provides a better assessment of the overall risk of developing cardiovascular disease[44] (Nakamura *et al*., 2006). The oxidation of LDL-C is a gradual process leading to oxidized LDL formation thus playing an important role in initiating the formation of atheriosclerotic plaques[44,45]. In this study, there were a decrease in the total cholesterol, HDL and LDL of all treated animals with significance on HDL level at the dose of 240 mg/kg compared to the control. This would be due to the fact that this extract has no effect on lipid metabolism, since the index of arteriosclerosis was not affected. It therefore presents no risk of cardiovascular disease. According to Schaffer and Menche[38], excess "bad" cholesterol (LDL) and the lack of "good" cholesterol (HDL) are major risk factors for cardiovascular disease. This hypothesis is supported by the trigycerides level which did not significantly change in the experimental groups as compared to the control. Indeed, higher levels of triglycerides measured in a fasting specimen indicate a lack of clearance or over-production; it could increase the risk of developing cardiovascular disease[46]. This suggests that AESV has no effect on the cholesterol metabolism in the rat and consequently its consumption leads to no risk of developing cardiovascular disease.

CONCLUSION

Based on the results obtained in this work it becomes clear that the aqueous extract of *S. veronicifolia* is nontoxic when administered at doses lesser than or equal to 960 mg/kg. The present research provided firsthand information on acute and sub chronic toxicity of *S. veronicifolia*.

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