Toxicological evaluation of methanol extract of Aloe vera in rats

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ABSTRACT
The toxicity profile of the methanol extract of the Aloe vera (Aloe barbadensis) gel was studied in Wistar rats. A multiple oral administration of the extract at single dose of 4, 8, 16g/kg body weights for 14 days did not produce signs of toxicity, behavioral appearances, changes on gross appearance. The sub-acute toxicity was determined by administration of graded doses (1, 2, 4, 8 and 16g/kg b.wt orally) of the extract daily for 6 weeks and the effects on body weight, organ weight, histology as well as serum biochemical parameters were estimated. Body weight of dosed and control rats increased throughout the duration of treatment. Our data demonstrated significantly no difference in serum concentrations of aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total protein, albumin, urea, creatinine, total and direct bilirubin. Histological findings on liver, kidney, small intestine, heart and brain revealed normal architecture and no obvious pathology was noted. It may be concluded that the methanol extract of Aloe vera do not produce significant toxic effect in rats during acute and sub-acute treatment in rats. Hence, the extract can be utilized for nutraceuticals formulations.

Keywords: Aloe vera, amino transferase, alkaline phosphatase. Sub-acute

Introduction
The use of plants for healing purposes is getting increasingly popular as they are believed as being beneficial and free of side effect [1]. Aloe vera (Aloe barabdensis Mill) belonging to the family of: Liliaceae is a perennial herb with short stem, stout and thick. Leaves are sessile, crowded, lanceolate, pale green, fleshy, margins and spiny. The fresh gel or mucilage from Aloe vera is a handy home grown remedy that can be used both as a moisturizing agent and for the treatment of minor burns, skin abrasions and irritation [2-5].

Aloe vera gel has been used medicinally for several thousands of years with a long and illustrious history. The gel of Aloe vera contains about 99 to 99.5% water with pH in the range of 4.4 to 4.7. The major components in the gel are glucomannans, acemannan, minerals, flavonoids, tannic acid, alprogen, C-glucosyl chrmone etc. The solid material contains about 45 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinine or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid [6]. The organic extract of Aloe vera leaves provided anti-inflammatory activity in the experimental rats probably because of alprogen, an anti-allergic glycol protein and C-glycosyl chromone a novel anti-inflammatory compound [7]. Glucomannan and acemannan present in the plant can accelerate wound healing, activate macrophages and demonstrate antineoplastic, anti-inflammatory and antiviral effects [8-11]. The potency of the gel with respect to its capability in reducing chemically induced toxicity is also reported [12]. As this plant has potential health benefits, it can be better utilized for nutraceutical and functional food formulations. Hence a study has been conducted to evaluate methanol extract of the gel for its toxicity up to the level of 16g/kg body wt.

Materials and Methods
Plant material and extraction
Specimens of Aloe vera (L) Burn.fili were collected from Gokulum garden, identified by Prof.H.S. Prakash, Professor, Dept of Applied Botany, University of Mysore, Mysore, Karnataka, India and cultivated in the premises of our institution. In this study the fresh leaves of this cultivated plant were used. The gel of the Aloe vera leaves was scraped with sterilized knife. The gel is blended in an electric blender. The blended sample was (300g) was extracted with solvent methanol at room temperature. The extraction process was repeated till the solvents become colorless. The extracts were then filtered using Whatmann No.1 paper. The filtrate were concentrated in vacuum at 50°C ± 1°C in a rotary evaporator to obtain the crude extracts. The percentage yield of the extract was 15.78gms. The extract was dissolved in a known volume of water to feed rats.

Experimental design
Adult male Albino rats (wistar strains) weighing (100-150g) were used in the study. All the animals were maintained in a controlled environment condition of temperature (24 ± 1º C) an alternative 12h light/ dark
cycles. The animals were fed with prepared diet and water *ad libitum*. The experiment protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

**Acute toxicity study**

The acute oral toxicity was evaluated following the World Health Organization (WHO) guideline \(^\text{13}\) and the Organization of Economic Co-operation and Development (OECD) guideline for chemical testing \(^\text{14}\). Rats were divided into four groups. The treated group was orally given the methanol extract with a single dose of 8, 16, 20g/kg b.wt. while the control group received only water vehicle. The animals were monitored for apparent signs of toxicity for 14 days.

**Sub acute toxicity**

The method was performed following the WHO guideline \(^\text{13}\) and the OECD guideline (15). Rats were divided into six groups. The treated group was orally given the extract at a dose of 1(dose 1), 2(dose 2), 4(dose 3), 8(dose 4), and 16(dose 5) g/kg body weight daily for 42 days, while the control group received the vehicle at the same volume. All the rats were observed for apparent signs of toxicity or behavioral alterations during the experimental period.

**Liver and kidney functional tests**

Weekly blood was drawn from the orbital plexuses; blood samples were taken in clean sterile tubes and left till clotting occurred. Serum was collected after centrifugation at 3000rpm for 15 minutes. Serum was kept at -20°C until used. Chemical analysis carried out on serum to assay the state of liver and kidney. This includes blood urea \(^\text{[16]}\), creatinine \(^\text{[17]}\), total protein \(^\text{[18]}\), albumin \(^\text{[19]}\), total bilirubin \(^\text{[20]}\), direct bilirubin \(^\text{[21]}\), alkaline phosphatase (ALP) \(^\text{[22]}\), aspartate amino transferase (APT) \(^\text{[23]}\) and alanine amino transferase (ALT) \(^\text{[24]}\) measured. Weekly body weights were also noted. At the end of the experiment, all the rats were fasted for 12hrs, sacrificed under anesthetic condition.

**Histopathological studies**

All the sacrificed rats were necropsied. Specimens viz.liver, kidney, small intestine, heart were collected from different organs and fixed in 10% neutral buffer formalin. Paraffin sections (6-8microns) were prepared and stained with Harris Haematoxylin and eosin \(^\text{[25]}\) for microscopic examination.

**STATISTICAL ANALYSIS**

Results were expressed as mean ± standard deviation (S.D). Statistical significance determined by one-way analysis of variance (ANOVA) (Graph Pad Prism version 3 software). The data obtained from acute toxicity studies was analyzed for (P values less than 0.05) were considered significant.

**Results and Discussion**

**Acute toxicity**

The present study reports the acute and sub-acute toxicity studies of *Aloe vera* up to the maximum dose of 16g/kg body wt. of rats. The rats were orally given a multiple dose of the methanol extract from the gel of *Aloe vera* at 4, 8, 16g/kg neither the signs of toxicity nor death of rats were observed during the 14 days of the acute toxicity study. It is known that the alterations of body weight gain and organ weights of rats would reflect the toxicity of the substance \(^\text{26}\). The significant difference in organ weights between treated and untreated (control) animals may also occur in the absence of any morphological changes \(^\text{27}\). In the study, the body weights were recorded as shown in (Figure 1) and no significant difference was noticed as compared to control group.

**Sub-acute toxicity**

The sub-acute toxicity study of methanol extract of *Aloe vera* with the above doses did not reveal any toxicity symptoms as revealed in body weights (Figure 2) and organ weights of rats (Figure 3). The body weights of experimental and control rats were increased through out the duration of oral feeding. There was also no change in organ weights during the oral feeding.

In this study, serum enzymes viz. APT, ALP and ALT in treated groups of rats did not show any change in comparison to the control groups (Table 1). Liver cell damage is characterized by a rise in serum enzymes viz. APT, ALP, ALT etc. \(^\text{[28]}\). Generally, SGPT concentrations are consistently higher than SGOT levels and this is expected as body cells generate more SGPT than SGOT \(^\text{[29]}\). Usually, about 80% of SGPT is found in mitochondria whereas SGOT is a purely cytosolic enzyme. Therefore, SGPT is found in mitochondria whereas SGOT is a purely cytosolic enzyme. Therefore, SGPT appears in higher concentrations in a number of tissues (liver, kidneys, heart and pancreas) and is released slowly in comparison to SGOT. But since SGOT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than SGPT and within its limits can provide a quantitative assessment of the degree of damage sustained by the liver \(^\text{[30]}\). Since SGOT and
SGPT activities are not showing any significant difference with the treatment of extracts, it does not indicate damage to liver cells.

The liver histology revealed evidence of normal hepatocytes (Table 2). The markers of renal functions viz. urea and creatinine levels were not changed in the group of rats treated with *Aloe vera* extracts. This view strengthened by the fact that the relative weight of the kidneys did not show any evidence of toxicity (Figure 3) and also histologically determines normal glomeruli and tubules. Also, total protein, albumin, globulin and bilirubin (total and direct) level of treated rats of all dose groups shows no changes in comparison to control group rats (Table 1). The relative body weight gain determines any toxicity of all doses administered in rats (Figure 2).

**Conclusions**

Acute and sub-acute toxicity study of *Aloe vera* in Wistar rats indicated that the methanol extract at the doses of 1, 2, 4, 8 16g/kg b.wt. do not produce significant dose related changes of biochemical parameters or histopathology of internal organs.

**REFERENCES**


[6] Peng, S.Y., Horman, J., Curtin, G., Corrier, D., McDaniel, R., Busbee, D., Acemannan., Zhang, L., Tizard, I.R., *Acute and sub-acute toxicity study of* *Aloe vera* *in Wistar rats indicated that the methanol extract at the doses of 1, 2, 4, 8 16g/kg b.wt. do not produce significant dose related changes of biochemical parameters or histopathology of internal organs.

**REFERENCES**


Figure 1: Body weight of rats in the acute toxicity study of the methanol extract from the Aloe vera gel. Values are expressed as Mean ± SD (n=6).

Figure 2: Body weight of rats in the sub acute toxicity study of the methanol extract from the Aloe vera gel. Values are expressed as Mean ± SD.
Figure 3: Effect of methanol extract of *Aloe vera* gel on the relative organ weights

Values are expressed as mean ± SD

Table 1. Histological observation on Wistar rats given methanol extract of *Aloe vera* of six weeks

<table>
<thead>
<tr>
<th>Treatment dose (g/kg)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Small intestine</th>
<th>Brain</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>normal histology</td>
<td>normal histology</td>
<td>normal histology</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 1</td>
<td>normal hepatocytes</td>
<td>normal</td>
<td>normal villi with lining columnar epithelium, mucosal glands</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>se 2</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 3</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 4</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 5</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
</tbody>
</table>
### Table 2. Blood parameters of rats fed with the methanol extract from *Aloe vera* gel

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>dose 1</th>
<th>dose 2</th>
<th>dose 3</th>
<th>dose 4</th>
<th>dose 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>3.60±0.06</td>
<td>3.75±0.03</td>
<td>3.67±0.10</td>
<td>3.70±0.07</td>
<td>3.74±0.06</td>
<td>3.68±0.06</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.50±0.03</td>
<td>6.45±0.05</td>
<td>6.44±0.11</td>
<td>6.59±0.20</td>
<td>6.74±0.07</td>
<td>6.69±0.19</td>
</tr>
<tr>
<td>APT (U/I)</td>
<td>14015±2.17</td>
<td>139.6±12.97</td>
<td>140.29±1.83</td>
<td>139.76±2.21</td>
<td>141.00±1.06</td>
<td>140.58±1.71</td>
</tr>
<tr>
<td>ALT (U/I)</td>
<td>34.52±0.15</td>
<td>34.58±0.33</td>
<td>34.05±0.27</td>
<td>34.21±0.41</td>
<td>33.85±0.51</td>
<td>33.9±0.39</td>
</tr>
<tr>
<td>ALP (U/I)</td>
<td>72.64±11.07</td>
<td>179.9±6.18</td>
<td>180.93±4.26</td>
<td>181.91±4.33</td>
<td>177.99±5.49</td>
<td>179.58±6.61</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.13±0.00</td>
<td>0.13±0.00</td>
<td>0.13±0.00</td>
<td>0.13±0.06</td>
<td>0.13±0.00</td>
<td>0.14±0.00</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.34±0.00</td>
<td>0.34±0.02</td>
<td>0.36±0.007</td>
<td>0.36±0.01</td>
<td>0.35±0.01</td>
<td>0.35±0.01</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD (n=6) (p<0.05)
Figure 4  Histopathological observations of rat organs

a) Section of kidney - control rat

b) Section of kidney - rat treated with *Aloe vera* gel extract (16g/kg b.wt)

c) Section of liver - control rat

d) Section of liver - rat treated with *Aloe vera* gel extract (16g/kg b.wt)

e) Section of small intestine - control rat

f) Section of small intestine - rat treated with *Aloe vera* gel extract (16g/kg b.wt)
g) Section of brain - control rat

h) Section of brain - rat treated with *Aloe vera* gel extract (16g/kg b.wt)

i) Section of heart - control rat

j) Section of heart - rat treated with *Aloe vera* gel extract (16g/kg b.wt)