Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 373-377
Received: 22-09-2019
Accepted: 24-10-2019

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Effect on the body weight, organ weight and haematology during sub-chronic lantadene toxicity and its amelioration with *Berberis lycium* and *Picrorhiza kurroa* in guinea pigs

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Abstract
Background: The objective of this study was to investigate the ameliorative effect of *Berberis lycium and Picrorhiza kurroa* on lantadene-induced sub-chronic toxicity in Guinea pigs.
Method: Total 42 Guinea pigs of either sex were divided into 7 groups: Group-C served as control, Group- L (Lantadene @ 24mg/kg bw), Group-L+B1 (*B. lycium* @ 100 mg/kg bw), Group- L+B2 (*B. lycium* @ 200 mg/kg bw), Group- L+P1 (*P. kurroa* @ 100 mg/kg bw), Group- L+P2 (*P. kurroa* @ 200 mg/kg bw), Group- L+ S (Silymarin @ 100 mg/kg bw). Each group except control received oral dose of lantadene (24 mg/kg bw) daily. Ameliorative groups were treated with daily oral dose of ameliorative plant extract one hour after lantadene treatment.
Results: Significant decrease in weekly body weights, Hb (gm/dl), PCV% and significant increase in absolute and relative weight of liver were recorded.

Keywords: Sub-chronic, lantadene toxicity, amelioration, *Berberis lycium*, *Picrorhiza kurroa*, guinea pigs

1. Introduction
India is blessed with a rich biological diversity and ranks 6th amongst the 17 mega biodiversity nations of the world [1]. Plants are the integral part of biodiversity; amongst them some plants are very noxious and produce toxicity in livestock. These toxic plants are of major concern to Veterinarians because of their harmful effects to livestock in terms of reducing productivity and causing mortality [2]. The severity of toxic effects of poisonous plants depend upon the variety among species, nature, part and amount of toxic component taken, environmental conditions, species, age, body size and body condition of the animals [3]. Among poisonous plants, *Lantana camara* is one of the most commonly known noxious [4] and invasive weed worldwide [5].
Lantana camara Linn. (common name lantana, wild sage; locally known as bara phulnoo in Himachal Pradesh), an ornamental shrub, belongs to the family Verbenaceae. The branches of the plant possess curved prickles, grows to a height of 2–3 m, and its spreaded branches cover an area of about 1 m^2 [6]. The most prevalent and most noxious variety of *L. camara* is red flower variety (*L. camara* var. *aculeata*), which causes severe toxicity in grazing animals [7]. It has been reported that it make animals ill severely after ingestion. Its foliage contains the toxic pentacyclic triterpenoids, called lantadenes. Major lantadenes are A, B, C, D and reduced lantadene A and reduced lantadene B. Lantadene A and Lantadene B cause hepatotoxicity and photosensitivity in grazing animals such as sheep, goats, bovines and horses [3].
In India, hepatoprotective medicinal plants and their formulations have been traditionally used in ayurveda for the prevention and treatment of liver diseases. *Berberis lycium* belongs to family Berberidiceae. *B. lycium* contains berberine, palmitine, berbamine and a number of other compounds [8]. *B. lycium* is reported to possess antibacterial, antifungal, anticoccidial, immunostimulant, antimutagenic, and antihyperlipidemic activities [9].
Picrorhiza kurroa is a well-known herb in the ayurveda and has traditionally been used to treat disorders of the liver and upper respiratory tract. It reduces fever and is also used in treating dyspepsia, chronic diarrhoea and scorpion sting. The medicinal importance of *P. kurroa* is due to its pharmacological properties like hepatoprotective, antioxidant (particularly in liver), antiallergic and antiasthamatic, anti cancerous activity particularly in liver and immunomodulatory effect [10].

Considering these facts, the study was planned to assess ameliorative effect of *Berberis lycium* and *Picrorhiza kurroa* against lantadene-induced sub-chronic toxicity in Guinea pigs.

2. Materials and Methods

2.1 Plant material

Leaves of red flower variety of *L. camara* were collected during the month of October-November from an area adjoining Palampur town (H.P.) located at an altitude of 1200 m above msl. The samples were oven dried at 55°C and ground to a fine powder of 1 mm particle size. The extraction of lantadenes was carried out by protocol described earlier [11]. The isolated lantadenes were stored in sealed vials at room temperature until further use. The purification was monitored by thin layer chromatography (TLC) [11].

Root bark of *B. lycium* and rhizome of *P. kurroa* were washed with water, shade-dried and ground to a fine power of 1 mm particle size using grinder. The dried powder of each plant material was extracted with methanol and was kept overnight at room temperature. The extract was filtered through coarse filter paper and filtrate of each plant material was concentrated on rotary evaporator (Heidolph Laborota 4000) at 40°C and freeze-dried (Lab tech, LFD-5508S) at high vacuum (250 torr) and at low temperature (-40°C) and was stored at -20°C until further use. The yield of the freeze-dried methanolic extract of ameliorative plants was calculated as percentage:

Total yield of extract (%) = (Weight of extract / Weight of sample) x100

2.2 Experimental design

Guinea pigs (200-250 g) of either sex were procured from Central Research Institute (CRI), Kasauli, H.P., and housed in clean polypropylene cages under standard laboratory conditions of humidity (55±10% RH) and temperature (25±2°C). All sanitary and hygienic measures were observed as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. After acclimatization for one week, the Guinea pigs were randomly divided into 7 groups with 6 animals in each group and were given the following treatments:

Treatment groups:

I) C- Control
II) L- Lantadene @ 24 mg/kg bw
III) L+ B1 (*B. lycium* @ 100 mg/kg bw)
IV) L+ B2 *(B. lycium* @ 200 mg/kg bw)
V) L + P1 (*P. kurroa* @ 100 mg/kg bw)
VI) L+ P2 (*P. kurroa* @ 200 mg/kg bw)
VII) L+ S (Silymarin @ 100 mg/ kg bw)

Each group except control received oral dose of lantadene (24 mg/kg bw) daily. Groups were treated daily with oral dose of ameliorative plant extract one hour after lantadene treatment. Silymarin was used as positive control [12].

2.3 Haematology

For haematological study, about 1 ml of blood sample was collected in to dry sterilized anticoagulant vials containing K2-EDTA from posterior vena cava at the time of euthanasia. The haematological estimations, such as haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) were carried out.

2.4 Absolute organ weight

Animals were euthanized after the completion of 90 days trial period and examined. Necropsy was performed to examine gross pathological lesions in various internal organs. Specific organs of interest were preserved in 10% neutral buffered formalin for further evaluation of histological anomalies. The organs, kidneys and liver were immediately taken out and weighed after being washed with cold normal saline.

2.5 Relative organ weight

The internal organs (liver and kidneys) were removed and weighed to calculate the relative organ weights (using the formula).

Relative organ weight (%) = (organ weight (g) / body weight of the animal on sacrifice day (g)) ×100

2.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to detect differences among groups and the means±S.E. were compared by Tukey's multiple comparison test using 0.05 (95% confidence and significance level). All analyses were performed with Graph Pad Prism software, 7.04 version (San Diego, CA). T-Test was used for analysis of percent yield of freeze-dried methanolic extract of ameliorative herbal plants.

3. Results

3.1 Percent yield and thin layer chromatography

In the present study, percent yields of freeze-dried methanolic extract of *B. lycium* and *P. kurroa* ameliorative herbal plants were 5.21±0.617 and 17.58±2.587, respectively (Table 1). In our study, the main components, lantadene A and lantadene B were present in appreciable levels in the leaves of lantana. The thin layer chromatogram for lantadene A and B is presented in Figure 1.

3.2. Clinical signs

No toxic symptoms or mortality was observed in any animal during the study period. All groups were normal and did not display significant changes in behaviour except reduced food intake in lantadene treated group.

3.3. Weekly body weight gain

The total treatment period was of 90 days. All throughout the experimental period, the lantadene treated animals maintained a continuous decrease in body weight. The body weight at 12th week in lantadene administered animals (340.50±2.35) showed statistically significant decline as compared to the animals of control (381.00±7.03) and ameliorative groups L+B1 (358.50±9.63), L+P1 (338.83±1.25), L+P2 (356.66±3.94) and L+S (349.66±6.83). Subsequently, when ameliorative groups were compared with lantadene treated group, there was significant increase in body weight in ameliorative groups as compared to lantadene treated group (Table 2).

3.4 Haematology

There was a significant decrease in Hb (gm/dl) and PCV (%) levels in animals of lantadene treated group (7.60 ± 0.29 g/dl and 39.85 ± 0.68%, respectively) as compared to control group (11.65±0.28 g/dl and 45.74±0.84%). The PCV levels of ameliorative plant groups L+B1 (50.77±0.68), L+B2 (47.03±0.83), L+P1 (47.63±.50), L+P2 (46.74±1.72), L+S (45.82±1.22) were also high as compared to control but did

not show any significant difference. The results of the present study revealed no significant difference in TEC, TLC and DLC levels amongst animals of all the groups. The results of haematological changes have been presented in Table 3.

3.5 Effect on absolute weight of organs

In absolute organ weight determination, results showed that there was statistically significant increase in the absolute liver weight of lantadene treated group (20.97±0.43) as compared to control (18.91±0.36) and ameliorative group L+B2 (18.48±0.42), L+P2 (18.77±0.36). There was reduction in absolute kidneys weight of lantadene treated group as compared to control and other ameliorative groups but it was not statistically significant (Table 4).

3.6 Effect on relative organ weight of organs

In this study, statistically significant increase in relative liver weight of lantadene treated Guinea pigs (7.72±0.42) as compared to control (5.04±0.08) and ameliorative groups L+B1(5.34±0.27), L+B2 (5.46±0.08), L+P1(5.41±0.11), L+P2 (5.55±0.11), L+S (5.21±0.17) was recorded. There were no any changes in relative kidney weight amongst all the groups (Table 5).

4. Discussion

Lantana camara is the most widespread species of genus *Lantana*, growing luxuriantly at elevations up to 2000 m in tropical, sub-tropical and temperate regions [13]. This weed leads to hepatotoxicity and photosensitization in grazing animals and has allelopathic effect on other vegetation [3]. This plant is capable to cause mortality in ruminant as well as non-ruminant species. Among the non-ruminants, Guinea pigs are the most susceptible species [14] and therefore have been used as a model in the present study. In the present study, amelioration of sub-chronic lantadene toxicity was attempted using methanolic herbal extracts of *B. lycium* and *P. kurroa*. An appreciable yield of extracts was obtained from *B. lycium* (5.21±0.617%) and *P. kurroa* (17.58±2.587%). The presence of lantadenes in the leaves of *L. camara* was confirmed by thin-layer chromatography.

The body weight changes serve as sensitive indicators of general health status of animals [15]. In the present study, there was a significant decrease in weekly body weight of the animals of lantadene-treated group as compared to control and ameliorative groups. Kumar and co-workers (2018) [16] also reported a significant decrease in body weight of lantadene treated Guinea pigs during sub-chronic toxicity.

The haematopoietic system is one of the most sensitive targets for toxic plants and an important index of physiological and pathological status in human and animals [17]. There was a significant decline in the values of Hb and PCV in the animals of lantadene-treated group as compared to control and ameliorative groups. Whereas decrease in the values of PCV indicated shrinkage in the size of erythrocytes due to lantadenes intoxication as was also reported earlier [18]. Kumar and co-workers (2018) [16] reported a significant decrease in Hb and PCV levels during sub-chronic lantadene toxicity in Guinea pigs. A progressive decrease in PCV, TEC and haemoglobin in acute lantana toxicity in goats has been reported [19]. Similar study by Kalra and co-workers (1984) [20] reported a decrease in PCV, TEC, Hb, heterophil number and leucocytosis in buffaloes and cattle in lantana poisoning. However, intoxication of Guinea pigs with acute dose of lantadenes resulted in significant elevation of Hb concentration and PCV [21].

Organ weights are widely accepted in the evaluation of test article-associated toxicities [22]. The evaluation of organ weights is fundamental to many biological studies. This is particularly true in the field of toxicological drug testing. Dose-related increases in liver weight are commonly observed in repeat-dose toxicity studies performed in rodents, dog or other large animals [23]. In this study, there was a statistically significant increase in the absolute liver weight of lantadene treated group as compared to control and other ameliorative groups. Administration of xenobiotics may alter renal weight, and as a consequence any renal weight changes in toxicity studies should be assessed. When increases in renal weight are manifestations of toxicity, they are frequently associated with macroscopic appearances of swelling and pallor of the kidney and evidence of significant damage [23]. In the present study, there were no significant changes in absolute kidney weight between control and lantadene treated group.

Relative organ weight may serve as an indicator of pathological and physiological status in animals. Toxic substances induce abnormal metabolic reactions that affect primary organs such as liver and kidneys [24]. Alteration in organ weight is a sign of impairment in the normal functioning of the body organs. Organ-body weight ratio may indicate organ swelling, atrophy or hypertrophy [25]. In the present study, there was a significant increase in the relative liver weight of lantadene treated group as compared to control group. Though the relative kidney weight was higher, but it was statistically not significant as compared to control group.

The results of our study indicated that the oral administration of toxic dose of lantadenes (24 mg/kg bw) showed significant reduction of haemoglobin concentration, packed cell volume, and significant decline in the body weights in Guinea pigs. During amelioration, it was observed that both *Berberis lycium* (@ 200 mg/kg bw) and *Picrorhiza kurroa* (@ 200 mg/kg bw) ameliorated the effect of lantadene-induced toxicity to the greatest extent. There was not much difference between *B. lycium* (@ 200 mg/kg bw) and *P. kurroa* (@ 200 mg/kg bw) on the basis of above parameters. Further studies need to be conducted to evaluate the mechanism of toxicity with more parameters and to validate these herbal plants extracts for therapeutic use.

5. Acknowledgements

The authors are grateful to the Director, ICAR-IVRI, Izatnagar, Bareilly for providing necessary facilities. This work was supported by the research fund of ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, India (Project Code: IVRI/PALAM/13–16/010).

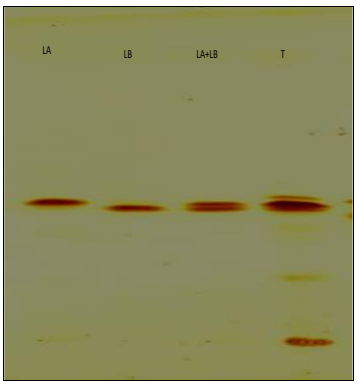

Fig 1: Thin layer chromatogram of lantadenes isolated from *Lantana camara* Linn. var. aculeate leaves. LA: lantadene A standard; LB: lantadene B standard; LA + LB: Mixture of lantadene A standard+ lantadene B standard; T: test sample

Table 1: Details of the plants, plant parts and percent yield of freeze-dried methanolic extract of different ameliorative herbal plants.

S. No.	Ameliorative herbal plant	Common Name	Plant part	Percent Yield (%)
1.	*Berberis lycium*	barberry	Roots	5.21±0.617
2.	*Picrorhiza kurroa*	kutki	Rhizome	17.58±2.587

Values are presented as mean ± SEM (n=3). Independent sample t-test was performed to analyze this dataset. $p<0.05$

Table 2: Effect on weekly body weight (gm) of control, lantadene treated and ameliorative groups (L+B1, L+B2, L+P1, L+P2, L+S) during sub-chronic toxicity study in Guinea pigs. Values are expressed on mean± S.E. for six animals in each group.

Days	C	L	L+B1	L+B2	L+P1	L+P2	L+S
Day 0	238.33±7.24	222.83±6.40	223.67±11.47	223.50±8.11	231.00±8.83	211.00±8.68	220.17±11.8
week 1	258.17±6.64	258.33±8.16	257.50±8.71	251.17±8.67	242.17±8.85	235.00±8.88a	233.00±6.00
week 2	281.00±6.90	265.83±7.67	268.67±8.23	268.83±11.39	270.67±8.82	164.00±10	257.83±8.52
week 3	290.33±6.21	273.00±7.81	283.17±9.88	281.66±9.86	277.67±6.98	273.50±8.2	272.50±6.82
week 4	298.33±6.15	277.84±7.17	289.00±9.51	291.17±8.30	286.83±5.12	283.33±6.19	281.50±7.49
week 5	306.33±5.39	284.83±7.49	297.67±8.75	301.50±8.31	295.67±5.39	294.67±5.31	292.00±7.57
week 6	317.33±4.69	291.83±6.91	306.67±8.32	309.67±7.09	302.83±5.49	303.83±4.71	300.50±7.11
week 7	325.67±4.68	301.00±6.64	314.50±8.23	317.16±7.12	310.16±4.28	311.16±3.69	308.83±6.39
week 8	333.00±4.96+	304.50±6.31*	321.00±9.01	326.66±5.98	320.66±3.04	321.33±1.85	319.50±6.55
week 9	339.00±4.33++	305.00±6.33**	328.33±9.46^{+}	333.16±5.89	328.33±2.84	326.16±1.72	326.16±6.61
week 10	347.83±4.51+++	303.16±7.61***	333.50±10.41+	339.66±6.01++	332.50±2.23+	336.16±2.02+	332.83±6.89
week 11	356.66±4.99+++	315.67±4.19***	354.16±9.41+++	347.00±5.70++	336.00±1.96	345.16±1.72+	341.83±7.02+
week 12	381.00±7.03+++	340.50±2.35***	358.50±9.63+++	357.50±4.66++	338.83±1.25	356.66±3.94+	349.66±6.83+

***$P<0.0002$ vs Control +++ $P<0.0002$ vs Lantadene
** $P<0.0021$ ++ $P<0.0021$
*$P<0.0332$ +$P<0.0332$

Table 3: Effect on haematological values of lantadene treated and ameliorative groups (L+B1, L+B2, L+P1, L+P2, L+S) during sub-chronic toxicity study in Guinea pigs. Values are expressed on mean± S.E.

Organ	C	L	L+B1	L+B2	L+P1	L+P2	L+S
TEC (×10^6 /µl)	6.71±0.25	5.80±0.23	5.91±0.29	5.59±0.23*	5.98±0.16	6.02±0.19	5.92±0.29
TLC (×10^3/µl)	7.25±0.9	9.50±0.44	10.95±0.43	10.65±0.17	10.60±0.46	10.30±0.31	10.25±0.56
Hb (g/dl)	11.65±0.28+	7.60±0.29*	8.55±0.33	8.45±0.22	8.45±0.47	8.95±0.55	8.80±0.45
PCV (%)	45.75±0.84++	39.85±0.68**	50.76±0.68*$^{++++}$	47.02±0.83+++	47.62±0.50+++	46.74±1.72^{++}	45.81±1.22++
Lymphocytes (%)	59.00±0.71	61.50±0.64	58.25±0.85	57.00±0.91	57.25±1.11	58.00±0.41	58.25±0.63
Heterophils (%)	32.25±0.85	35.00±0.41	31.00±1.58	34.75±0.75	31.50±0.96	33.75±0.85	32.75±1.11
Monocytes (%)	4.00±0.41	4.25±0.95	5.75±1.18	5.25±0.85	6.50±0.96	5.50±1.19	6.75±0.95
Eosinophils (%)	3.25±0.75	2.75±0.48	2.25±0.63	2.50±2.9	3.50±0.29	2.00±0.41	2.00±0.41
Basophils (%)	1.00±0.41	0.75±0.48	1.00±0.41	0.50±0.29	0.25±0.25	0.75±0.25	0.25±0.25

** $P<0.0021$ ++++$P<0.0001$ vs Lantadene
*$P<0.0332$ +++ $P<0.0002$
++ $P<0.0021$
+$P<0.0332$

Table 4: Effect on organ weight (gm) of lantadene treated, L+B1, L+B2, L+P1, L+P2, L+S groups during sub-chronic toxicity study in Guinea pigs. Values are expressed on mean± S.E. for six animals in each group.

Organ	C	L	L+B1	L+B2	L+P1	L+P2	L+S
Liver	18.91±0.36+	20.97±0.43*	19.28±0.36	18.48±0.42++	19.28±0.49	18.77±0.36++	18.17±0.53++
Kidneys	4.22±0.21	3.90±0.27	4.50±0.22	4.13±0.22	4.27±0.29	4.33±0.24	4.48±0.23

*$P<0.0332$ vs Control ++ $P<0.0021$ vs Lantadene
+$P<0.0332$

Table 5: Effect on relative organ weight (gm) of lantadene treated and ameliorative groups (L+B1, L+B2, L+P1, L+P2, L+S) during sub-chronic toxicity study in Guinea pigs. Values are expressed on mean± S.E. for six animals in each group.

Organ	C	L	L+B1	L+B2	L+P1	L+P2	L+S
Liver	5.04±0.08++++	7.72±0.42****	5.34±0.08^{++++}	5.46±0.27^{++++}	5.41±0.11^{++++}	5.55±0.11^{++++}	5.21±0.17^{++++}
Kidneys	1.110±0.64	1.145±0.75	1.261±0.72	1.155±0.57	1.26±0.85	1.21±0.59	1.28±0.04

****$P<0.0001$ vs Control ++++$P<0.0001$ vs Lantadene

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