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Original Article

Toxicological Study of n-hexane and Chloroform Extracts of *Lantana camara* L. in Experimental Animals

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ABSTRACT

Aim: The aim of the current study is to investigate the acute, sub-acute and sub-chronic toxicity study of extracts (n-hexane and chloroform) of *L. camara* in Wistar rats. **Materials and Methods:** HPTLC and GC-MS analysis were carried out to analyze different constituents present in *L. camara* extract. Acute toxicity study was conducted as per OECD 420 guidelines, while sub-acute and sub-chronic studies were conducted according to OECD 407 and 408 guidelines respectively. Toxicopathological effects of both extracts were tested with the help of hemotoxylin and eosin staining of vital organs (liver, kidney, brain, testes, and ovaries) in experimental animals. Antioxidant and behavioral tests were also conducted on both the extracts, so as to evaluate the possible effects of toxicity. **Results:** The toxicity investigation of chloroform and n-hexane extracts exhibits no toxicity in terms of hematological ($p<0.01$) and biochemical parameters ($p<0.05$). Histopathological sections also confirmed the non-toxic effects on the liver, brain, kidney and on reproductive organs. **Conclusion:** The n-hexane and chloroform extracts of *L. camara* can be safely used for therapeutic purposes.

Keywords: *Lantana camara*, GC-MS, HPTLC, Toxicity, Histopathology, Antioxidant, PI uptake.

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INTRODUCTION

Poisonous plants are of major concern worldwide and particularly for veterinarians, as they are responsible for harmful effects (reduction in productivity or mortality) on livestock. The toxicity of poisonous plants varies between different species and also depends upon different environmental conditions, on nature, part and concentration of toxic constituents present in plants and either on species, size, age and body conditions of animals.[1] Toxic plants are a threat to livestock and along with these; they have noxious effects on biodiversity and the ecosystem. It is being investigated that the poisonous plants exhibit certain beneficial effects also and can aid in the cure of different ailments in humans.[2] Therefore, these noxious plants or weeds are closely monitored for their toxicity and in case of emergent situations or where cure with other modalities failed, these were used for treatment purposes. For this, a scientific validation study is needed to have data on the exact mechanism of toxicity and which constituents are mainly responsible for lethality.

Among toxic plants, *Lantana camara* L. is one such noxious, lethal and invasive weed that is distributed in tropical and sub-tropical areas worldwide. *L. camara* is an ornamental shrub that was introduced by the britishers in the year 1809 at the botanical garden, Calcutta. *L. camara* is derived from the latin word 'Lantana means *lento* which means "to bend". Local names of this weed is termed as red wild sage, bunchberry and baraphulnoo. One of the unique characteristics of this plant is that there is the change in the inflorescence with season and age, so it is quite difficult to classify it according to taxonomics. In the year 1753, Linnaeus coined its binomial name and based on the color of different flowers, its toxicity alters. Important varieties include pink *L. camara*, red *L. camara*, orange *L. camara*, white *L. camara*, pink edged red *L. camara*. Among all, red flower variety i.e *L. camaravar.* Aculeate is the most toxic whereas the pink variety is nontoxic and often consumed by animals in New Zealand. *L. camara* is reported to be in the list of top 100 most notorious weeds distributed widely throughout the world and found to be a major weed in 12 countries.[3] In India, this weed invaded in Tamil Nadu, covered the Western Ghats in South

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India, and in Garhwal. A study conducted by Kumar *et al.* 1984 reported that in Himachal Pradesh there is the heavy outbreak of lantana toxicity and sporadic cases of toxicity from cattle, buffaloes and ruminants. Lantadenes, pentacyclic triterpenes are majorly contributors as a toxic substance present in the *L. camara* weed. Lantadenes are responsible for jaundice, hepatotoxicity and photosensitization and certain other compounds such as naphthoquinones, iridoid glycosides, oligosaccharides and oil constituents are of lesser importance in terms of toxicity.[4] Plants contain various constituents such as oleanolic acid, ursolic acid that are responsible for hepatoprotective, antihyperlipidemic activity; verbascoside responsible for protein kinase C inhibition; Oleanonic acid responsible for anti-inflammatory; 22-beta-hydroxy-3-oxolean-12-en-28-oic acid for antiviral; apigenin, eupafolin and cirsilineol for antiproliferative activity. This plant is used for the treatment of different ailments due to other constituents present. Moreover despite its toxicity, it is widely consumed by livestock in field conditions. Although numerous studies conducted so far has described the toxic effects of this plant weed in grazing animals.[5,6] so generally it is not used as fodder but in case of fodder scarcity, drought and flood, it is consumed while grazing or either in small quantities it is mixed with regular fodder. The plant possess anticancer, antifungal, antidiabetic, analgesic, anti-feedant, anti-bacterial, antimotility, larvae repellent, antiulcer, anticonvulsant and antioxidant activities.[7] Numerous studies reported so far that *L. camara* leaf extract on acute or sub-acute toxicity studies showed that animals died within 2-4 days while dose-dependent mortality was observed in sub-acute studies. LD_{50} of lantadene in sheep was found to be 1-3 mg/kg body weight via the intravenous route and 60 mg/kg via the oral route (slow absorption by GI tract). 25 mg/kg dose of lantadenes did not show mortality in guinea pigs but led to hepatoxicity and nephrotoxicity, as evident from biochemical and histopathological alterations.[4] Though toxicity studies conducted on lantadenes or on leaf extract of *L. camara*, have been reported till the complete scientific validation of the toxic effects of this weed is lacking. The present study deals with the acute, sub-acute and sub-chronic toxicity study and antioxidant activity of n-hexane and chloroform extracts of *L. camara* and phytochemical investigation by GC-MS and HPTLC analysis.

MATERIALS AND METHODS

Animals

Toxicity studies and antioxidant assay were carried out on Wistar albino rats with an average weight between 150-250 g. Study protocols were approved by the Institutional Animal Ethical Committee of Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh (1157/PO/Re/S/07/CPCSEA). All experimental procedures on animals were conducted as per the guidelines of the Indian National Science Academy. Laboratory animals were procured from the animal house facility of Central Drug Research Institute, Lucknow. All animals were acclimatized for 48 hr before the experiment. Standard laboratory conditions were followed for housing experimental animals with a constant temperature of 25±2°C and humidity of 60±5%.

Chemicals

All the chemicals used in this study were of synthetic grade and procured from Merck specialties Pvt. Ltd. (Mumbai, India) and Himedia Laboratories (Nashik, India). The enzymatic antioxidant assay was performed with the help of a UV Spectrophotometer (JASCO V-630).

Test Drug

Lantana camara L. was collected from different locations of Khordha district in the month of March 2017 and authenticated visually according to a taxonomic method by Dr. K.B. Satapathy of Centurion University of Technology and Management, Bhubaneswar, Odisha, India. A sample voucher (UDPS-Dash-1006) was submitted at the herbarium of the University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Odisha, India.

The plant material (whole plant) was collected, washed and dried under shade. After that, this was converted to coarse powder in a mixer grinder. This powder material was used for the preparation of extract. The plant material was extracted successively with n-hexane and chloroform as solvents separately. The obtained extract was filtered and concentrated in a rotary evaporator (IKA Model RV 10D S96) to remove the excess solvent. Concentrated semisolid extract was stored in desiccators till further use.

HPTLC Profiling and GC-MS analysis of test drug

HPTLC study was carried out by CAMAG (Muttenz, Switzerland) HPTLC system, consisting of a Linomat V Automatic Sample Spotter (Camag Muttenz, Switzerland); syringe (100 µL) (Hamilton); developing chamber (CAMAG glass twin trough chamber). The TLC plate was precoated with silica gel 60 F_{254} (size 20 × 10 cm). Benzene: Chloroform: Ethyl acetate (6:3:1) was used as a solvent system. The plate was scanned at 254nm before spraying at 600nm and after spraying with detection reagent (p-Anisidine-Sulfuric acid) after that the plate was heated at 100°C for 5 min. The R_f values and color intensity of the bands were recorded.

GC-MS analysis of the test drug was carried out using a Scion 436-GC (Bruker) coupled with a triple quadrupole mass spectrometer (TRACE 1310/ISQ–LT; Thermo Scientific) equipped with a BR-5 MS (5% Diphenyl/95% Dimethyl poly siloxane) capillary column (30m ×0.25 mm, 0.25 µm particle size), at a constant flow of 1.0 mL/min and injection volume of 2µL was employed at a split ratio 10:1. The temperature of the oven was maintained at 110°C for 3.5 min, then this was increased to 200°C at a rate of 10°C/min, followed by 280°C at a rate of 5°C/min.

Mass spectra collected in the positive electron-ion mode with 70 eV ionization energy were recorded at 0.50 sec scan interval, at a scanning range of 50 to 500 amu. The source temperature and the inlet line temperature were 250°C and 290°C respectively. For the identification of metabolites NIST reference mass spectral library, having more than 62000 patterns was used.[8]

In vitro cytotoxicity study

In vitro cytotoxicity study of the test drug was carried out on l9p2 cell lines using a flow cytometer (BD FACS Acury, NJ, USA). The cells were seeded in 12 well plates with the density of 0.065X106 cells/well, at 37°C in a CO_2 incubator (Thermo Scientific BB150). Cells were treated with both the plant extract (5 µl each) and incubated for 2 hr. After incubation, cells were washed with the PBS buffer (pH 7.4) thrice to remove the excess of plant extract and trypsinized to make a cell suspension. Finally, the suspended cell solution was treated with 5µ Mpropidium iodide for 30 min (at 37°C), in PBS buffer which was supplemented with 1mM calcium chloride, magnesium chloride and 5mM glucose. Similarly, a set of untreated cells with both plant's extracts was taken as control. Fluorescence was recorded for the quantification of intracellular propidium iodide.

Acute toxicity study

Acute toxicity studies of *L. camara* (chloroform and n-hexane extracts) were carried out using OECD 420 guidelines in adult Wister albino rats (150-250g) of both sexes. The sighting study was done in an ascending order of dose (5, 50, 300 and 2000 mg/kg) followed by special observation for the initial 4 hr to all the animals for toxicity signs and behavioral changes. Thereafter the albino rats were observed for 14 days for any evident toxicity. Due to the absence of toxicity signs in the sighting study, the main studies were carried out at 2000mg/kg including 5 more rats. No toxicity signs and behavioral changes were observed. Hence, the drugs were assigned to GHS category 5 drugs.[9]

Sub-acute 28-days toxicity study

The sub-acute oral toxicity study was carried out in rodents for 28 days using OECD guidelines 407. Animals were divided into three groups (Group 1 as normal control; Group II as n-hexane extract and group III as chloroform extract) (n=10, five animals/sex/group). The test product was administered orally (200 mg/kg) once daily. At the 28th days, the animals were anesthetized and blood was withdrawn and estimated for various hematological parameters. Serum was separated and biochemical parameters were estimated. Finally, the animals were sacrificed and vital organs were excised for histopathological analysis. The excised organs were stored in 10% formalin till further analysis. During the study, change in body weight was observed at every 5 days interval.[10]

Sub-chronic-90 days toxicity study

The subchronic 90-day oral toxicity study was carried out as per the recommendation of OECD 408. Three groups of 10 male and 10 female Wistar rats received a dose of 200 mg/kg body weight at daily gavage for 90 consecutive days. Group 1 as normal control; Group II as n-hexane extract and group III as chloroform extract. Observations (mortality and changes in behavior) were made twice daily. Change in body weight was recorded every week. Alterations in hematological and biochemical parameters were also noted at the end of the study. Histopathological studies of excised organs were conducted to determine the damage at the organ level, if any for a longer period of time.[11]

Antioxidant assay

Antioxidant assay of *L. camara* (chloroform and n-hexane extracts) were carried out using Wistar albino rats of both sexes (150-200 g). Animals were acclimatized at least for 48 hr before the study and were housed in 12 hr day-night cycle. The animals were divided into four groups i.e., normal, FST, Group I and Group II. Group I was treated with chloroform extract, whereas Group II was treated with n-hexane extract at the dose level of 200 mg/kg body weight. All the animals were subjected to force swimming except normal.

The enzymatic antioxidant assay (SOD and MDA) was conducted in samples of experimental animals (serum, plasma and brain homogenate) in accordance to subsequent methods.

Statistical analysis

All the data were expressed as mean ±SD. Treatment group data were compared with control using Student's *t*-test. One-way ANOVA followed by Tukey's multiple-tests was used to compare the data at different time intervals.

RESULTS

Identification of plant metabolites by HPTLC and GC-MS

HPTLC analysis

The HPTLC chromatogram of both the extracts (n-hexane and chloroform) of *L. camara* can be distinguished at UV 254nm before derivatization. After derivation the both the extracts showed purple colored bands. The florescent and deep blue color bands (under 366 nm) evidenced 10 spots each with their corresponding ascending order of R_f values, 0.02, 0.11, 0.16, 0.19, 0.27, 0.51, 0.69, 0.82, 0.88, and 0.91 for n-hexane extract and with the R_f values in the ascending order of 0.04, 0.13, 0.18, 0.20, 0.29, 0.57, 0.71, 0.75, 0.85 and 0.90 for chloroform extract respectively conforms about the presence of different constituents (Figure 1).

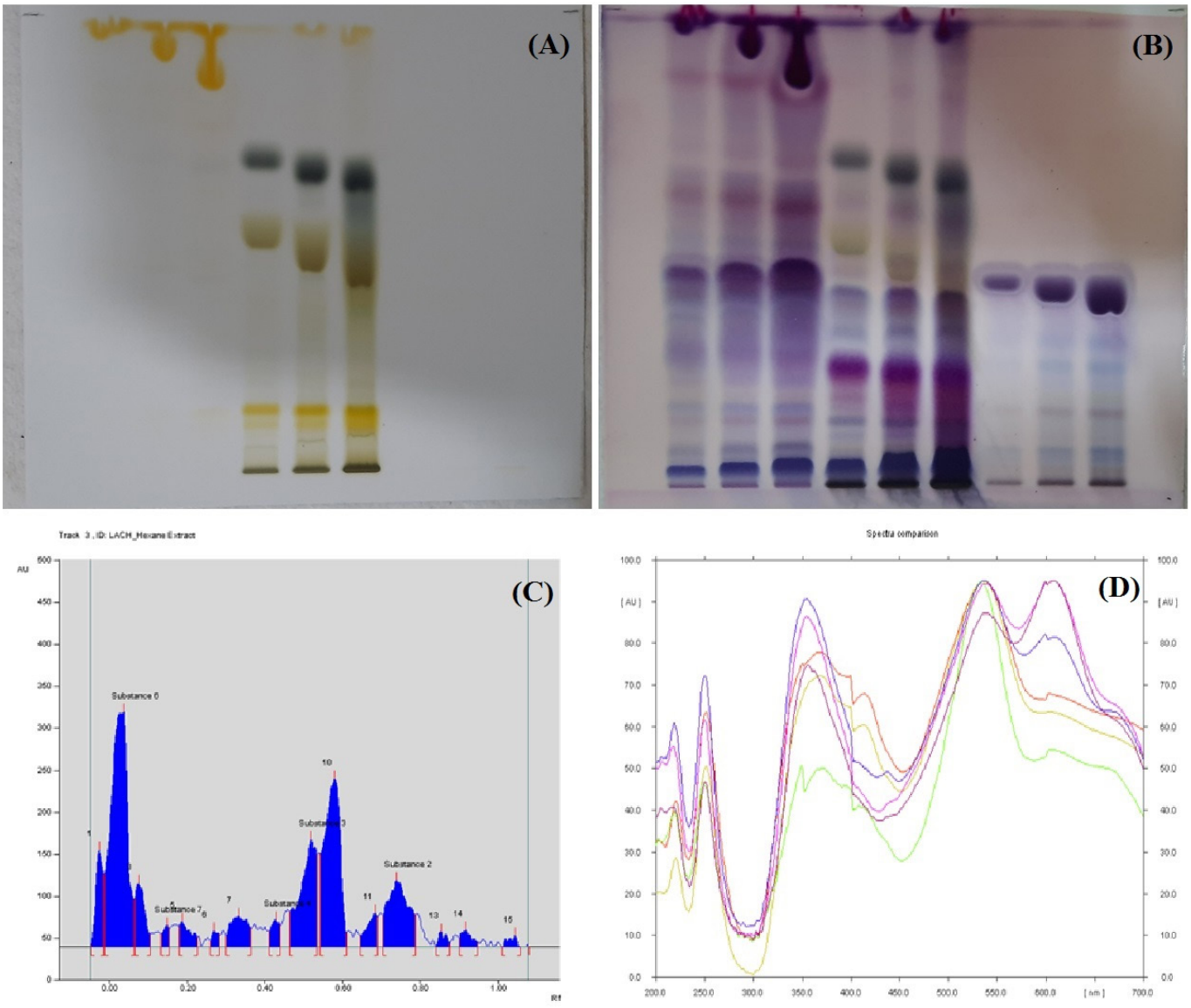

Figure 1: HPTLC profiling of n-hexane (A) and chloroform (B) fraction of *Lantana camara.*

GC-MS analysis

The obtained data of GC-MS with their retention time (R_t) and molecular formula are represented in (Tables 1 and 2). 25 compounds were identified in n-hexane extract of *L. camara* which are 1b,5,5,6a-Tetramethyl-octahydro-1 -oxa-cyclopropa[a]inden-6-one, Cholestan-3-ol, 2-methylene- ,(3β,5α),2-Methylenecholestan-3-ol, Retinal, cis-13-Eicosenoic acid etc. Similarly, 19 compounds were identified in chloroform extract which are Methane-oxybis-dichloro, Phenol, 2,4-bis(1,1-dimethylethyl)-, Cholestan-3-ol, 2-methylene-, (3β,5α)-, 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro, 2,5-Octadecadiynoic acid, methyl ester etc. The mass spectra of identified compounds from *L. camara* are presented in Figure 2.

In vitro cytotoxicity Study

In-vitro cytotoxicity study of n-hexane and chloroform extract of plant *Lantana camara* L. was performed using l9p2 cell lines. Obtained data are depicted in Figure 3. Data reveals that intracellular uptake of propidium iodide was found 7.35±0.55 and 9.03±0.82 in case of plant extract samples extracted in n-hexane and chloroform respectively as compared to the control. Cellular toxicity data is clearly indicated that plant extract was found safe for the l9p2 cell line. Maximum cells were found to be safe after exposure to both types of plant extract.

Acute toxicity study

In acute toxicity study conducted for a period of 14 days showed that no of toxicity signs in sighting study, the main studies were carried out at 2000mg/kg including 5 more rats. No toxicity signs

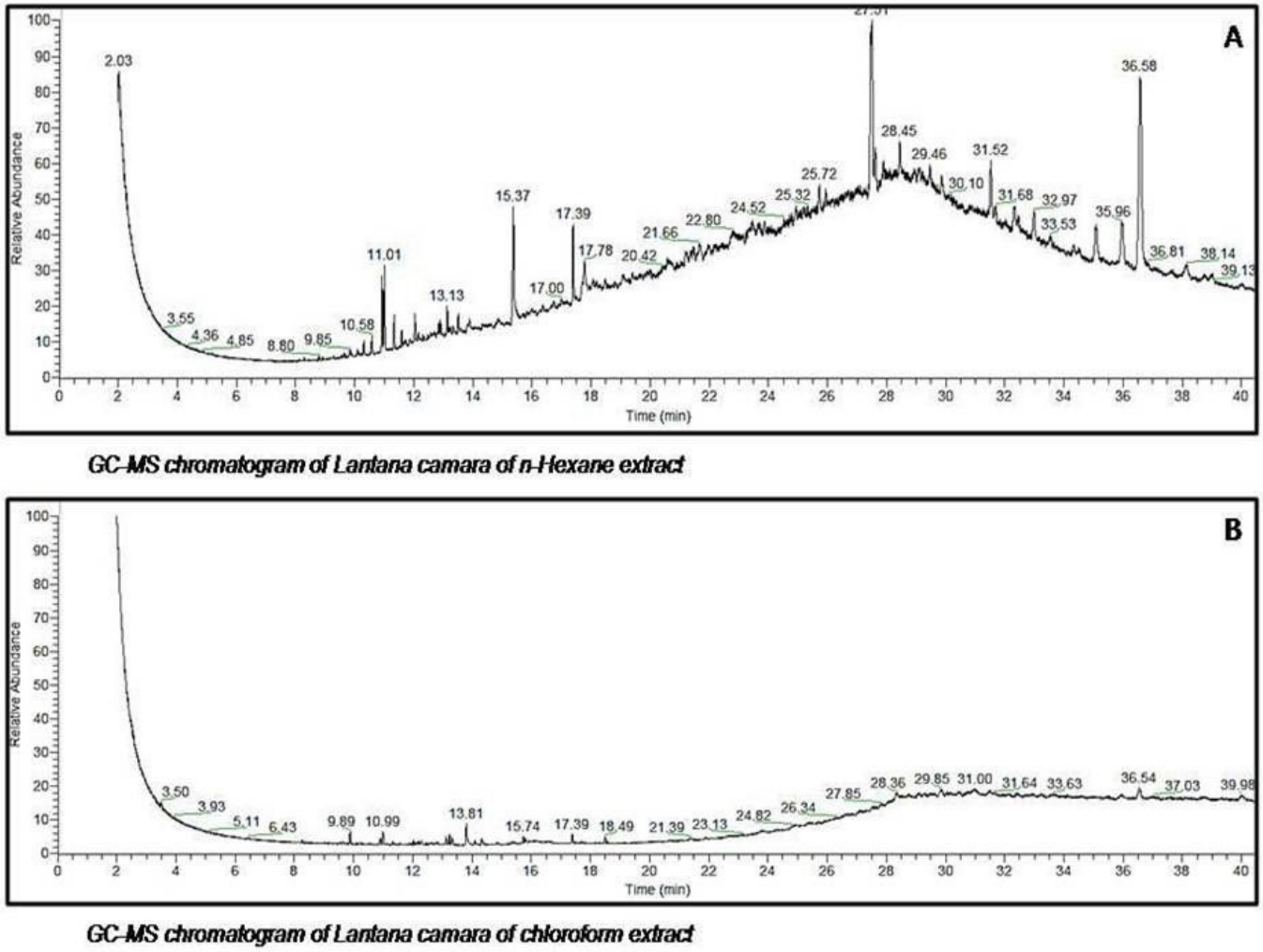

Figure 2: GC-MS analysis of n-hexane (A) and chloroform fraction (B) of *Lantana camara.*

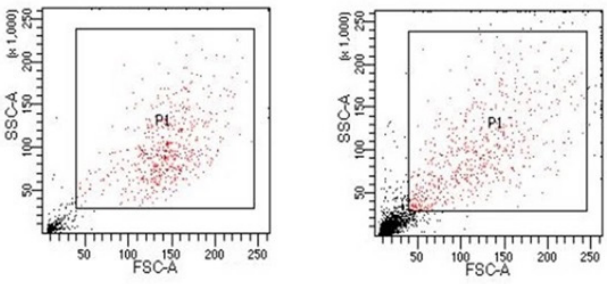

Figure 3: PI Uptake in l9p2 cells.

and behavioral changes were observed in both the extracts of *L. camara*.

Sub-acute toxicity study

In order to perform the sub-acute toxicity of n-hexane and chloroform extract of *L. camara,* the toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored for 28 days. It was noticed that n-hexane extract was found to cause alterations in biochemical ($p>0.01$) and hematological ($p>0.05$) parameters (Table 3 and 4). Some behavioral parameters such as aggressiveness, alopecia, alertness, grip strength, lacrimation and other reflexes were also observed at the end of 28 days showed no toxic signs at dose of 200 mg/kg chloroform extract of *L. camara* (Table 5). Though, no significant change ($p>0.05$) in body weight was recorded in any of the treatment groups.

Sub-chronic toxicity study

Sub-chronic study for a period of 90 days exhibits no toxic signs in any of the treatment groups i.e in n-hexane and chloroform extracts. Hematological ($p<0.01$), biochemical ($p<0.05$) and histopathological (Figure 4) findings revealed no alterations noted at the end of the study period (on the 90th day). Dose of 200 mg/kg n-hexane and chloroform extracts was safely administered to Wistar rats for 90 days. This long-term toxicity study exhibited no toxic symptoms on any organs and therefore we concluded that both extracts are safe on long-term administration.

Table 1: Compounds identified in n-Hexane extract of *Lantana camara*.

Sl. No.	Compound Name	Formula	R_t
1	1b, 5, 5, 6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	10.31
2	Cholestan-3-ol, 2-methylene-, (3β,5α)	$C_{28}H_{48}O$	10.58
3	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-	$C_{15}H_{24}O$	10.92
4	Caryophyllene oxide	$C_{15}H_{24}O$	11.01
5	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	11.34
6	2-Methylenecholestan-3-ol	$C_{28}H_{48}O$	11.59
7	5α-Cholestan-3β-ol, 2-methylene	$C_{28}H_{48}O$	12.04
8	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	12.90
9	Retinal	$C_{20}H_{28}O$	13.13
10	5α-Cholestan-3β-ol, 2-methylene	$C_{28}H_{48}O$	13.50
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	15.37
12	2-Hexadecen-1-ol, 3,7, 11, 15-tetramethyl	$C_{20}H_{40}O$	17.39
13	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	17.78
14	2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin	$C_{15}H_{24}O_4$	24.94
15	Oleic acid	$C_{39}H_{76}O_3$	25.72
16	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	25.92
17	1.2, 2, 4-Trimethyl-3-[(3E,7E,11E)-3,8,12,16-tetramethyl-3,7,11,15-heptadecatetraenyl]cyclohexanol	$C_{30}H_{52}O$	27.47
18	Tetrapentacontane, 1,54-dibromo	$C_{54}H_{108}Br_2$	28.45
19	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$	29.46
20	17-Pentatriacontene	$C_{35}H_{70}$	31.52
21	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-7,10-dihydroxy-1, 1, 3, 6, 9-pentamethyl-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester,	$C_{27}H_{38}O_8$	32.45
21A	Tetrapentacontane, 1,54-dibromo	$C_{54}H_{108}Br_2$	32.97
22	7, 8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{54}O_4$	34.32
23	Stigmasterol trimethylsilyl ether	$C_{32}H_{56}OSi$	35.09
23A	Tetrapentacontane, 1,54-dibromo	$C_{54}H_{108}Br_2$	35.96
24	β-Sitosterol	$C_{29}H_{50}O$	36.58
25	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	38.14

Antioxidant assays

Antioxidant assays conducted on samples of experimental animals (serum, plasma and brain homogenate) revealed no toxicity-related alterations in MDA and SOD content in both the extracts. MDA level tends to decrease in n-hexane ($p>0.05$) and chloroform ($p>0.05$) extracts as compared to normal. MDA is an oxidant marker that tends to decrease resulting in antioxidant activity. SOD is a pro-oxidant marker which increases in n-hexane ($p>0.05$) and chloroform ($p>0.01$) extracts. This showed that both extracts contain certain phytochemicals that are responsible for the antioxidant effect (Figure 5).

DISCUSSION

Toxicity studies assess the undesirable or detrimental effects of substances or plant extracts in bolus or repeated exposure. These studies provide information related to organ toxicity and also identify the adverse effect level.[12] Identification of unknown plant phytochemicals plays a crucial role in the modernization, medicinal development and quality control of herbal formulation. The study of medicinal plants provides a comprehensive idea of plant phytochemicals with their usage and also helps to protect humans or animals from natural poisons.[13] Hence, the present study was undertaken with the aim to find out the phytochemicals present in both n-hexane and chloroform extracts of *L. camara* by using gas chromatography and mass spectrometry. HPTLC fingerprint of both the extracts showed the separation of different

Table 2: Compounds identified in chloroform extract of *Lantana camara*.

Sl. No.	Compound Name	Formula	R_t
1	Methane, oxybis[dichloro	$C_2H_2Cl_4O$	3.50
2	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	9.89
3	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	10.99
4	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro	$C_{22}H_{40}O_2$	11.32
5	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	12.02
6	9,10-Secocholesta-5,7,10(19)-triene-3,25,26-triol / 7-Methyl-Z-tetradecen-1-ol acetate	$C_{27}H_{44}O_{3}$ / $C_{17}H_{32}O_2$	13.13
6A	1-Hexadecanol, 2-methyl	$C_{17}H_{36}O$	13.24
6B	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	13.32
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	13.81
8, 8A	Ethanol, 2-(9-octadecenyloxy)	$C_{20}H_{40}O_2$	14.11 /14.33
9	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	15.74
10	2-Nonadecanone 2,4-dinitrophenylhydrazine	$C_{25}H_{42}N_4O_4$	15.82
11	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy	$C_{23}H_{30}N_2O_5$	17.39
12	2-Hexadecanol	$C_{16}H_{34}O$	18.49
13	Oleic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	21.39
14	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	28.36
15	Linoleic acid, 2,3-bis-(O-TMS)-propyl ester	$C_{27}H_{54}O_4Si_2$	29.85
16	9,12-Octadecadienoic acid (Z,Z),2,3-bis[(trimethylsilyl)oxy]propyl ester	$C_{27}H_{54}O_4Si_2$	31.00
17, 18	2,3-Bis[(trimethylsilyl)oxy]propyl (9Z,12Z)-9,12-octadecadienoate	$C_{27}H_{54}O_4Si_2$	31.50 /31.64
19	8,14-Seco-3,19-epoxyandrostane-8,14-dione,17-acetoxy-3β-methoxy-4,4-dimethyl	$C_{24}H_{36}O_6$	36.54

Table 3: Biochemical Parameters.

	28 days			90 days		
Biochemical parameter	**Control**	**n-Hexane**	**Chloroform**	**Control**	**n-Hexane**	**Chloroform**
Creatinine (mg/dL)	0.5890±0.079	0.6600 ± 0.049	0.5540 ± 0.074	0.46±0.06	0.42±0.05	0.44±0.07
Urea (mg/dL)	15.30 ± 0.47	14.50 ± 0.40	15.20 ±0.57	15.30±0.51	15.10± 0.67	16.31±0.10
Triglycerides (mg/dL)	52.20±1.13	51.40±1.08	47.10±1.62	54.03± 0.66	53.51±0.17	54.89± 0.23
Total Cholesterol (mg/dL)	46.60±1.21	51.40±1.08	54.03±1.67	54.16± 0.19	55.12 ±0.34	56.11±0.27
Total protein (mg/dL)	4.40±0.26	4.20±0.35	3.70±0.26	4.50± 0.21	5.30± 0.43	5.50 ±0.87
Albumin (g/dL)	3.20±0.41	3.70±0.33	3.20±0.29	3.71± 0.11	4.17± 0.91	4.54± 0.97
AST (IU/L)	121.41±2.68	121.3±1.65	116.61±2.045	120.12± 1.10	121.45±1.20	125.13± 1.45
ALT (IU/L)	69.40±1.57	67.60±1.301	68.60±1.108	68.16± 1.12	69.18± 1.16	7034 ±1.78
ALP (IU/L)	112.6±4.67	117.01±0.714	117.41±0.718	115.10±2.10	116.23±1.12	117.21± 1.36
T. Bilirubin (mg/dL)	0.2569±0.32	0.267±0.029	0.254±0.023	0.28± 0.01	0.30± 0.04	0.31± 0.07

Data are expressed as mean ± SEM.

Table 4: Hematological Parameter.

	28 days			90 days		
Hematological parameter	**Control**	**n-Hexane**	**Chloroform**	**Control**	**n-Hexane**	**Chloroform**
Total R.B.C. count ($\times 10^6$ mm−3).	9.09±0.15	8.90±0.12	9.11±0.16	8.26±0.09	8.00±0.14	8.22±0.14
Total W.B.C. Count ($\times 10^3$ mm−3).	12.67±0.22	12.35±0.15	11.23±0.23	13.46±1.01	13.06±1.11	14.21±0.45
Haemoglobin (Hb) (g/dL)	15.61±0.36	14.07±0.30	15.63±0.36	12.88±0.46	13.70±0.51	13.30±0.58
Hematocrit (%).	44.21±1.01	43.61± 1.72	36.4±1.36	40.35±0.85	45.16±0.12	38.19±1.23
Platelets (×103 mm−3).	834.91±24.01	867.21±23.25	739.81±26.86	37.88±1.20	35.22±1.31	34.28±1.20
Lymphocytes(%).	84.7±1.32	81.8±1.33	72.8±1.43	80.12±1.12	83.42±1.45	78.14±1.34
Neutrophils (%).	20.6±0.65	12.6±0.52	19.2±0.91	19.65±0.35	23.67±1.20	25.17±1.64

Data are expressed as mean ± SEM.

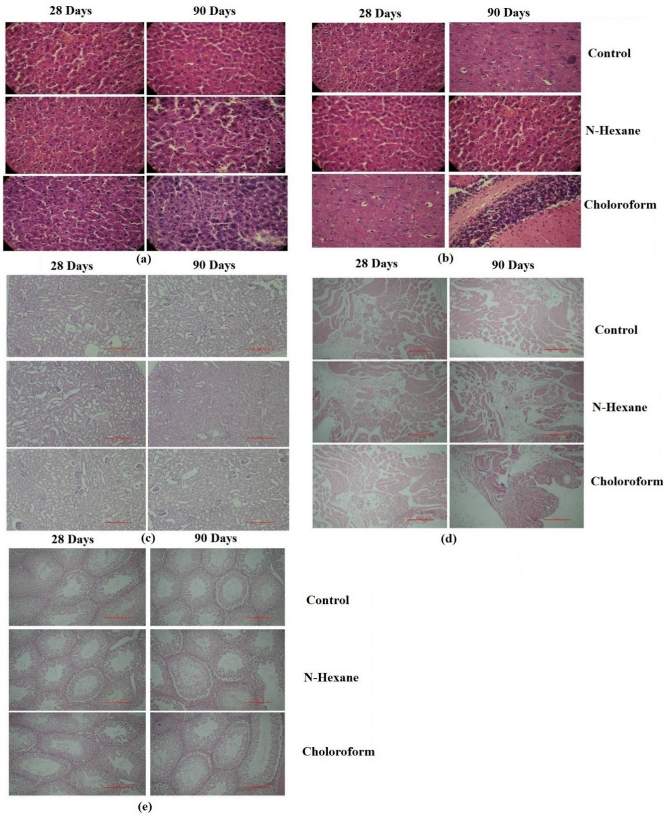

Figure 4: Histopathological findings of n-hexane and chloroform fraction of *Lantana camara* in brain (a), Liver (b), Kidney (c), Ovaries (d) and Testes (e) at the end of 28th and 90th day.

constituents and their R_f values are reported. GC-MS analysis of n-hexane and chloroform extracts of *L. camara* revealed the presence of 25 and 19 compounds respectively. Some of the compounds identified by this study include, fattyacids, sterols, eicosanoids, terpenoids etc. are which endowed with biological activities. The weed, *L. camara* is toxic due to the presence of certain constituents while some constituents are responsible for protective effects in different ailments. Therefore, the toxicity study has been carried out on *L. camara* extracts in order to assume that the weed is safe on oral administration. In the acute toxicity study, we found that both the extracts did not produce any mortality and thus were safe during the study period of 14 days of observation. No significant change in body weight or behavioral alteration was noted during the observation period. Considering the findings of the sub-acute toxicity study for 28 days, we found that no momentous change was seen in body weight and weight of liver, kidney, heart and reproductive organs, suggesting that administration of both extracts of *L. camara* at the sub-acute dose of 200 mg/kg had no effect on normal growth. The measure of body weight and organ weight are important indicators as they provide information pertaining to toxicity, enzyme induction, acute injury or any other physiologic perturbations. Organ toxicity correlates with histopathological changes.[14] The histopathological findings depict the anatomical or pathological alterations in vital organs. The findings of the present investigation depict that the vital organs such as kidney, liver, heart, ovaries and testes were not adversely affected throughout the treatment period. No noteworthy reduction was noted in biochemical and hematological parameters in treated animals at the dose tested, we conclude that n-hexane and chloroform extracts have no toxicity in analyzed organs. Serum hematology and clinical biochemistry analysis were performed to evaluate any possible alteration involved in renal and hepatic functions influenced by both extracts. Liver and kidney function evaluations are the foremost analysis, as they are major organs that are involved in metabolism and elimination.[12] Increase in ALT, AST and ALP levels demonstrate signs of hepatotoxicity (Parimoo and Sharma 2014). n-Hexane and chloroform extracts did not show any toxicity-related signs in sub-acute toxicity studies. As we all are aware of the fact that weed is most noxious and poisonous, therefore, a sub-chronic study was performed to confirm the correct toxicological profile of these extracts.[4,15] Parameters observed during the treatment period of 90 days were weekly monitoring of body weight, feed and water intake, behavioral changes and on the last day i.e 90th day, analysis of hematological and biochemical parameters. Histopathological

Table 5: Signs of toxicity in sub acute toxicity (28 days).

Parameters observed	Day-2	Day-4	Day-6	Day-8	Day-10	Day-12	Day-14	Day-16	Day-18	Day-20	Day-22	Day-24	Day-26	Day-28
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reflex														
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

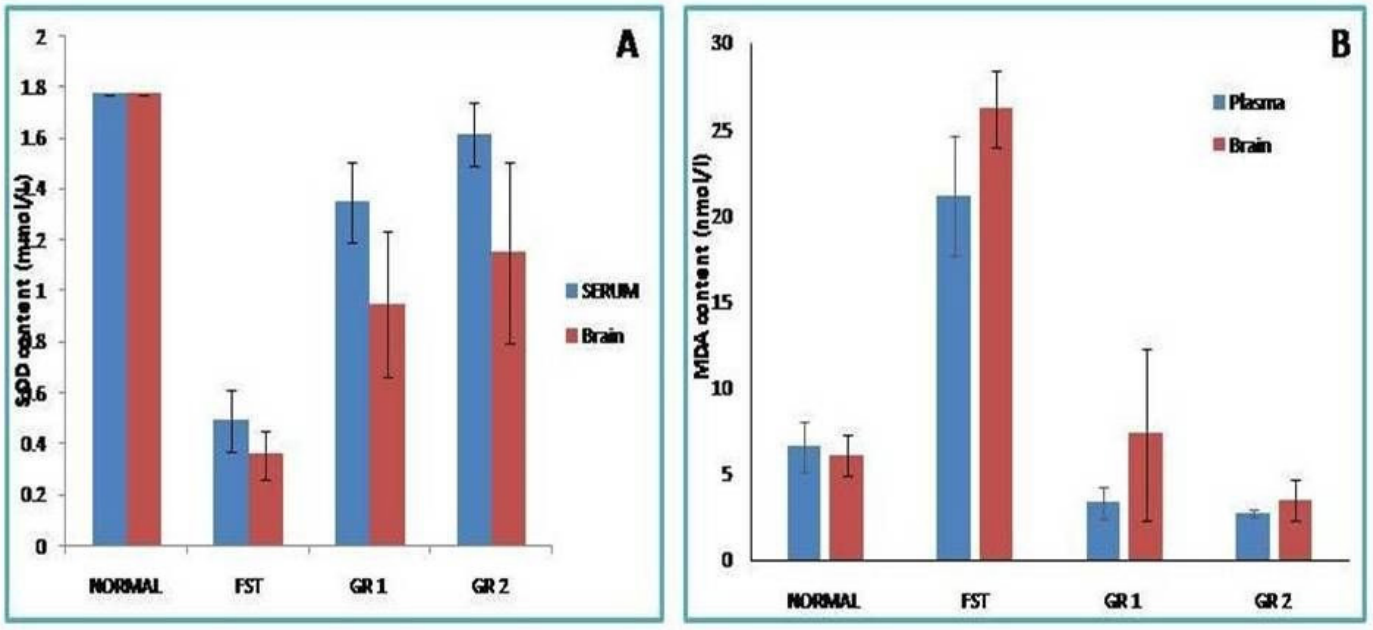

Figure 5: Level of antioxidant in n-hexane and chloroform fractions of *Lantana camara*.

findings were also noted down after the sacrifice of animals on the last day. All the parameters studied during 90 days protocol revealed no toxic signs and symptoms in experimental animals. No significant changes in body weight were noted. Both extracts administered at a tested dose for a longer duration did not produce any organ toxicity or mortality. Our research group effectively demonstrates in this study that both extracts record no deaths or serious toxic symptoms. No morphological and histopathological changes were observed in any organ which confirms that both extract are safe while administered orally.

In summary, acute (14-days), sub-acute (28 days), and sub-chromic (90 days) studies depict that n-hexane and chloroform extracts are safe on single bolus administration and on continuous repeated exposure for a longer duration of time.

CONCLUSION

n-Hexane and chloroform extracts of *L. camara* were tested for their toxicity. From the current study, the extracts were found to be non-toxic. *In vivo* studies also revealed the lack of toxicity in both extracts. Therefore, these extracts can be used safely to cure different ailments.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPTLC: High-Performance Thin Layer Chromatography; **GC-MS:** Gas Chromatography-Mass Spectrophotometry; **OECD:** Organization for Economic Cooperation and Development; ***L. camara*:** *Lantana camara;* **GI tract:** Gastro Intestinal Tract; **TLC:** Thin Layer Chromatography; **CO_2:** Carbon di Oxide; **PBS:** Phosphate Buffer Saline; **GHS:** Globally Harmonized System; **SOD:** Superoxide dismutase; **MDA:** Malondialdehyde; **ALT:** Alanine transaminase; **AST:** Aspartate aminotransferase; **ALP:** Alkaline phosphatase.

REFERENCES

1. Pour BM, Latha LY, Sasidharan S. Cytotoxicity and oral acute toxicity studies of *Lantana camara* leaf extract. Molecules. 2011;16(5):3663-74. doi: 10.3390/molecules16053663, PMID 21540795.
2. Verdeguer M, Blázquez MA, Boira H. Phytotoxic effects of *Lantana camara, Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. Biochem Syst Ecol. 2009;37(4):362-9. doi: 10.1016/j.bse.2009.06.003.
3. Kumar R, Katiyar R, Kumar S, Kumar T, Singh V. *Lantana camara*: An alien weed, its impact on animal health and strategies to control. J Exp Biol. 2016;4:3S.
4. Kumar R, Sharma R, Patil RD, Mal G, Kumar A, Patial V, Kumar P, Singh B. Sub-chronic toxicopathological study of lantadenes of *Lantana camara* weed in Guinea pigs. BMC Vet Res. 2018;14(1):129. doi: 10.1186/s12917-018-1444-x, PMID 29653586.
5. Parimoo HA, Sharma R. Orally induced sub-acute toxicity of Lantadenes of *Lantana camara* in guinea pigs: A haematological study. J Pathol. 2014;1(2):12-5.
6. Pass MA, Pollitt S, Goosem MW, McSweeney CS. The pathogenesis of lantana poisoning. Queensland poisonous plants committee, Yeerongpilly, Australia. Plant Toxicol. 1985:487-94.

7. Ganai GN, Jha GJ. Immunosuppression due to chronic *Lantana camara*, L. Toxicity in sheep. Indian J Exp Biol. 1991;29(8):762-6. PMID 1769720.
8. Pakkirisamy M, Kalakandan SK, Ravichandran K. Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacogn J. 2017;9(6):956-2.
9. OECD [Organization for Economic Co-operation and Development]. Paris: OECD; 1992. Guideline 420: Acute oral toxicity—Fixed dose procedure.
10. Organization of economic co-operation and development (OECD). The OECD guideline for testing of chemicals: 408 Subchronic Oral Toxicity—Rodent: 90 Day Study. Paris, France: OECD, 1998.
11. Pati AK, Subudhi BB, Sahu PK. Chronic forced swimming induced stress alters behavioural, histological and anti-oxidant status. Indian Drugs. 2017;54(06):06.
12. Isbrucker RA, Edwards JA, Wolz E, Davidovich A, Bausch J. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: Dermal, acute and short-term toxicity studies. Food Chem Toxicol. 2006;44(5):636-50. doi: 10.1016/j.fct.2005.11.003, PMID 16387402.
13. Sousa EO, Costa JGM. Genus Lantana: Chemical aspects and biological activities. Rev Bras Farmacognosia. 2012;22(5):1115-80. doi: 10.1590/S0102-695X2012005000058.
14. Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Vet Pathol. 2013;50(6):1007-15. doi: 10.1177/0300985813485099, PMID 23558974.
15. Sharma OP, Sharma S, Pattabhi V, Mahato SB, Sharma PD. A review of the hepatotoxic plant *Lantana camara*. Crit Rev Toxicol. 2007;37(4):313-52. doi: 10.1080/10408440601177863, PMID 17453937.

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