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Research Article

Acute and sub-chronic toxicity study of methanol leaf extract of *Cardiospermum halicacabum* L and *Vitex negundo* L in rats

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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 upto 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 practioners in India prescribe the leaves of CH and VN to the patients without knowing the possible adverse effects.[1] 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-arthritic herbs CH and VN for the treatment of arthritis used in Indian traditional practice.

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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[2] CH known to contain arachidic acid, apigenin, apigenin-7-O-glucuronide, chysoeriol-7-O-glucuronide, luteolin-7-O-glucuronide, saponin, quebrachitol, proanthocyanidin, beta sitosterol and stigmosterol.[3-7] 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[8-12] and Sri Lankan communities.[13] 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.[14] valayar tribal community of kundry hill area Erode district, Tamilnadu, India for the treatment of seasonal infections.[15] CH has been used in Indian traditional medicine for a long time in the treatment of rheumatism, stiffness of the limbs and snakebite.[16,17] Malayali tribes in villages located in the forest area of Chitteri Hills, Dharmapuri district Tamilnadu, India use leaf juice of CH orally for a period of 2 days to arrest dysentery.[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.[19] Flavonoids such as casticin, orientin, isoorientin, luteolin, lutecin-7-O-glucoside, corymbosin; gardenins A and B reported reported to be present in VN.[20] Besides these, many glycosidic iridoids, alkaloids, and terpenoids have also been isolated from VN.[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.[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.[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[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 authenticated 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-

iour pattern. Occurance of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma was noted.

Sub-chronic toxicity study

The sub-chronic toxicity assessment of MLECH and MLEVN was accomplished by performing studies as per OECD Guideline 407.[30] 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 were carried out in a auto analyser (Photometer 5010 V5+, Robert Riely, Berlin) using Piramal healthcare limited reagent kit. The animals were sacrificed by cervical dislocation and the organs thymus, heart, liver, spleen, and kidneys were isolated and weighed after separating the superficial fat. The isolated organs were observed histopathologically.

Statistical analysis

The results are expressed as the mean ± SEM. The significance of the difference was evaluated by one-way ANOVA followed by Dunnett'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, convulsion 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).

Organ weight

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

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					
Treatment	Body weight (g)				
	0 day	15th day	30th day	45th day	Weight gain on 45th day
Control (1 % w/v SLS)	124.5 ± 1.40	147.2 ± 1.44	173.0 ± 2.14	186.5 ± 1.33	62.0 ± 0.73
MLECH 400 mg/kg	125.6 ± 1.52	149.2 ± 2.38a	173.2 ± 3.34 a	189.5 ± 1.76 a	63.2 ± 0.60 a
MLEVN 400 mg/kg	127.2 ± 1.52	153.8 ± 2.50 a	177.8 ± 2.42 a	189.8 ± 2.05 a	61.5 ± 0.42 a
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_{50} 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, mucous 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[31] 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.[32,33]

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.[34] 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.[35] 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

Groups	Total RBC (X106cells/ mm3)	Total Hb (g/dl)	Total WBC (X106cells/ mm3)	Platelet Count (X106cells/ mm3)	PCV (%ia)	Differential Count			
						Polymorphs (%)	Lymphocytes (%)	Monocytes (%)	Eosnophils (%)
Control	8.34 ± 0.12	16.25±0.19	10.93±1.58	806.83±79.03	50.01±1.58	2.5±0.42	89.83±1.60	4.1±0.70	2.5±0.42
MLECH 400 mg/kg	8.78 ± 0.23a	16.23±0.24a	8.80±0.86a	791±25.11a	51.31±0.92a	3.33±0.55a	90.33±1.72a	4.0±0.93a	2.33±0.49a
MLEVN 400 mg/kg	8.20 ± 0.26a	16.05±0.30a	8.75±1.70a	842.5±42.36a	50.13±1.03a	1.66±0.21a	88.66±1.82a	2.16±0.30a	1.66±0.33a

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

MPV (fL)	MCV fL)	MCH (pg)	MCHC (g/dL)	RDW (%)
7.26±0.08	68.41±1.00	19.83±0.27	29.20 ± 0.42	21.06±0.52
7.13±0.12a	64.70±1.50a	18.48±0.53a	29.61 ± 0.48a	21.23±0.51a
7.15±0.15a	66.61±1.03a	19.41±0.47a	29.15 ± 0.44a	21.15±0.30a

Table 3. Effect of MLECH and MLEVN on lipid profile of animals						
Groups	**Dose mg/kg (p.o.)**	**Cholesterol (mg/dl)**	**Triglycerides (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
Control	1% w/v of SLS (5 ml/kg)	158.03±14.32	148.8±12.84	26.70 ± 3.68	101.3± 16.61	30.03 ± 1.95
MLECH	400	115.15±13.06a	164.4±14.23a	28.75± 3.29a	65.36 ± 5.30a	32.25 ± 2.84a
MLEVN	400	165.7±11.37a	150.0±8.08a	38.30± 5.56a	97.21 ± 11.18a	30.18 ± 1.58a
Data provided as mean ± SEM (n=6); [a] p<o.05 treated groups vs control						

Table 4. Effect of MLECH and MLEVN on liver function test of animals					
Groups	**Dose mg/kg (p.o.)**	**SGOT (U/L)**	**SGPT (U/L)**	**ALP (U/L)**	**Total Bilirubin (mg/dl)**
Control	1% w/v of SLS (5 ml/kg)	26.33±1.66	39.66±5.51	110.66±3.97	0.50±0.13
MLECH	400	26.00±2.25a	39.00±3.54a	111.00±3.47a	0.3±0.12a
MLEVN	400	30.83±2.10a	39.00±3.15a	105.83±1.62a	0.35±0.08a
Data provided as mean ± SEM (n=6); [a] p>o.05 treated groups vs control					

Table 5. Effect of MLECH and MLEVN on kidney function test of animals				
Groups	**Dose mg/kg (p.o.)**	**Creatinine (mg/dl)**	**Urea (mg/dl)**	**Uric acid (mg/dl)**
Control	1% w/v of SLS (5 ml/kg)	0.63±0.08	43.18±1.75	3.01±0.29
MLECH	400	0.71 ±0.08a	46.40±1.95a	2.78±0.43a
MLEVN	400	0.61±0.03a	36.65±3.167a	2.48±0.22a
Data provided as mean ± SEM (n=6); [a] p>o.05 treated groups vs control				

Table 6. Effect of MLECH and MLEVN on organ weight									
Groups	**Thymus (g)**	**Heart (g)**	**Lungs (g)**	**Liver (g)**	**Spleen (g)**	**Left adrenal (g)**	**Right adrenal (g)**	**Left kidney (g)**	**Right kidney (g)**
Control	0.23 ±0.01	0.74 ± 0.04	1.68 ± 0.19	5.50 ± 0.25	0.74 ±0.01	0.038± 0.005	0.039 ±0.004	0.65 ±0.04	0.67 ±0.04
MLECH 400 mg/kg	0.25 ±0.03	0.73 ± 0.06	3.20 ± 0.99	4.53 ± 0.25	0.77 ±0.05a	0.036 ±0.004a	0.035 ±0.005a	0.60 ±0.02a	0.61 ±0.03a
MLEVN 400 mg/kg	0.25 ±0.02	0.77 ± 0.04a	1.59 ±0.14a	4.66 ±0.53a	0.74 ±0.05a	0.052 ±0.003a	0.051 ±0.003a	0.64 ±0.01a	0.64 ±0.01a
Data provided as mean ± SEM (n=6); [a] p>o.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.[36] 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.

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CONFLICT OF INTEREST

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

REFERENCES

1. Venkatesh Babu KC, Krishnakumari S. Cardiospermum halicacabum suppresses the production of TNF-α and NO by human peripheral blood mononuclear cells. Afr J Biomed Res. 2006; 9: 95–9.
2. Joshi SK, Sharma BD, Bhatia CR, Singh RV, Thakur RS. The Wealth of India. Raw Materials (Revised), Vol III: Council of Scientific and Industrial Research Publication, New Delhi; 1992; 562.
3. Dass AK. Chemical examination of Cardiospermum helicacabum Linn. Bull. Bot. Surv. India. 1966; 8: 357-8.
4. Khan MSY, Arya M, Javed K, Khan MH. Chemical examination of Cardiospermum halicacabum Linn. Indian Drugs. 1990; 27: 257–8.
5. Satyavathi VV. Medicinal plants of India. Vol 1: Indian Council of Medical Research, New Delhi; 1995. 83.
6. Srinivas K, Choudary KA, Rao SS, Satyanarayana T, Krishna Rao RV. Phytochemical examination of Cardiospermum halicacabum Linn. Indian J Nat Prod. 1998; 1427: 24-7.
7. Subramanyam R, Newmaster SG, Gopinathan P, Newmaster CB. Exploring Ethnobiological Classifications for Novel Alternative Medicine: A case study of Cardiospermum halicacabum L. (Modakathon, Balloon Vine) as a traditional herb for treating rheumatoid arthritis. Ethnobotany. 2007; 1927: 1-16.
8. Chopra RN. Glossary of Indian medicinal plants, Council for Scientific and industrial research, New Delhi; 1992. 269.
9. Kirtikar KR, Basu BD. Indian Medicinal Plants. Allahabad, India; 2001. pp. 444-6.
10. Nadkarni KM. Indian Materia Medica. 3rd ed, Vol 1, Popular Prakashan, Mumbai; 2005. pp.271.
11. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kanchipuram district of Tamilnadu, India. J Ethnobiol Ethnomed. 2006; 2: 43-6.
12. Newmaster SG, Ragupathy S, Ivanhoff RF, Nirmala CB. Mechanisms of ethnobiological classifications. Ethnobotany. 2006; 18: 4-26.
13. Jayaweera DMA. Medicinal Plants Used in Ceylon. Parts I - IV, National Science Council of Sri Lanka Publication, Colombo; 1982. Pp. 73.
14. Sutha S, Mohan VR, Kumaresan S, Murugan C, Athiperumalsami T. Ethnomedicinal plants used by the tribals of Kalakad-Mundanthurai Tiger Reserve (KMTR), Western Ghats, Tamil Nadu for the treatment of rheumatism. Indian J Traditional Knowledge. 2010; 9(3): 502-9.
15. Ramachandran VS, Nair NC. Ethnobotanical observations on Irulars of Tamil Nadu, India. J. Econ.Tax. Bot. 1981; 2: 183–90.
16. Sadique J, Begum VH, Thenmozhi V, Elango V. Hypoglycemic effect of cottonseed aqueous extract in alloxan induced diabetes mellitus in rats. Biochem. Med. Metabol. Biol. 1987; 38: 104–10.
17. Chandra T, Sadique J. Anti-arthritic effect of Cardiospermum halicacabum in rats. Indian Medicine. 1989; 1: 12-20.
18. Kadhirvel K, Ramya S, Palin Sathya Sudha T, Veera Ravi A, Rajasekaran C, Vanitha Selvi R, Jayakumararaj R. Ethnomedicinal Survey on Plants used by Tribals in Chitteri Hills Environ. We Int. J. Sci. Tech. 2010; 5: 35-46.
19. Banerji A, Chadha MS, Malshet VG. Isolation of 5 hydroxy 3, 6, 7 3', 4' pentamethoxy flavone from Vitex negundo. Phytochemistry. 1968; 8(2): 511-2.
20. Achari B, Chowdhury US, Dutta PK, Pakrashi SC. Two isomeric flavonones from *Vitex negundo*. Phytochemistry. 1984; 23: 703-4.
21. Nair CKN, Mohenan N. Medicinal plants in India with special reference to Ayurveda. NAG Publisher, Delhi, India; 1998. pp. 414.
22. Ignacimuthu, S, Ayyanar M, Sivaraman K. Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). J Ethnobiol Ethnomed. 2006; 2: 25-31.
23. Rajadurai M, Vidhya V, Ramya M, Bhaskar A. Ethno-medicinal plants used by the traditional healers of Pachamalai hills, Tamil nadu. India', Ethnomedicine. 2009; 3: 39-41.
24. Eswar Kumar K, Mastan SK, Amrander Reddy G, Ragunandan N, Sreekanth N,Chaitanya G. Anti-arthritic property of the ethanolic leaf extracts of *Cardiospermum helicacabum* Linn. Biomed Pharmacol J. 2008; 1: 2.
25. Gautam LN, Shrestha SL, Wagle P, Tamrakar BM. Chemical constituents from *Vitex negundo* (Linn.) of Nepalese origin. Scientific World. 2008; 6(6): 27-32.
26. Chadha YR. The Wealth of India - A dictionary of Indian raw materials and industrial products. Publication Information directorate, CSIR, New Delhi; 2003. pp. 206-7.
27. Chandramu C, Manohar RD, krupadanam DGL, Dashavamtha RV. Isolation,characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. Phytother Res. 2003; 17(2): 129-34.
28. Guha G, Rajkumar V, Ashok Kumar R. Polyphenolic constituents of methanolic and aqueous extracts of *Vitex negundo* render protection to Hep3B cells against oxidative cytotoxicity. Food Chem Toxicol. 2010; 48(8): 2133-8.
29. Organisation for Economic Co-Operation and Development (OECD) Guidelines for testing of chemicals 423. Acute oral toxicity – acute toxic class method. #423, Paris, France; Dec 2001.
30. Organisation for Economic Co-Operation and Development (OECD), Guidelines for Testing of Chemicals, Repeated Dose 28-days Oral Toxicity Study in Rodents #407, Paris, France; 1995.
31. Sireeratawong, S, Lertprasertsuke N, Srisawat U, Thuppia A, Ngamjariyawat A, Suwanlikhid N, Jaijoy K. Acute and subchronic toxicity study of the water extract from Tiliacora triandra (Colebr.) Diels in rats. Sonklanakarin J Sci Tech. 2008; 30(5): 729-37.
32. Tan PV, Mezui C, Orock GK, Nijikam N, Dimo T, Bitolog P. Teratogenic effects, acute and sub-chronic toxicity of leaf aqueous extracts of *Ocimum suave* Wild (Lamiaceae) in rats. J Ethnopharmacol.. 2008; 115(2): 232-7.
33. Mukinda JT, Eagkes FK. Acute and sub-chronic oral toxicity profile of the aqueous extract of *Polygala fruicosa* in female mice and rats. J Ethnopharmacol. 2010; 128(1): 236–40.
34. Mansura A. Effect of Peristrophe bicalyculata on lipid profile of P-407 induced hyperlipidemic Wistar rats. J Med Plants Res. 2011; 5(4): 490-4.
35. Thapa BR, Walia A. Liver function test and their interpretation. Indian J Paediatr. 2007; 74(7): 663-71.
36. Almdal TP, Vilstrup H. Effect of streptozotocin induced diabetes and diet on nitrogen loss from organs and the capacity of urea synthesis in rats. Diabetologia. 1987; 30(12): 952-6.