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

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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 analyzed 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 people¹. 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 needs²,³. 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 advantageous⁴. Many studies have reported the toxic effects of herbal medicines⁵-⁷. S. veronicifolia Lam is a plant of Malvaceae family which presents long unicellular acicular trichomes and small 5-armed stellate hairs⁸. It is used in folkloric medicine as abortifacient, pain-killer, ecobic, land conservator, analgesic during labour, shorter of postpartum bleeding as well as in the treatment of pregnancy ailments, dysmenorrhea, male infertility (poor erection), gonorrhea, malnutrition and debility⁹-¹¹. 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. veronicifolia¹² and the presence of antioxidant active compound in S. veronicifolia extract¹³. Although many belonging Sida plants sp, especially S. Acuta; S. rhombifolia¹², S. cordifolia¹⁵,¹⁶ and S.

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veronicifolia\textsuperscript{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\textsuperscript{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, alkaline phosphatase, total and direct bilirubin, total cholesterol, HDL-cholesterol, triglyceride, creatinine, and urea were obtained from commercial firm.

**Acute toxicity study**

The doses adjustment method of OCDE\textsuperscript{12} for acute toxicity studies was used for the estimation of the lethal dose (LD\textsubscript{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\textsuperscript{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\textsuperscript{19,20}. After the first day following the treatment, animals were supplied with food and water \textit{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) \textit{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...
(Germany) kits in accordance with the procedures described by Schumann and Klauke. Urinary proteins were measured by the Bradford method while total serum and tissues (liver and kidney) protein levels were measured by the Biuret method. Ions (Na$^+$ and K$^+$) concentrations were measured by Flame photometry. Low Density Lipoprotein (LDL) level and Arteriosclerosis Index (AI) were calculated as described respectively by Roeschla and Ibrahim using the following formulas:

$$\text{LDL} = \frac{T_C - T_G}{5} - \text{HDL}$$

$$\text{IA} = \frac{(C_T - \text{HDL})}{\text{HDL}}$$

Histological Cut

Tissue cross sections were done on liver and kidneys fixed in 10% formalin as described by Vanhulle et al. 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).

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

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 blood 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
Validation of medicinal plants

in alkaline phosphatase

The effect of our plant extract, which is

sticky, behavior and organs after

36 hours of serum aminotransferases

s indicate of their

changes or histological damage in the liver (which is the

main organ of elimination) and/or the kidney (which is

the target organ of metabolism) and/or to many laws regulating

subchronic toxicity 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. veronicaefolia on hematologic parameters of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Doses (mg/kg)</th>
<th>0</th>
<th>60</th>
<th>240</th>
<th>960</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x 10^3/µl)</td>
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<tr>
<td></td>
<td>7.26±0.68</td>
<td>6.86±1.03</td>
<td>5.98±1.09</td>
<td>5.92±0.42</td>
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<tr>
<td>RBC (x 10^6/µl)</td>
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<tr>
<td></td>
<td>6.19±1.26</td>
<td>6.31±0.23</td>
<td>5.98±0.43</td>
<td>6.52±0.42</td>
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<tr>
<td>Haemoglobin (g/dl)</td>
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<tr>
<td></td>
<td>14.14±0.93</td>
<td>12.42±1.47</td>
<td>13.30±1.20</td>
<td>14.88±0.87</td>
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<tr>
<td>Hematocrit (%)</td>
<td></td>
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<tr>
<td></td>
<td>32.70±5.67</td>
<td>34.67±1.19</td>
<td>32.30±2.20</td>
<td>34.92±2.64</td>
<td></td>
</tr>
<tr>
<td>Platelets (x 10^3/µl)</td>
<td>219.76±52.86</td>
<td>447.00±43.73*</td>
<td>353.60±35.85</td>
<td>354.00±45.86</td>
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</tr>
<tr>
<td>Lymphocytes (%)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>84.54±2.14</td>
<td>65.68±2.62**</td>
<td>67.58±4.51*</td>
<td>71.38±3.16*</td>
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<tr>
<td>Granulocytes (%)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>9.62±1.52</td>
<td>26.70±2.23***</td>
<td>24.92±3.87**</td>
<td>22.64±2.34**</td>
<td></td>
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<tr>
<td>MCV (fl)</td>
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</tr>
<tr>
<td></td>
<td>57.84±6.13</td>
<td>55.20±1.63</td>
<td>54.24±1.42</td>
<td>53.56±1.75</td>
<td></td>
</tr>
</tbody>
</table>

*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; WBC, Red blood cells; RBC, white blood cells; MCV, mean corpuscular volume.

derived products, including extracts forms an essential part
of scientific validation of medicinal plants. 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
macrosopic anatopathological studies, AESV can then
be classified as orally nontoxic substances according to
the scale of Hodge and Sterner 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.
Then, the weight gains and food intake observed in all animals 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). Liver injury can be

ALP and bilirubin. Hepatocellular or cytolytic injury
involves predominantly a serum aminotransferase levels
elution, usually preceding increases in total bilirubin
level and modest increases in alkaline phosphatase
level and modest increase in alanine transaminase
level (ALT) and modest increase in aspartate transaminase
level. 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. Generally, mixed types of injuries, involving both
hepatocellular and cholestatic mechanisms, occur.
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. 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 Creatininc, urea and
Kidney malfunction causes a rise above the normal threshold of serum creatinine and decreased urinary levels. Thus, this results recorded suggest that AESV did not affect the renal function since changes in the haematological system of an animal are relevant to the assessment of the toxicity of a substance, especially in foreign substances including plant extracts on biological systems. These tests are indicative of human toxicity. 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 constituents of the blood. Kidney malfunction causes a rise above the normal threshold of serum creatinine and decreased urinary levels. Thus, this results recorded suggest that AESV did not affect the renal function since changes in the haematological system of an animal are relevant to the assessment of the toxicity of a substance, especially in foreign substances including plant extracts on biological systems. These tests are indicative of human toxicity. 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 constituents of the blood.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0</th>
<th>60</th>
<th>240</th>
<th>960</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein level (mg/ml)</td>
<td>45.28±2.92</td>
<td>49.38±2.39</td>
<td>48.66±2.62</td>
<td>52.00±2.64</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>14.19±2.13</td>
<td>12.56±1.98</td>
<td>15.12±1.50</td>
<td>15.59±1.45</td>
</tr>
<tr>
<td>AST (UI/L)</td>
<td>66.89±9.04</td>
<td>86.67±8.67</td>
<td>92.72±5.55*</td>
<td>69.91±7.86</td>
</tr>
<tr>
<td>ALP (UI/L)</td>
<td>22.42±3.37</td>
<td>19.85±3.13</td>
<td>23.89±2.38</td>
<td>24.63±3.30</td>
</tr>
<tr>
<td>ALT/ALP</td>
<td>0.63</td>
<td>0.6</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Total Bilirubin (µmol/l)</td>
<td>2.53±0.37</td>
<td>1.83±0.53</td>
<td>2.85±0.50</td>
<td>1.92±0.31</td>
</tr>
<tr>
<td>Direct Bilirubin (µmol/l)</td>
<td>1.08±0.21</td>
<td>0.64±0.10</td>
<td>1.11±0.12</td>
<td>1.23±0.27</td>
</tr>
<tr>
<td>Indirect Bilirubin (µmol/l)</td>
<td>1.44±0.55</td>
<td>1.19±0.59</td>
<td>1.74±0.51</td>
<td>0.78±0.31</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.31±0.07</td>
<td>0.46±0.05</td>
<td>0.51±0.09</td>
<td>0.49±0.17</td>
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<tr>
<td>Urea (mg/dl)</td>
<td>54.29±3.64</td>
<td>52.86±1.75</td>
<td>47.14±2.86</td>
<td>46.43±1.60*</td>
</tr>
<tr>
<td>Sodium (mg/dl)</td>
<td>49.42±0.24</td>
<td>49.42±0.24</td>
<td>49.42±0.24</td>
<td>49.42±0.24</td>
</tr>
<tr>
<td>Potassium (mg/dl)</td>
<td>3.45±0.55</td>
<td>3.45±0.55</td>
<td>3.45±0.55</td>
<td>3.45±0.55</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>69.85±3.45</td>
<td>67.23±2.90</td>
<td>60.34±9.81</td>
<td>56.21±10.21</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>17.01±1.03</td>
<td>14.46±1.38</td>
<td>11.12±2.20*</td>
<td>14.98±1.62</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>52.84±2.90</td>
<td>52.77±2.11</td>
<td>49.22±11.07</td>
<td>41.23±8.85</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>76.55±4.81</td>
<td>74.16±11.82</td>
<td>103.90±14.08</td>
<td>79.70±7.32</td>
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<tr>
<td>AI</td>
<td>3.14±0.20</td>
<td>3.77±0.34</td>
<td>5.54±1.73</td>
<td>2.65±0.38</td>
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<tr>
<td>Urose</td>
<td></td>
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<tr>
<td>Protein level (mg/ml)</td>
<td>0.32±0.03</td>
<td>0.38±0.02</td>
<td>0.50±0.07**</td>
<td>0.30±0.03</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>12.26±0.75</td>
<td>15.57±1.17</td>
<td>16.06±2.50</td>
<td>11.03±3.04</td>
</tr>
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<td>Urea (g/l)</td>
<td>69.74±3.48</td>
<td>101.00±12.91*</td>
<td>86.57±8.22</td>
<td>68.54±8.07</td>
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<td>Sodium (mg/dl)</td>
<td>13.17±0.65</td>
<td>13.17±1.13</td>
<td>14.63±0.00</td>
<td>11.71±0.93</td>
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<td>Potassium (mg/dl)</td>
<td>84.30±4.23</td>
<td>78.81±8.26</td>
<td>87.97±2.59</td>
<td>59.56±1.36*</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
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<tr>
<td>Protein levels (mg/g)</td>
<td>80.96±3.48</td>
<td>83.55±10.60</td>
<td>98.66±12.46</td>
<td>88.25±16.66</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>1.86±0.34</td>
<td>2.56±0.35</td>
<td>2.33±0.32</td>
<td>2.09±0.51</td>
</tr>
<tr>
<td>AST (UI/L)</td>
<td>35.34±2.64</td>
<td>46.53±14.73</td>
<td>38.19±6.91</td>
<td>86.7±9.64*</td>
</tr>
<tr>
<td>Kidneys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein level (mg/g)</td>
<td>8.90±0.60</td>
<td>8.96±0.66</td>
<td>7.82±0.79</td>
<td>6.85±0.33*</td>
</tr>
</tbody>
</table>

*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.
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\(^4\) (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\(^35\), excess “bad” cholesterol (LDL) and the lack of “good” cholesterol (HDL) are major risk factors for cardiovascular disease. This hypothesis is supported by the triglycerides 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 lower than or equal to 960 mg/kg. The present research provided fisthand information on acute and sub chronic toxicity of *S. veronicifolia*.

**AKNOLEDGEMENTS**

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**REFERENCES**


