ABSTRACT

Introduction: *Cardiospermum halicacabum* L and *Vitex negundo* L are distributed throughout the plains of India and used in traditional practice for the treatment of inflammation and rheumatic disorders. The aim of this study is to evaluate acute and sub-chronic toxicity of methanol leaf extracts of *Cardiospermum halicacabum* (MLECH) and methanol leaf extract of *Vitex negundo* (MLEVN). MLECH and MLEVN were evaluated for acute and sub-chronic toxicity in mice and rats respectively. Materials and Methods: The acute oral toxicity study was carried out as per the OECD guidelines 423 and the sub-chronic toxicity was carried out as per the guidelines set by OECD 407 with slight modification. Body weight, food consumption, water consumption, hematological parameters, biochemical parameters, organ weight and histopathological analysis were carried out. Results: No sign of toxicity viz. lethargy, jerk, convulsion and mortality was observed up to 2000 mg/kg for MLECH and MLEVN in acute toxicity test. For sub-chronic toxicity test, single individual (400 mg/kg) and combined doses of MLECH and MLEVN (400 mg/kg each) in equal proportion not exhibited any signs of toxicity and mortality. Conclusion: In acute oral toxicity study, the oral administration of MLECH and MLEVN in mice was found to be safe up to a dose of 2000 mg/kg and in sub-chronic toxicity study no signs of toxicity observed at a dose of 400 mg/kg in rats.

Key words: sub-chronic toxicity, *Cardiospermum halicacabum*, *Vitex negundo*.

INTRODUCTION

*Cardiospermum halicacabum* L (CH) and *Vitex negundo* L (VN) are the most commonly used medicinal plants in Indian traditional practice for the treatment of inflammation and rheumatoid arthritis (RA). The traditional practitioners in India prescribe the leaves of CH and VN to the patients without knowing the possible adverse effects. In view of this fact, we screened the methanol leaf extract CH and VN for acute and sub-chronic toxicity, before we make an attempt to develop a formulation using anti-artritic herbs CH and VN for the treatment of arthritis used in Indian traditional practice.

*Cardiospermum halicacabum* (CH) is a climber (Figure 1) belongs to the family Sapindaceae locally known as balloon vine and called as “modakathon” in Tamil. “Modaku” means crippling joint pain; “thon” means remedy. It is a deciduous, branched, herbaceous climber distributed through out the plains of India CH known to contain arachidic acid, apigenin, apigenin-7-O-glucuronide, chysoeriol-7-O-glucuronide, luteolin-7-O-glucuronide, sapo- nin, quebrachitol, proanthocyanidin, beta sitosterol and stigmosterol. Irulas in Thanjavur, Tamilnadu, India, provided evidence for the use of this plant as a traditional remedy that has been used for centuries to treat RA and it is still used by some locals to treat RA in Asian and Sri Lankan communities. Ethnopharmacological studies revealed that CH was used by the tribals (Kanikkar) of...
Kalakad-Mundanthurai Tiger Reserve (KMTR), Western Ghats, Tamil Nadu for the treatment of rheumatism.\textsuperscript{14} Valayar tribal community of kundy hill area Erode district, Tamilnadu, India for the treatment of seasonal infections.\textsuperscript{15} CH has been used in Indian traditional medicine for a long time in the treatment of rheumatism, stiffness of the limbs and snakebite.\textsuperscript{16,17} Malayali tribes in villages located in the forest area of Chitterri hills, Dharmapuri district Tamilnadu, India use leaf juice of CH orally for a period of 2 days to arrest dysentery.\textsuperscript{18}

*Vitex negundo* (VN) (family: Verbenaceae) known as Nirgundi in Hindi and Nochi in Tamil, grows gregariously in wastelands and is also planted as a hedge-plant. It is an erect, 2–5 m in height, slender tree with quadrangular branchlets distributed throughout India. The leaves (Figure 2) have five leaflets in a palmately arrangement, which are lanceolate, 4–10 cm long, hairy beneath and pointed at both ends with bluish purple flowers.\textsuperscript{19} Flavonoids such as casticin, orientin, isoorientin, luteolin, lutein-7-O-glucoside, corymbosin; gardenins A and B reported reported to be present in VN.\textsuperscript{20} Besides these, many glycosidic iridoids, alkaloids, and terpenoids have also been isolated from VN.\textsuperscript{21} From roots to fruits, all parts of VN is used in Indian traditional practice and folk system of medicine due to the presence of various Secondary metabolites in the plant.

Traditional healers in Kanchipuram district of Tamilnadu, India used VN for the treatment of head ache, fever, cold and cough.\textsuperscript{11} Ethnopharmacological survey revealed that VN is used for the treatment of respiratory disorders, fever and head ache by the tribals of Madurai district and traditional healers of Pachamali hills (Salem and Tiruchirapalli) of Tamilnadu, India.\textsuperscript{22,23}

Though all parts of CH and VN are used in ayurvedic medicine, no report is available for acute and sub-chronic study of these plants. Since leaves of these plants are used majorly for treating arthritis in Indian traditional medicine\textsuperscript{24–28} the acute and sub-chronic study was carried out in leaf extract. Further, the aim of this study is to evaluate the toxicity of MLECH and MLEVN administered orally to Wistar rats, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

**MATERIALS AND METHODS**

**Plant materials**

Both the plants CH and VN were collected by Ramasamy Arivukkarasu in November 2010 from hilly areas of Palakkad district, Kerala, India. The plants were authenti- cated by GVS. Murthy, Director of Botanical survey of India, Coimbatore, India and reconfirmed by S. John Britto of St Joseph College Tiruchirappalli, Tamilnadu, India. Voucher specimens (KMCH/Dr.TP/DST-DPRP/ COG/Ch-Pal/2010/01; KMCH/Dr.TP/DST-DPRP/ COG/Vn-Pal/2010/02) of the plants were deposited at the Department of Pharmacognosy, KMCH College of Pharmacy, Coimbatore for future reference.

**Preparation of extracts**

Fresh mature leaves of CH and VN were processed to remove foreign, earthy matter and residual materials carefully from the leaves and cleaned. It was then shade dried at room temperature (32 ±2°C) for 10 days, pulverized to coarse powder, passed through a #40 mesh sieve and both CH and VN was extracted separately with methanol in a soxhlet apparatus for 72 h. Methanol extract of CH and VN was filtered and concentrated separately under reduced pressure using IKA Rotary evaporator (Model No RN 10 digital V, ILMAC Germany) at 40°C. Percentage yields of methanol leaf extracts of CH and VN are 12.4 % and 8.4 % w/w respectively.

**Animals**

Wistar female Albino mice (7–8 weeks old, 20-30 g) and Wistar strains of female rats (10-12 weeks old, 150-200 g) were obtained from the animal house of Kovai medical center research and educational trust, Coimbatore, India. The animals were kept in polypropylene cages at a temperature of 25 ± 2°C, humidity (50±5) RH and 12 h light and dark cycles. They were fed with standard laboratory animal diet and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. Experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC) (KMCRET/DST/01/2011, dt.16/07/2011).

**Acute toxicity study**

Acute toxicity test was performed according to OECD29 guideline for testing of chemicals (2001). Healthy young adult albino nulliparous, non-pregnant female mice weighing about 20-30 g were administered as a single dose (1 ml) orally using oral feeding needle with 5, 50, 300 and 2000 mg/kg of MLECH and MLEVN separately in 1% w/v SLS suspension. Animals were observed individually for first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days to observe toxicity signs like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behav-
Sub-chronic toxicity study

The sub-chronic toxicity assessment of MLECH and MLEVN was accomplished by performing studies as per OECD Guideline 407. A Wistar strain of rats (150-200 g) was divided into three groups, each consisting of six animals. Group 1 received 5 ml/kg of 1% w/v of sodium lauryl sulphate was considered as normal control. MLECH and MLEVN suspension were prepared freshly in 1% w/v sodium lauryl sulphate before oral administration. MLECH at a dose of 400 mg/kg and MLEVN at a dose of 400 mg/kg were administered to groups 2 and 3 respectively. All the treatments were done orally using rat oral feeding needles, daily for 45 days. During the treatment period the body weight of animals were monitored on 0, 7th, 14th, 21st, 28th, 35th, 42nd and 45th day. Food and water intake examination for all the groups were observed daily from 0 to 45 days.

On 45th day, the overnight fasted animals were anaesthetized with ketamine (20 mg/kg, i.p) followed by blood samples withdrawal from retro-orbital sinus and the collected blood samples were evaluated for hematological parameters viz. red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), platelet count, packed cell volume (PCV), differential count, Mean Platelet Volume (MPV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Blood Cell Distribution Width (RDW). A portion of the blood samples were centrifuged at 10000 rpm for 10 min and the separated serum was analyzed for biochemical parameters viz. Cholesterol, Triglycerides, HDL and LDL levels. Biochemical investigations was performed in order to assess the lipid profile of animals and to evaluate for any toxic effects of CH and VN on liver and kidney. There was no significant alteration in Cholesterol, Triglycerides, HDL and LDL levels in treated groups when compared with control group of rats (Table 3). No significant change observed in SGOT, SGPT, ALP and total bilirubin content of treated group animals when compared with control group animals (Table 4). There was no significant alteration observed in creatinine, urea and uric acid levels of treated group animals when compared with control group animals (Table 5).

Organ weight
No abnormal change in the internal organs weight of rats was observed when compared to control group as shown in (Table 6).

Statistical analysis
The results are expressed as the mean ± SEM. The significance of the difference was evaluated by one-way ANOVA followed by Dunnert’s test. Data were considered statistically significant if p < 0.05.

RESULTS

Acute Toxicity Study
No change in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern of the animals observed. Absence of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma also noted. Thus in acute toxicity study, no sign of toxicity viz. lethargy, jerk, convolution and mortality was observed upto 2000 mg/kg for MLECH and MLEVN.

Sub-chronic toxicity study

Body weight
Gain in body weight was observed not only for control group animals but also for MLECH and MLEVN group treated animals throughout the study period (Table 1).

Food and water consumption
Food and water consumption of the rats were continuously monitored for 45 days, where there is no change in consumption was observed for treated groups and the control group animals (Figure 3 and Figure 4).

Hematological analysis

The results of hematological investigations (Table 2) conducted on day 45 revealed no significant changes in the values of RBC, WBC, Hb, platelet count, PCV, differential count, MPV, MCV, MCH, MCHC and RDW of treated groups when compared with the respective control rats.

Biochemical Investigations

Biochemical investigations was performed in order to assess the lipid profile of animals and to evaluate for any toxic effects of CH and VN on liver and kidney. There was no significant alteration in Cholesterol, Triglycerides, HDL and LDL levels in treated groups when compared with control group of rats (Table 3). No significant change observed in SGOT, SGPT, ALP and total bilirubin content of treated group animals when compared with control group animals (Table 4). There was no significant alteration observed in creatinine, urea and uric acid levels of treated group animals when compared with control group animals (Table 5).

Histopathological investigation

Thymus
Section from the thymus of control group, MLECH 400 mg/kg treated group and MLEVN 400 mg/kg treated group showed normal lymphoid follicle with ductal germinal centre separated by fibro vascular connective tissue. No evidence of toxic signs observed (Figure 5).

Heart
Section from the heart of control group, MLECH 400 mg/kg treated group and MLEVN 400 mg/kg treated
Table 1. Effect of sub-chronic doses of MLECH and MLEVN on body weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>Weight gain on 45th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1 % w/v SLS)</td>
<td>124.5 ± 1.40</td>
<td>147.2 ± 1.44</td>
<td>173.0 ± 2.14</td>
<td>186.5 ± 1.33</td>
<td>62.0 ± 0.73</td>
</tr>
<tr>
<td>MLECH 400 mg/kg</td>
<td>125.6 ± 1.52</td>
<td>149.2 ± 2.38a</td>
<td>173.2 ± 3.34a</td>
<td>189.5 ± 1.76a</td>
<td>63.2 ± 0.60 a</td>
</tr>
<tr>
<td>MLEVN 400 mg/kg</td>
<td>127.2 ± 1.52</td>
<td>153.8 ± 2.50a</td>
<td>177.8 ± 2.42a</td>
<td>189.8 ± 2.05a</td>
<td>61.5 ± 0.42 a</td>
</tr>
</tbody>
</table>

Data provided as mean ± SEM (n=6); ap>0.05 treated groups Vs control

group, showed normal cardiac muscle. No inflammation, edema or necrosis was observed (Figure 6).

Liver
Histopathological section of liver in control group, MLECH 400 mg/kg treated group, and MLEVN 400 mg/kg treated group showed normal lobular architecture. The portal tracts, hepatocytes, central veins, sinusoids are found to be normal. No evidence of toxic signs observed as there is no inflammation, fatty change or fibrosis (Figure 7).

Spleen
Section from the spleen of control group, MLECH 400 mg/kg treated group and MLEVN 400 mg/kg treated group of rats showed normal capsule and trabeculae. No change was observed in white pulp, pencillar artery and the red pulp (Figure 8).

Kidney
Sections from kidney of control group, MLECH 400 mg/kg treated group and MLEVN 400 mg/kg treated group showed normal cortex, medulla and pelvicalyceal system. The cortex showed normal glomeruli and proximal convoluted tubules. The interstitium and distal convoluted tubules are found to be normal. No inflammation or tubular necrosis was observed (Figure 9).

DISCUSSION

The toxic effect of chemicals or drugs or natural products can be assessed by three different toxicological studies viz. acute toxicity, sub-chronic toxicity and chronic toxicity study. Acute toxicity study is generally carried out to determine the LD<sub>50</sub> of the test substance. In sub-chronic toxicity study, repeated dose of test substance is given to determine its effect on biochemical parameters and to assess the toxicological risk of potential target organs. In chronic toxicity study, repeated oral dose of the test substance is given for a period of 90 days to over a year to assess the carcinogenic and mutagenic potential of the test substance. Female sex of mice and rats were used since they were found to be slightly more sensitive than male sex.

Safety profile of MLECH and MLEVN in experimental animals was demonstrated in the present study. No mortality was observed in acute toxicity study. No changes in skin, fur, respiratory, eyes, mucus membranes, autonomic, central nervous systems, circulatory somatomotor activity, behaviour pattern was observed. Observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma also concluded the normal behavior of the animals.

Body weight is one of the critical parameter for the evaluation of first sign of toxicity<sup>31</sup> and hence this investigation on monitoring the body weight was carried out throughout the study period. No significant difference in body weight was observed for all the treated group when compared with the control group. This may be co-related with no alterations observed in food and water consumption of animals.

Hematological investigation was carried out as hematopoietic system is the most sensitive target for toxic substances and it is the important index of physiological and pathological status. Further blood profile provides vital information on the response for body injury or stress.<sup>32,33</sup>

Assessment of lipid profile was performed to predict the risk of cardiovascular diseases as this may cause elevated serum levels of triglycerides, cholesterol and VLDL.<sup>34</sup> There was no significant alteration in Cholesterol, Triglycerides, HDL and LDL levels in treated groups when compared with control group of rats.

Liver function test was carried out for the animals treated with MLECH and MLEVN as liver toxicity can be easily predicted from the variation in SGOT, SGPT, ALP and bilirubin levels.<sup>35</sup> No significant changes in SGOT, SGPT, ALP and bilirubin level was observed in all treated group animals.
Table 2. Effect of MLECH and MLEVN on hematological parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total RBC (X106 cells/mm³)</th>
<th>Total Hb (g/dl)</th>
<th>Total WBC (X106 cells/mm³)</th>
<th>Platelet Count (X106 cells/mm³)</th>
<th>PCV (%a)</th>
<th>Differential Count</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>Polymorphs (%)</td>
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<td></td>
<td>Lymphocytes (%)</td>
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<td></td>
<td></td>
<td>Monocytes (%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Eosinophils (%)</td>
</tr>
<tr>
<td>Control</td>
<td>8.34 ± 0.12</td>
<td>16.25±0.19</td>
<td>10.93±1.58</td>
<td>806.83±79.03</td>
<td>50.01±1.58</td>
<td>2.5±0.42</td>
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<td></td>
<td>89.83±1.60</td>
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<td></td>
<td>4.1±0.70</td>
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<td></td>
<td></td>
<td>2.5±0.42</td>
</tr>
<tr>
<td>MLECH 400 mg/kg</td>
<td>8.78 ± 0.23a</td>
<td>16.23±0.24a</td>
<td>8.80±0.86a</td>
<td>791±25.11a</td>
<td>51.31±0.92a</td>
<td>3.33±0.55a</td>
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<td></td>
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<td></td>
<td>90.33±1.72a</td>
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<td></td>
<td></td>
<td>4.0±0.93a</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>2.33±0.49a</td>
</tr>
<tr>
<td>MLEVN 400 mg/kg</td>
<td>8.20 ± 0.26a</td>
<td>16.05±0.30a</td>
<td>8.75±1.70a</td>
<td>842.5±42.36a</td>
<td>50.13±1.03a</td>
<td>1.66±0.21a</td>
</tr>
<tr>
<td></td>
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<td>88.66±1.82a</td>
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<td></td>
<td>2.16±0.30a</td>
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<td></td>
<td></td>
<td>1.66±0.33a</td>
</tr>
</tbody>
</table>

Data provided as mean ± SEM (n=6); ap>0.05 treated groups vs control

<table>
<thead>
<tr>
<th></th>
<th>MPV (fL)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>RDW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.26±0.08</td>
<td>68.41±1.00</td>
<td>19.83±0.27</td>
<td>29.20 ± 0.42</td>
<td>21.06±0.52</td>
</tr>
<tr>
<td>MLECH 400 mg/kg</td>
<td>7.13±0.12a</td>
<td>64.70±1.50a</td>
<td>18.48±0.53a</td>
<td>29.61 ± 0.48a</td>
<td>21.23±0.51a</td>
</tr>
<tr>
<td>MLEVN 400 mg/kg</td>
<td>7.15±0.15a</td>
<td>66.61±1.03a</td>
<td>19.41±0.47a</td>
<td>29.15 ± 0.44a</td>
<td>21.15±0.30a</td>
</tr>
</tbody>
</table>
Table 3. Effect of MLECH and MLEVN on lipid profile of animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg (p.o.)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1% w/v of SLS (5 ml/kg)</td>
<td>158.03±14.32</td>
<td>148.8±12.84</td>
<td>26.70±3.68</td>
<td>101.3±16.61</td>
<td>30.03±1.95</td>
</tr>
<tr>
<td>MLECH</td>
<td>400</td>
<td>115.15±13.06a</td>
<td>164.4±14.23a</td>
<td>28.75±3.29a</td>
<td>65.36±5.30a</td>
<td>32.25±2.84a</td>
</tr>
<tr>
<td>MLEVN</td>
<td>400</td>
<td>165.7±11.37a</td>
<td>150.0±8.08a</td>
<td>38.30±5.56a</td>
<td>97.21±11.18a</td>
<td>30.18±1.58a</td>
</tr>
</tbody>
</table>

Data provided as mean ± SEM (n=6); *p<0.05 treated groups vs control

Table 4. Effect of MLECH and MLEVN on liver function test of animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg (p.o.)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1% w/v of SLS (5 ml/kg)</td>
<td>26.33±1.66</td>
<td>39.66±5.51</td>
<td>110.66±3.97</td>
<td>0.50±0.13</td>
</tr>
<tr>
<td>MLECH</td>
<td>400</td>
<td>26.00±2.25a</td>
<td>39.00±3.54a</td>
<td>111.00±3.47a</td>
<td>0.3±0.12a</td>
</tr>
<tr>
<td>MLEVN</td>
<td>400</td>
<td>30.83±2.10a</td>
<td>39.00±3.15a</td>
<td>105.83±1.62a</td>
<td>0.35±0.08a</td>
</tr>
</tbody>
</table>

Data provided as mean ± SEM (n=6); *p>0.05 treated groups vs control

Table 5. Effect of MLECH and MLEVN on kidney function test of animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg (p.o.)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1% w/v of SLS (5 ml/kg)</td>
<td>0.63±0.08</td>
<td>43.18±1.75</td>
<td>3.01±0.29</td>
</tr>
<tr>
<td>MLECH</td>
<td>400</td>
<td>0.71±0.08a</td>
<td>46.40±1.95a</td>
<td>2.78±0.43a</td>
</tr>
<tr>
<td>MLEVN</td>
<td>400</td>
<td>0.61±0.03a</td>
<td>36.65±3.167a</td>
<td>2.48±0.22a</td>
</tr>
</tbody>
</table>

Data provided as mean ± SEM (n=6); *p>0.05 treated groups vs control

Kidney function test was performed to assess whether treatment of MLECH and MLEVN to rats causes any damage to kidney. Urea, uric acid and creatinine levels were determined as they are considered as important markers of renal dysfunction. There was no significant alteration observed in creatinine, urea and uric acid levels of treated group animals when compared with control group animals.

CONCLUSION

Based on our results, we conclude that MLECH and MLEVN were found to be safe up to a dose of 2000 mg/kg/day. The study provided significant data on the sub-chronic toxicity profile of MLECH and MLEVN which may be valuable in the clinical study of medicinal herbs CH and VN.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

REFERENCES

21. Nair CKN, Mohenan N. Medicinal plants in India with special reference to Ayurveda. NAG Publisher, Delhi, India; 1998. pp. 414.