Safety Assessment of *Centella asiatica* in albino rats

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**ABSTRACT**

Aim: Present study was designed to assess the safety levels of *Centella asiatica* (aerial pars) after 30 days oral administration in albino rats. **Materials & Methods:** Control group (I) received distilled water and test groups (II, III and IV) received graded dosage of 250, 500, 1,000 mg/kg b.wt orally respectively for a period of 30 days. Changes in body weight were recorded at 10 days interval. On 32nd day blood samples were collected and the whole blood was used for hematological studies, DNA fragmentation assay and marker enzymes levels were assessed in serum. The vital tissues (Liver, Kidney, Heart, Spleen, and Brain) were dissected out and utilized for viability assay (Trypan blue dye exclusion test), evaluation of apoptosis (fluorescence microscopy) and histopathological studies. **Results:** Group III and IV animals showed a significant increase in serum biomarkers (AST, ALT, BUN, Creatinine) and apoptotic index. There was statistically significant decrease in viability count in treatment groups in comparison to the control group. Histopathology also revealed a significant hepatic damage and a moderate degree of changes in the renal tissue. **Conclusion:** Based on the above observations it was concluded that the administration of *Centella asiatica* @1,000 mg/kg b.wt for a period of 30 days may cause a significant damage to liver tissue in rats. **Significance & Impact of the study:** This study signifies the organ specific toxicity of *Centella asiatica*.

**Key words:** Mandookaparni, Toxicity, Apoptosis, Herbal Medicine

**INTRODUCTION**

India is very rich in natural resources and the knowledge of traditional medicine and the use of plants as a source of medicine is innate and very important component of the health care system. The Indian system of medicine has identified 1,500 medicinal plants of which 500 are commonly used. According to a recent estimate of WHO, 70-80% of the world population especially in developing countries relies on traditional medicine mostly plant drugs for their primary health care needs.[1]

India is the fourth largest producer of pharmaceuticals in the world and an important consumer of medicines too.

Each day, new drugs are introduced and prescribed. The safety of drugs is of paramount importance to patients and health care professionals. The pharmaceutical industry has been an ethical and legal responsibility to ensure that the products they sell will not harm the patients they are intended for.

Patients taking prescription drugs and therapeutic herbs may be at risk for adverse drug-herb interaction, including that alters bio-availability and efficacy of prescription drugs. Drug interaction and adverse effects from herbal medicine are more likely to occur among patients who have chronic medical conditions, such as liver, heart, or kidney disease. Older patients have more comorbid illness and may be more susceptible to complications caused by herbal medicine. Several recent publications report on renal failure caused by Chinese herbs. The use of most herbal medicine is not evidence based and the risk clearly outweighs the benefit.

*Centella asiatica* (Sanskrit: - Mandooka parni, English: - Indian penny wort) is a medicinal plant with long history of therapeutic use in Indian system of medicine. It is a...
creeping plant which has its origin in tropical and sub tropical climates. In south Asian countries such as India and Indonesia it has long history of therapeutic use, healing wounds and slowing the progression of leprosy. Furthermore, it is considered to prolong life and increase energy.

No information is available in regard to toxicological consequences of *Centella asiatica* with particular reference to their effects on apoptosis and marker enzyme levels in rats. In view of the dramatic and tremendous increase in the use of herbal drugs and in particular ever increasing exposure of humans to the *Centella asiatica* has become a reality. Despite these facts, the toxicological effects of *Centella asiatica* have not been investigated.

Therefore, the present study is designed in such a way to assess the toxic effects of *Centella asiatica* after short term oral administration in rats. The outcome of the study will provide necessary and useful information regarding the usage of *Centella asiatica* and there by improving its acceptability and safety in the world of alternative medicine.

**MATERIALS AND METHODS**

**Collection of Plant**
*Centella asiatica* was procured from the herbal garden maintained by the Department of Pharmacology and Toxicology, R.V.C, Ranchi, authenticated with botanist. Shade dried, powdered, muslinised, aerial parts were used along with gum acacia (binding agent) for oral administration in rats.

**Experimental design**
A total of 24 Wistar albino rats of either sex were randomly grouped in to 4 groups of 6 in each group. All the animals were allowed for acclimatization period of seven days before study. A set of two rats were housed in a propylene cage with 12 h: 12 h dark-light cycle, with feed and water ad libitum. The experimental protocol was approved by The Institutional Animal Ethical Committee (IAEC).

Rats in group I were fed with normal diet and kept as controls. Groups II, III and IV were fed with *Centella asiatica* @ the doses of 250, 500, and 1,000 mg/kg b.wt. respectively for 30 days. Average body weight gain was measured at 10 days interval. All the animals were examined for clinical signs, gross behavioural changes, morbidity, and mortality once daily throughout the experimental period.

**Sample collection**
Blood samples were collected by heart puncture in to EDTA as well as plain vials at the end of the study. Various organs like spleen, heart, brain, liver, and kidney were collected and weighed and further samples were stored for further histopathological analysis.

**Reagents and Chemicals**
All the reagents and chemicals used in the experiment were procured from Sigma Chemicals Co.St. Louis, USA, E.Merck (India), SISCO Laboratories and, Erba Transasia bio-chemicals Ltd (assay kits for enzyme estimation).

**Hematology**
Total leucocytes count, RBC, Platelets, Hemoglobin, MCV & MCHC were analyzed on “Sysmex® KX-21 automated hematology analyzer”.

**Biochemical Estimation**
Aspartate transaminase (AST), Alanine transaminase (ALT), Blood Urea Nitrogen (BUN), Creatinine level in the blood was measured (by kits from ERBA chemicals) on auto analyzer.

**Apoptosis Related Parameters**
**Ex Vivo Viability Count:** Pieces of liver, kidney and heart were collected from the rats of different groups and tissues were further chopped and washed in chilled normal saline (0.9% NaCl solution). Then cells were disaggregated by gently rubbing and pressing the chopped tissue through a wire mesh (200-300gauge). The cell suspension was adjusted to contain 1,00,000-1,20,000 cells per ml and were adjusted to viability count by following two methods.

**Trypan Blue Dye Exclusion Test (TBDET)**
One drop each of hepatocyte, kidney and heart cells suspension (containing approximately 10000-12000 cells) were separately mixed with three drops each of trypan blue solution (0.2%). The unstained viable cells were distinguished from the blue stained dead cells particularly due to damage of cell membrane and increased cellular permeability. The unstained viable cells were counted under a microscope and the percentage calculated accordingly.[13]

**Fluorescence Microscopy**
25 µl of cell suspension was taken in a PCR tube and 5 µl of dye mixture (equal volumes of acridine orange (100 µg/ml) + ethidium bromide (100 µg/ml) were added. 10 µl of stained cell suspension was taken on a clean microscope glass slide and covered with a 22 mm sq cover slip. The cells were examined under a fluorescent microscope with a 40X to 100X objective and 200 total cells/slides were counted and normal versus apoptic cells were recorded. Apoptotic cells were identified based upon chromatin condensation, nuclear fragmentation, membrane...
blebbing, cell shrinkage and apoptotic body formation and apoptotic index was calculated as

\[
\text{Apoptotic index} = \frac{\text{Number of apoptotic cells}}{\text{Total number of cells counted}} \times 100
\]

**DNA Fragmentation Assay**

Briefly, the isolated leucocytes were washed with Tris-buffered saline (TBS) buffer (pH 7.6) and pelleted by centrifugation at 8000 rpm for 5 minutes. The cell pellet was treated with 200 µl of lysis buffer and vortexed. Supernatant was collected in another micro centrifuge tube (MCT). The pellet was again treated with 200 µl of lysis buffer and supernatant was collected similarly. 35 µl of 10% Sodium dodecyl sulfate (SDS) was added to the supernatant and kept at 37°C for overnight. DNA was precipitated with 0.5 volume of 10M Ammonium acetate at 50°C. The lysate was then extracted with equal volumes of phenol/CHCl3/isoamyl alcohol (25:24:01). DNA was pelleted by centrifugation and washed with 70% ethanol at 1200 rpm for 5 min. The pellet was dissolved in 20 µl of TE buffer. 1 µl of RNase-A (10 mg/ml) was added and kept at room temperature for 1 hr. A 1.6% agarose gel (with ethidium bromide) was prepared with tri-borate-EDTA buffer (TBE) pH 8.0 and the sample was run at 60V for 1 hr. The presence of a ladder pattern indicates an apoptotic cell population.

**Necropsy and Histopathology**

The postmortem examination of sacrificed rats was carried out for the presence of gross lesions, if any. Pieces of liver, kidney, heart, brain, lungs, and spleen were collected and fixed in 10% buffered formalin for histopathological examination. All the formalin fixed tissues were mentioned organs were routinely processed, cut at 5 µm and stained with H&E stain.

**Statistical Analysis**

Data was expressed as Mean ± Standard Error (SE) and examined for statistical significance of difference with student’s ‘t’ test, p value of <0.05 being considered statistically significant.

**RESULTS**

Oral administration of Centella asiatica in Wistar albino rats in different doses (250, 500 and 1,000 mg/kg/b.wt) over a period of 30 days did not produce any clinical signs of toxicity, morbidity, mortality with no effect on gross behavioural effects. All the treated rats exhibited normal activities as that of the control group. All the four groups (control & treatment) of rats showed a steady gain in their body weight during the entire study period. (Table: 1). the changes in hematological parameters between the control and treatment groups were statistically significant but, all are in normal physiological range. (Table: 2).

**Serum Bio-Markers:** The effect of short-term exposure of 30 days oral administration of Centella asiatica resulted in a significant increase in the levels of ALT, AST in a dose dependent manner. The concentration of tissue total protein in groups II, III and IV were also significantly higher than the control group. The concentration of BUN and Creatinine in II, III and IV were also higher than the control group (Table: 3).

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**Table 1: Effect of Centella asiatica on the Body weight (gms) during the 30-Days oral administration in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kgwt)</th>
<th>O-Day</th>
<th>10-Days</th>
<th>20-Days</th>
<th>30-Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Control</td>
<td>104.32 ± 8.58</td>
<td>116.64 ± 12.91</td>
<td>120.37 ± 13.37</td>
<td>128.21 ± 13.68</td>
</tr>
<tr>
<td>Group-II</td>
<td>250</td>
<td>111.07 ± 7.78</td>
<td>123.47 ± 7.57</td>
<td>136.20 ± 8.38</td>
<td>140.78 ± 8.52</td>
</tr>
<tr>
<td>Group-III</td>
<td>500</td>
<td>125.05 ± 11.61</td>
<td>137.35 ± 6.96</td>
<td>140.93 ± 9.07</td>
<td>147.21 ± 6.21</td>
</tr>
<tr>
<td>Group-IV</td>
<td>1,000</td>
<td>119.53 ± 8.97</td>
<td>133.01 ± 9.70</td>
<td>139.48 ± 1.24</td>
<td>146.91 ± 1.01</td>
</tr>
</tbody>
</table>

*Values are given as mean ± S.E n = 6 in each group *P < 0.05 as compared to control

**Table 2: Effect of Centella asiatica on the Hematological parameters after 30Days oral administration in rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group-I</th>
<th>Group-II</th>
<th>Group-III</th>
<th>Group-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10⁶/µl)</td>
<td>7.60 ± 0.31*</td>
<td>7.36 ± 0.59*</td>
<td>7.43 ± 0.32*</td>
<td>10.03 ± 0.28*</td>
</tr>
<tr>
<td>WBC (10³/µl)</td>
<td>7.87 ± 0.30*</td>
<td>6.08 ± 0.29*</td>
<td>7.42 ± 0.60*</td>
<td>6.60 ± 0.11*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.50 ± 0.69*</td>
<td>13.28 ± 1.29*</td>
<td>13.28 ± 0.48*</td>
<td>13.28 ± 0.39*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>58.47 ± 0.83*</td>
<td>57.08 ± 0.76*</td>
<td>57.08 ± 0.76*</td>
<td>56.62 ± 0.54*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>30.85 ± 0.12*</td>
<td>31.42 ± 0.19*</td>
<td>32.00 ± 0.21*</td>
<td>31.73 ± 0.37*</td>
</tr>
<tr>
<td>PLT (10³/µl)</td>
<td>565.67 ± 8.82*</td>
<td>496.67 ± 25.02*</td>
<td>551.83 ± 9.16*</td>
<td>634.00 ± 1.46*</td>
</tr>
</tbody>
</table>

*Values are given as mean ± S.E n = 6 in each group *P < 0.05 as compared to control
Table 3: Effect of *Centella asiatica* on the Marker Enzyme level in serum after 30Days oral administration in rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group-I</th>
<th>Group-II</th>
<th>Group-III</th>
<th>Group-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>56.62 ± 0.70a</td>
<td>159.80 ± 2.08b</td>
<td>179.95 ± 2.0c</td>
<td>268.98 ± 12.24d</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>18.78 ± 0.5a</td>
<td>24.63 ± 0.26b</td>
<td>28.57 ± 0.54c</td>
<td>42.83 ± 1.8e</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>19.69 ± 1.6a</td>
<td>23.45 ± 1.04b</td>
<td>26.91 ± 1.01c</td>
<td>31.13 ± 1.98e</td>
</tr>
<tr>
<td>CREA (mg/dl)</td>
<td>0.81 ± 0.04a</td>
<td>1.10 ± 0.06b</td>
<td>1.03 ± 0.11c</td>
<td>1.54 ± 0.11c</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E n = 6 in each group *P < 0.05 as compared to control

Table 4: Effect of *Centella asiatica* on the TBDET of different vital organs after 30-Days oral administration in rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Group-I</th>
<th>Group-II</th>
<th>Group-III</th>
<th>Group-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>92.75 ± 0.85a</td>
<td>93.75 ± 0.63a</td>
<td>89.25 ± 0.85a</td>
<td>83.00 ± 0.91a</td>
</tr>
<tr>
<td>Kidney</td>
<td>94.50 ± 0.65a</td>
<td>94.25 ± 0.48a</td>
<td>91.50 ± 0.65a</td>
<td>90.25 ± 0.48a</td>
</tr>
<tr>
<td>Heart</td>
<td>94.00 ± 0.82a</td>
<td>95.00 ± 0.41a</td>
<td>94.00 ± 0.41a</td>
<td>93.50 ± 1.19a</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E n = 6 in each group *P < 0.05 as compared to control

Apoptosis related parameters were studied immediately at the end of the experiment and they are as follows

i. **TBDET:** The mean values of viable cell count in liver, kidney and heart tissues (%) by trypan blue dye exclusion test at the end of the trial period have been presented in Table-4. Viable hepatocyte count in group-III, IV was 89.25 ± 0.85 and 83.00 ± 0.91 (%) respectively. Which are significantly lower when compared with the group-I 92.75 ± 0.85 (%). Moreover, the viable count of kidney cells in group-I, II, III and IV was 94.50 ± 0.65, 94.25 ± 0.48, 91.50 ± 0.65 and 90.25 ± 0.48 (%) respectively.

Whereas, the viable count of cardiac cells in group-I, II, III and IV was 94.00 ± 0.82, 95.00 ± 0.41, 94.00 ± 0.41 and 93.50 ± 1.19 respectively. No significance difference was recorded in the viable cell count of cardiac cells.

ii. **Flourescence microscopy:** The morphology of cells under fluorescence was characterized in Figures: 1 and 2. The apoptotic index was calculated and presented in the Table 5. Apoptotic index of hepatic tissue was significantly increased in group-III and group-IV as compared to control group. However, the increase in apoptotic index was marginal in group-II which was fed with *Centella asiatica* @ 250 mg/kg b.wt.

Furthermore, kidney cells also recorded the increase in apoptotic index in all three treatment groups as compared to that of control group. There is no significant change/increase in the apoptotic index was observed in the cardiac tissue.

iii. **DNA fragmentation assay:** DNA fragmentation assay of the leucocytes at the end of the experimental period showed clear intact pattern of DNA in all the four groups of rats (Figure: 3).

**Gross Pathology, Organ weight to body weight ratios and Histopathology**

Effect of *Centella asiatica* on weights of liver, kidney, heart, spleen and brain (gms) of the rats are presented in the Table 6. At necropsy, treatment groups did not show any gross...
Table 5: Effect of Centella asiatica on the apoptotic index of different vital organs during the 30-Days oral administration in rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Group-I</th>
<th>Group-II</th>
<th>Group-III</th>
<th>Group-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3.30 ± 0.33*</td>
<td>4.25 ± 0.38*</td>
<td>7.05 ± 0.50*</td>
<td>9.44 ± 0.96*</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.38 ± 0.24</td>
<td>1.25 ± 0.14</td>
<td>2.13 ± 0.38</td>
<td>1.38 ± 0.24</td>
</tr>
<tr>
<td>Heart</td>
<td>1.75 ± 0.32</td>
<td>1.88 ± 0.31</td>
<td>1.63 ± 0.13</td>
<td>1.50 ± 0.20</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E n = 6 in each group * P < 0.05 as compared to control

Table 6: Effect of Centella asiatica on the Organ to body weight ratio (%) after 30- Days oral administration in rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Group-I</th>
<th>Group-II</th>
<th>Group-III</th>
<th>Group-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.83 ± 0.69</td>
<td>4.99 ± 0.37</td>
<td>5.02 ± 0.17</td>
<td>5.83 ± 0.47</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.705 ± 0.15</td>
<td>0.733 ± 0.10</td>
<td>0.985 ± 0.22</td>
<td>0.968 ± 0.26</td>
</tr>
<tr>
<td>Heart</td>
<td>0.36 ± 0.04</td>
<td>0.32 ± 0.05</td>
<td>0.45 ± 0.05</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.38 ± 0.03*</td>
<td>0.44 ± 0.04**</td>
<td>0.53 ± 0.03*</td>
<td>0.52 ± 0.03*</td>
</tr>
<tr>
<td>Brain</td>
<td>0.89 ± 0.06</td>
<td>0.78 ± 0.10</td>
<td>0.92 ± 0.07</td>
<td>0.89 ± 0.31</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E n = 6 in each group * P < 0.05 as compared to control


pathological lesions in any of the organs and no effect on organ weights and their ratios, except the spleen which has shown an increase in gross weight in a dose related manner.

Liver revealed consistent tissue alterations showing granular to vascular changes prominently in perilobular hepatocytes and comparatively milder in centrilobular hepatocytes. Some foci of the perilobular hepatocytes also showed perinuclear cytolysis as well as pyknotic nuclei. Moreover there was marked infiltration of mononuclear cells and proliferation of bile ducts in portal areas, proliferation of central vein in portal areas and sinusoids in some focal areas were also consistently observed. (Figures: 4, 5).

Histopathological examination of kidney of the rats fed with Centella asiatica at a dose of 1,000mg/kg b.wt revealed...
consistent tissue alteration showing granular, vacuolar and as well as desquamative changes in tubular epithelial cells of proximal convoluted tubules (Figure: 6).

At places, the lumen of the tubules showed albuminous precipitates. Mild congestion of intestinal blood vessels was also seen at some places. The bowman's area also showed congestive changes as well as hypercellularity.

The microscopic section of spleen showed hypercellularity in both red and white pulp, in rats fed with Centella asiatica at a dose rate of 1,000 mg/kg b.wt in comparison to the spleen of normal control group rats. (Figure: 7). Likewise, the spleen of rats fed with Centella asiatica at a dose rate of 500 mg/kg b.wt also showed mild increase in cellularity in red and white pulp.

Figure 6: Micro photograph of renal tissue showing degeneration of tubular epithelial cells leading to desquamation at some places

Figure 7: Spleen-Micro photograph of malphigian corpuscles showing increased mononuclear population

The microscopic section of brain and Heart resembled almost normal in all the groups of experimental rats which felt to reveal pathological changes of significance.

DISCUSSION

Centella asiatica administration at any of the tested dose levels did not affected the body weight gain. There were no abnormalities/ adverse effects on hematology. The gross behavioral changes such as ataxia, catalepsy, pilo-errection, salivation, hyperactivity, cyanosis, lacrimation, righting and pinnal reflexes were found to be normal throughout the entire experimental period.

Liver being the main detoxification organ and kidney the major excretory organ, these are very susceptible to the toxicities by drugs. Damage to liver is known to result in cellular changes in the tissues and alterations in activities of enzymes in tissues and serum.[7] Damage of the liver results in entry of these particular enzymes in to the circulation. Therefore, the increased serum levels of GOT
and GPT are used as indicators of liver damage observed significant elevation in the serum levels of aspartate and alanine transaminase after oral administration of *Centella asiatica* in human patients.\[8\]

The extent of liver injury is associated with increased serum levels of AST, ALT. The injuries may be acute/ chronic, reversible or irreversible. Of these enzymes, ALT is thought to be more specific to hepatic injury because it is mainly present in the cytosol of the liver cells and is in low concentrations elsewhere.

Renal parameters were also evaluated in the present study. Kidney eliminates waste product of metabolism from the body. In renal failure, waste products particularly nitrogenous substances like Non protein nitrogen, urea, uric acid accumulates. Creatinine is the least variable nitrogenous constituent of blood. Creatinine increases in early nephritis & in chronic hemmorhagic nephritis with uremia. In the present study, *Centella asiatica* increased both blood urea nitrogen and Creatinine values in a dose dependent manner.

Any significant loss of a cell population or particular cell type may result into dysfunctioning of an organ. Induction of apoptosis could impair the steady state kinetics of healthy tissues in an organism resulting in deregulatory cellular responses.\[9, 10\] Apoptosis can be assessed by biochemical (DNA fragmentation assay) and morphological (fluorescence microscopy) assays.

**TBDET viability count method** was designed for ascertaining the initial event of cellular injury is based on the fact that living cell membrane is able to selectively exclude certain substances where as dead cells permit these substances to enter in side. The trypan blue is a dye which is also excluded by the living cells whose membrane does not allow the dye to enter inside the cell. However, cells whose membranes become permeable, trypan blue enters in side the cell and stains them. In this way, this dye distinguishes between permeable and non permeable cells there by identifying the live and dead cells.\[11\] Liver tissue of group-III and IV rats showed a significant decrease in the viability count when compared to normal control group.

**Flourescence Microscopy** – using acridine orange (AO) and Ethidium bromide visualized the characteristic features of apoptosis i.e. cell shrinkage and karyorhexis in the hepatocytes.

**Histopathology**

The consistent development of prominent perinuclear cytolysis, pyknotic nuclei in perilobular hepatocytes, marked infiltration of mononuclear cells and proliferation of bile ducts in portal areas, proliferation of central vein in portal areas and sinusoids in some focal areas were indicating the hepatotoxicity of the *Centella asiatica*.

Degeneration or necrosis of epithelial cells of the renal tubules, albuminous precipitates in the lumen, indicate nephrotoxicity of *Centella asiatica*. The hypercellularity and Hyperplasia of spleen could be correlated to immunosuppressive effect of *Centella asiatica*. No significant microscopic alterations could be seen in brain, heart and lungs.

**CONCLUSION**

*Centella asiatica* in the doses (250,500 and 1,000 mg/kg bwt) used in the present study over a period of 30 days produced no apparent toxicity and alterations in the body weight gain. But significant increase in the weight of the spleen in the group of rats fed with *Centella asiatica* @ 1,000 mg/kg b.wt was observed. The level of ALT, AST in the serum which indicates the liver functionality was significantly raised in all the three treatment groups. And the increase in BUN and CREATINNIE levels in the serum suggested the possible damage of renal tissue. Though the hematological parameters are showing significant change between groups, all those values are in normal physiological range. *Centella asiatica* @ 1,000 mg/kg b.wt caused induction of higher levels of apoptosis in hepatic tissue and a marginal increase in the renal tissue.

The result of present study clearly indicated that when *Centella asiatica* was orally administered in different doses in rats for 30 days the various biochemical parameters of the rats were affected. The apoptosis induced by *Centella asiatica* in liver shows a promising way for future studies on its role in tumor management. More detailed studies with multiple parameters on dose time relationship in various other species of animals will help in completion of the *Centella asiatica* toxicity on biological systems.

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**REFERENCES**
