Orally Induced Sub-Acute Toxicity of Lantadenes of *Lantana camara* in Guinea Pigs: A Haematological Study

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Abstract

A sub-acute toxicity study of lantadenes of *Lantana camara* was conducted in guinea pigs, where a total of 20 guinea pigs of either sex were divided into 5 groups. Group I served as control, while groups II, III, IV and V were orally administered lantadenes at the dose levels of 100, 50, 25 and 12.5 mg/kg body weight, respectively daily for 28 days in gelatin capsules. All the animals from group II exhibited severe acute toxicity within 48-72 hrs of the study period with a significantly reduced total erythrocyte count, heterophilia and the resultant lymphocytopenia. However, in group IV, the oral administration of lantadenes for 28 days resulted in significantly raised values of haemoglobin concentration and packed cell volume in guinea pigs. It is concluded that the oral administration of lantadenes produced dose-dependent toxicity in the guinea pigs and the dose level of 25 mg/kg body weight of lantadenes was found to produce haematological changes during the sub-acute exposure. This is probably the first report on the haematological alterations during sub-acute toxicity of lantadenes in guinea pigs.

Keywords: Lantadenes, Sub-acute toxicity, Guinea pig, Haematology.

1. Introduction

*Lantana camara* Linn commonly known as wild sage belongs to the genus *Lantana*, family Verbenaceae, growing luxuriantly at elevations up to 2000 m in tropical, sub-tropical and temperate regions (Ghisalberti, 2000). It is most prolific in areas receiving average annual rainfall of at least 900 mm (Ensbey, 2003). It has been suggested that global warming facilitates the invasion of *L. camara* due to significant increases in growth along with physiological and allelopathic effects (Zhang *et al*., 2014). *Lantana camara* toxicity caused by lantadenes is characterized by intrahepatic cholestasis, associated liver damage and photosensitization. Both ruminants including cattle, sheep, buffaloes, goats, and non-ruminants like horses, guinea pigs, rabbits, female rats are susceptible to lantana leaf toxicity. In tropical countries, the ripe blue-black berries are eaten, but ingestion of the green berry has led to human fatalities (Ghisalberti, 2000). *L. camara* poisoning has also been reported in ostriches in Zimbabwe as well as in Kangaroos, although there were no previous reports of lantana toxicity in ratites (Johnson and Jensen, 1998; Cooper, 2007). Heavy outbreaks of lantana toxicity occur during drought or flood conditions when fodder is scarce (Sharma and Makkar, 1981).

Lantadenes present in *L. camara* causes hepatotoxicity, intra-hepatic cholestasis and nephrotoxicity. Intra-hepatic cholestasis causes retention of phylloerythrin in blood which in turn causes photosensitization (Sharma *et al*., 2007). Assessment of haematological profile becomes a pre-requisite to understand the normal functioning of the system and to further confirm the toxic nature of the administered plant extract or any drug. Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic chemicals (Hoffbrand and Pettit, 1997). To the best of the knowledge of authors, this is probably the first report on the haematological alterations induced by oral administration of lantadenes during sub-acute exposure in guinea pigs.

2. Materials and Methods

2.1 Collection of *L. camara* Leaves and Isolation of Lantadenes
L. camara leaves were collected during the month of September from an area adjoining Palampur town located at an altitude of 1200 m above mean sea level. The plant material was collected from the same source to rule out phytochemical variations caused due to regional and climatic conditions. Fresh leaves of L. camara were cleaned and washed thoroughly with water and re-washed with distilled water. Washed fresh leaves were oven dried at 55°C, ground to a fine powder followed by isolation of lantadenses using standard protocol (Parimoo et al., 2014). Lantadene A was found to be 72.82% by reversed-phase high performance liquid chromatography analysis (Parimoo et al., 2014).

2.2 Experimental Animals
Twenty guinea pigs of around 45 days old, weighing 200-250 gm and of either sex were procured from Laboratory Animal Resource Section, ICAR-IVRI, Izatnagar. Clean water and *ad libitum* access to standard laboratory animal diet supplemented with vitamin C (Limcee, 1000 mg/kg feed) was provided to animals throughout the period of the experimental trial. All the experimental procedures were conducted as per the guidelines of the Institute Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3 Experimental Design
After the acclimatization period, all the animals were weighed and divided randomly into 5 groups. Group I served as control while Groups II, III, IV and V were administered lantadenses at the dose levels of 100, 50, 25 and 12.5 mg/kg body weight, respectively, daily, orally via gelatin capsules, for a period of 28 days. Group I received empty gelatin capsules throughout the experiment.

2.4 Haematology
Animals that became moribund during the trial and those which survived the trial period were sacrificed using diethyl ether. For haematological study, about 1 ml of blood sample was collected into dry sterilized anticoagulant vials containing K$_2$-EDTA from posterior vena cava at the time of sacrifice. 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 in duplicate.

2.5 Statistical Analyses
The data were subjected to one-way analysis of variance (ANOVA) to test the effect of groups on different variables under study. The group means were compared at 5 per cent level of significance (P<0.05) as per standard procedures (Snedecor and Cochran, 1968).

3. Results and Discussion
During the experimental trial, a striking difference in the mortality pattern of different groups was observed. All the animals of group II became moribund within 48-72 hrs of the beginning of trial and were sacrificed. In group III, 2 animals became moribund on 9th day and one on 13th day of trial and were sacrificed on respective days. One animal of group III survived up to the end of trial. All the animals of groups IV and V survived up to the end of trial. The results of the present study revealed no significant difference in haemoglobin levels amongst animals of groups I (13.55±0.15), II (14.15±0.12) and V (13.90±0.17). However, there was a significant increase in haemoglobin levels of animals of groups III (17.60±0.67) and IV (15.75±0.20) as compared to other groups. Haemoglobin values were expressed as g/dl. Packed cell volume levels did not show any significant difference amongst animals of groups I (48.00±0.81), II (48.25±0.62), III (51.25±1.37) and V (50.50±2.78). However, there was a significant increase in PCV levels of animals of group IV (55.00±1.78) as compared to groups I and II. PCV values were expressed in percent (%). Total erythrocyte count of group II (6.24±0.13) was significantly raised than other groups (I, 4.98±0.17; III, 5.14±0.13; IV, 5.20±0.29 and V, 5.12±0.25). There was no significant difference in TEC values amongst animals of other groups. TEC values were expressed as ×10$^3$/µl. There was no significant difference in total leucocyte count values of different groups as compared to control group. However, relatively increased values were observed in group IV animals at the end of study. TLC of group I was 5.76±0.19; II, 6.59±1.57; III, 6.81±0.44; IV, 8.20±0.58 and V, 6.38±0.71. TLC values were expressed as ×10$^3$/µl. The differential leucocytic counts did not show any significant difference in the number of monocytes (I, 5.00±0.40; II, 4.75±0.47; III, 4.25±0.47; IV, 4.50±0.86 and V, 4.25±0.25) and basophils (I, 0.75±0.25; II, 0.75±0.25; III, 1.00±0.00; IV, 0.50±0.28 and V, 0.50±0.28) amongst different groups. However, animals of groups II (37.25±2.49) and III (34.50±2.72) showed significant increase in heterophils number compared to groups IV (25.50±2.90) and V (25.75±2.49). Group III (5.50±0.64) also showed significant increase in number of eosinophils than other groups (I, 3.25±0.62; II, 4.00±0.40; IV, 3.75±0.62 and V, 3.75±0.47). Lymphocytic number of groups V (65.75±2.62) and IV (65.75±3.11) did not show any significant difference amongst themselves but were significantly higher than...
II* 48.25±0.62
I 48.00±0.81
Groups         PCV

V 50.50±2.78
IV 55.00±1.78
III** 51.25±1.37

were of significance only in group IV. The TEC levels
also elevated in all the groups compared to control  but
groups (II and III). Similar to our studies, earlie r
particularly in higher dose lantadenes administered
groups. However, heterophils were also elevated
any significant difference amongst animals of different
haemoconcentration. The TLC values did not exhibit
particularly in group IV, probably due to
haemoglobin and packed cell volume were elevated
significantly elevated compared to control. Thus,
group II (exhibited acute toxicity) where, TEC was
were not significantly different from control excep t in
of all the groups were elevated but the increased v alues
only in groups III and IV. Similarly, PCV values we re
control but this increase in value was of significance

14.15±0.12
15.75±0.20
17.60±0.67
5.20±0.29
8.20±0.58
25.50±2.90
65.75±3.11
3.75±0.62
0.50±0.28
4.50±0.86

Table 1: Haematological parameters (Mean±SEM) in different groups during sub-acute lantadenes toxicity in guinea pigs

Groups | PCV (%) | Hb (g/dl) | TEC (x10^3/µl) | TLC (x10^3/µl) | Heterophils | Lymphocytes | Eosinophils | Basophils | Monocytes |
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
I | 48.00±0.81* | 13.55±0.15* | 4.98±0.17* | 5.76±0.19* | 29.25±0.47* | 61.75±0.85* | 3.25±0.62* | 0.75±0.25* | 5.00±0.40* |
II* | 48.25±0.62* | 14.15±0.12* | 6.24±0.13* | 6.59±1.57* | 37.25±2.49* | 53.00±2.70* | 4.00±0.40* | 0.75±0.25* | 4.75±0.47* |
III** | 51.25±1.37** | 17.60±0.67** | 5.14±0.13** | 6.81±0.44** | 34.50±2.72** | 54.75±3.40** | 5.50±0.64** | 1.00±0.00** | 4.25±0.47** |
IV | 55.00±1.78** | 15.75±0.20** | 5.20±0.29** | 8.20±0.58** | 25.50±2.90** | 65.75±3.11** | 3.75±0.62** | 0.50±0.28** | 4.50±0.86** |
V | 50.50±2.78** | 13.90±0.17** | 5.12±0.25** | 6.38±0.71** | 25.75±2.49** | 65.75±2.62** | 3.75±0.47* | 0.50±0.28** | 4.25±0.25** |

Values within columns (between groups I, II, III, IV and V) with different superscripts are significantly different by ANOVA (P<0.05) (N=4).

Group I=Control; Group II= lantadenes (100 mg/kg bw); Group III=lantadenes (50 mg/kg bw); Group IV=lan tadenes (25 mg/kg bw); Group V=lantadenes (12.5 mg/kg bw).

Earlier studies using oral administration of L. camara leaf powder resulted in transient increase in the hematocrit values and heterophil numbers and a decline
in number of thrombocytes in sheep blood (Seawright, 1963). Similarly, oral administration of crude lantadenes to sheep caused significant increase in coagulation time, prothrombin time and serum bilirubin content and decrease in erythrocyte sedimentation rate (Uppal and Paul, 1978). Studies by early researchers reported elevation in blood clotting time and hematocrit values and a decrease in erythrocyte sedimentation rate in lantana intoxicated buffaloes and cattle (Hussain and Roychoudhury, 1992). In acute lantana toxicity in goats, a progressive decrease in PCV, TEC and haemoglobin levels has been reported (Ali et al., 1995). Similarly, studies by Kalra and co-workers reported fall in packed cell volume, total erythrocyte count, hemoglobin, heterophil number and leukocytosis in buffaloes and cattle in lantana poisoning (Kalra et al., 1984).

4. Conclusion
The results of our study indicated that the oral administration of sub-acute toxic dose of lantadenes (25 mg/kg body weight) resulted in significant elevation of haemoglobin concentration and packed cell volume in guinea pigs.

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References


Parimoo HA (2013). Toxicopathological effects of lantadenes of Lantana camara and amelioration with different herbal plants in guinea pigs. *M.V.Sc. Thesis Submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, UP, India.*


