Hematological and Clinical Changes in Rabbits Exposed to Lantana Camara under Experimental Conditions

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Abstract: Lantana camara is well known to cure several diseases and used in various folk medicinal preparations. The objective of this study was to investigate the chronic toxicity of Lantana camara, in rabbits exposed to fruit and leaf of the plant in powder form in feed from hematological and clinical points of views. Methods to evaluate the chronic toxicity, a fixed dose of 5 g / kg body weight of L. camara fruit and leaf in powder forms were giving with feed as pellet daily for 14 days. The main parameters depended in the experiment were; body weight, respiratory rates, heart rates and body temperature from clinical point of view. In addition to determination of bleeding and clotting times, with estimation of erythrocytes counts, Hemoglobin concentration, Packed cell volume, and erythrocytes indices (MCV, MCH, MCHC), leucocytes counts, differential leucocytes count (lymphocytes, Heterophils, Eosinophils, Monocytes and Basophils %)

Results: in 28 days L. camara leaf and fruit powder showed no obvious chronic toxicity. The rabbits exposed to lantana showed changes in clinical parameters, in addition to the hematological parameters. As they showed, loss body weight, decreased respiratory rates, heart rates. With decreased counts of erythrocytes, Hb concentration, PCV values, prolongation of bleeding and clotting times. While the total and differential count did not show significant changes.

Keywords: Lantana camara; Hematological; Clinical changes; rabbits; Iraq

1. Introduction

Herbs have recently attracted attention as health beneficial foods and as source materials for drug development. Lantana camara L. is one of the most prevalent and noxious weed belong to pyrrolizidine alkaloids, family Verbenaceae family, causing hepatotoxicity in grazing animals (1). Some metabolites isolated from their leaves possess antithrombin activity (2), and antipyretic activity (3-4). L. camara leaves have been reported to make animals ill after ingestion and its berries are toxic before they become ripe (5-7).

The hemolytic activity of L. camara aqueous extract and its solvent fractions exhibited very low hemolytic activity towards the human erythrocytes (8). The active substance in lantana camara leaves is triterpentine acid (9-13). (14)

2. Materials and Methods

2.1 Plant Materials

The leaves and fruits of Lantana camara were collected from August to December 2013, from gardens in Baqubah city, Diyala, Iraq. The fruits and leaves, dry in shade, then were powdered by electric blender.

2.2 Test Animals

Fifteen healthy local breeds' rabbits from either sex, weighing 1-1.5 kg, of 1-2 years old, were used for the study. The animals were housed in cages, in college of Veterinary Medicine, University of Diyala. They were acclimatized to laboratory conditions for 15 days prior to exposure. The temperature in the animal room was maintained between 25 ± 2 °c, with illumination cycle set to 12 h light and 12 h dark. The rabbits were fed with concentrated feed and left ad libitum for water.

They were divided into three groups of 5 rabbits each, the first group (I) was exposed to lantana camara fruits in powder forms mixed with feed, while the second group (II) was exposed to Lantana camara leaf in powder form mixed with feed, at a dose rate of 5 g / day for 14 days for both fruit and leaf. The third group (III) was left without exposure as control group.

The main parameters depended in the experiment were, the clinical signs; body weight, respiratory, rates, heart rates and body temperature. With monitoring the animals for any abnormal signs appear during the study. In addition to collection of blood samples in vials containing EDTA as anticoagulant, and submitted to blood examinations which included, total erythrocytes counts and Hb concentration, PCV estimated by using Mission Hb strips (Germany).erythrocytes indices
MCV, MCH, MCHC, leucocytes count, Differential leucocytes count (Lymphocytes, Heterohils, Eosinophils, Basophils, and Monocytes %), with estimation bleeding time and clotting time according to (20).

2.3 Statistical Analysis

All the values are expressed as mean ± S.E.M. for groups of five animals each. The values were analyzed by one way ANOVA. The values are statistically significant at level P < 0.05 (21).

3. Result

This study observed no significant toxic signs or death during the 28 day observation period. None of the rabbits showed clinical toxic signs such as anorexia, depression, lethargy, jaundice, dermatitis, and also, no mortality happened throughout the study.

The results revealed that body weight in group exposed to lantana fruit were decreased during the 7th, 14th, 21st and 28th days post exposure to fruit, in comparison with pre exposure level (1.540 ± 0.057 kg), the lowest body weight was in 21st day (1.178± 0.077 kg). The weights were significantly lower than that of control group in the same days. While in group exposed to lantana leaf the body weight were decreased significantly, in comparison with pre exposure level (1.508 ± 0.056 kg), the lowest level was in 14th day (1.220 ± 0.041 kg) which was significantly differ from that of control group. There was no significant difference in body weight between those exposed to leaf or fruit. Body weight of control group significantly increased during 14th, 21st, and 28th days in comparison with zero time of study (1.490 ± 0.157 kg), the highest increase was during 28th day (1.739 ± 0.231 kg), during 7th day no changes (Table -1).

Respiratory rates in group exposed to lantana fruits declined during experiment in comparison with pre exposure (140.2 ± 9.46 / minute), the lowest rate was in 14th day (72± 4.9 / minute). In group exposed to Lantana leaf, the same thing happen as respiratory rates declined during experiment in comparison with pre exposure value (143.6± 21.0 / minute), the lowest rate was in 14th day (74 ± 12.06 / minute). Respiratory rates in control group was not significantly changed (Table -1).

Heart beat in group exposed to Lantana fruit declined significantly in 7th, 14th, 21st days, and non-significantly decreased in 28th day, in comparison with pre exposure (193.2± 12.52), lowest level was in 14th day (139 ± 8.54). While in group exposed to Lantana leaf heart rates declined significantly only in 7th day, with no significant decreases in 14th, 21st and 28th days, in comparison with pre exposure (177.6 ± 6.76), the lowest rates was in 7th day (135 ± 7.46). Heart beat in control group was not significantly changed in comparison with zero time during 7th, 14th and 21th days, while in 28th day it increased (Table -1).

Body temperature of group exposed to Lantana fruits decreased in 7th day (36.8 ± 0.17°C), in comparison with zero time (38.16 ± 0.21°C), in 28th day increased to (38.17 ± 0.17°C). While in those exposed to Lantana leaf decreased in 7th, 14th, 21st, and 28th days, in comparison with zero time (38.24 ± 0.56°C), the lowest level was in 7th day (36.66 ± 0.32°C) which was significantly different. Body temperature in control group showed no changes during experiment (Table -1).

Table 1: Clinical signs in rabbits exposed to Lantana camara

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Days</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
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<tbody>
<tr>
<td>Body weight (Kg)</td>
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<td></td>
<td>1.54±0.057</td>
<td>1.31±0.055</td>
<td>1.18±0.042</td>
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<td></td>
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<td>1.31±0.067</td>
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<tr>
<td></td>
<td>III</td>
<td></td>
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<td>1.40±0.158</td>
<td>1.53±0.199</td>
<td>1.56±0.196</td>
<td>1.73±0.231</td>
</tr>
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<td>Respiratory Rates</td>
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<td>111±19.8</td>
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<td>(per minute)</td>
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<td>143.6±21.0</td>
<td>82.8±11.2</td>
<td>74.0±12.0</td>
<td>132.4±16.5</td>
<td>100.5±15.7</td>
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<tr>
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<td>III</td>
<td></td>
<td>135.4±20.6</td>
<td>88.8±4.08</td>
<td>100±15.5</td>
<td>111.6±7.39</td>
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<td>172±10.83</td>
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<td>185±19.84</td>
<td>156±8.85</td>
<td>141.3±10.41</td>
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<td>230.0±25.1</td>
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<td>Body temp (°C)</td>
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<td>38.17±0.52</td>
<td>38.43±0.19</td>
<td>38.13±0.57</td>
</tr>
</tbody>
</table>

I. group exposed to fruit; II. Group exposed to leaf; III. Control group

Values are Mean ± S.E.M.; * significant at P< 0.05

Bleeding time in group exposed to Lantana fruit prolonged in 7th, 14th, 21 days, in comparison with zero time (33 ± 3.39 seconds), the longest prolongation was in 7th day (43.75 ± 5.91 seconds), in the 28th day declined to (23.33± 3.33seconds). Bleeding time in group exposed to Lantana leaf showed prolongation during 7th, 14th and 28th days, in comparison with pre exposure (26 ± 4.7). The longest period was in 14th day (35± 7.36), then declined in 21st day (23.75 ± 3.15). In control group bleeding time showed no changes (Table -2-).

Clotting time in group exposed to Lantana fruit prolonged in 7th, 14th, and 21st day, in comparison with zero time (54 ± 8.28seconds). the longest period was in 21st day (105 ± 17.44), in 28th day it declined to (28.33 ± 0.61). Clotting time in group exposed to Lantana leaf prolonged in 7th, 14th, 21st days, in comparison with pre exposure (46 ± 6.78), the longest period was in 14th day (87.5 ± 42.5), the shortest period was in 28th day (40 ± 10.61). Clotting time in control group none significantly changed (Table -2-).
Total erythrocytes count in group exposed to Lantana fruit increased in 7th day to (6.49 ± 0.72 x 10^6) cmm, in comparison with zero time (5.16 ± 0.22 x 10^6 cmm), then declined in 14th day, and 21th, the lowest count was in 21th day (4.59 ± 0.39 x 10^6 / cmm). While in group exposed to Lantana leaf, total erythrocytes count decreased in 14th, 21th, and 28th days, comparison with zero time (5.29 ± 0.63 x 10^6 / cmm), the lowest count was in 28th day (3.87 ± 0.08 x 10^6 / cmm). Total erythrocytes count in control group did not significantly change (Table -3-).

Hemoglobin concentration in group exposed to Lantana fruits decreased in 14th, 21th, and 28th days, in comparison with zero time (12.04 ± 0.53), the lowest level in 21th day (10.78 ± 0.91). Hemoglobin concentration in group exposed to Lantana leaf decreased in 7th, 14th, 21th, and 28th days, in comparison with zero time (12.42 ± 0.14), the lowest in 28th day (10.4 ± 1.14). Hemoglobin concentration in control group did not show significant changes (Table -3-).

PCV in group exposed to Lantana fruit decline in 7th, 14th, 21th, and 28th days, in comparison with pre exposure (36.6 ± 0.24%), the lowest value was in 28th day (30.33 ± 3.48%). PCV in group exposed to Lantana leaf decreased in 14th, 21th, and 28th days, in comparison with zero time (35.5 ± 1.22 %), the lowest level was in 21th and 28th days (32 ± 0.91%). PCV in control group did not show significant changes (Table -3-).

MCHC in group exposed to Lantana fruit declined in 7th and 28th days, in comparison with pre exposure (24.27 ± 1.05), lowest declined in 28th day (21.04 ± 1.06), increased in 14th and 21th days, the highest in 21th day (26.08 ± 1.49). MCHC values in group exposed to Lantana leaf declined in 7th, 14th, 21th and 28th days, in comparison with pre exposure (26.01 ± 1.14), the lowest decline was in 7th day (21.04 ± 1.06) (Table -3-).

MCHC in group exposed to Lantana fruit increased in 14th day (34.09 ± 0.17), and 28th day (34.32 ± 0.22) in comparison with pre exposure (33.98 ± 0.16), decreased in 21th day (24.91 ± 5.12) MCHC in group exposed to Lantana leaf, declined in 7th and 14th day, in comparison with pre exposure (34.20 ± 0.07, the lowest in 14th day (33.73 ± 0.07), decreased in 21th day (40.16 ± 11.57), in 28th day no changes (Table -3-).
In the group exposed to Lantana fruit TLC increased in 7th, 21st and 28th days, in comparison with pre exposure time (4.364 ± 0.188x103 cmm), the highest level was during 7th day (4.880 ± 0.719X 103 cmm), in 14th day no changes. In group exposed to Lantana leaf, TLC decreased during 7th, 14th, 21st, and 28th days, in comparison with pre exposure (4.312 ± 0. 627x 103 cmm), the lowest level was during 28th day (2.672 ± 0.444 x 103 cmm), which was significantly differ in 28th and 7th days. The results revealed that total leucocytes count not changed in control group (Table -4-).

Lymphocytes % in group exposed to fruit decreased in 14th and 28th days while in those exposed to leaf non significantly increased in 21st and 28th days. In control group the % non-significantly changed (Table -4-).

Monocytes in those expose to fruit non significantly decreased in 7th, 21st, and 28th days, while in 14th day it increased in those exposed to leaf non significantly decreased in 7th and 28th days (Table -4-). Heterophiles in those exposed to fruit was not significantly increased. While in those exposed to leaf it significantly decreased in 21st and 28th days. In fruit group it non significantly increased, in control group it non significantly changed (Table -4-). Basophiles % in those exposed to fruits was not significantly changed, while in group exposed to leaf it increased significantly in 28th day. In control group it non significantly changed (Table -4-). Esinophiles % did not show any significant changes in control and those exposed to fruit. In group exposed to leaf it significantly decreased in 28th day (Table -4-)

<table>
<thead>
<tr>
<th>Parameter</th>
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<tr>
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<td></td>
<td>3</td>
<td>4.955±0.374</td>
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<td>Lymphocytes</td>
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<td>42.4±3.30</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>48.25±4.97</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>46±4.10</td>
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<tr>
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<tr>
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<td>2</td>
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<tr>
<td></td>
<td>3</td>
<td>3.4±1.30</td>
</tr>
</tbody>
</table>

I. group exposed to fruit; II. Group exposed to leaf; III. Control group

4. Discussions

Badakhshan et al, 2011(22) found that female mice is more sensitive to lantana camara than male mice. (23) Also found that female red kangaroo is more vulnerable to l. camara than the male animal. The results of the clinical part of the study revealed that body weight decreased in both groups that exposed to fruit and leaf. Respiratory rated decreased in both groups, heart rates decreased in group exposed to leaf only in 7th day, while in group exposed to fruits decreased during the study, body temperature decreased in group exposed to leaf, while in group exposed to fruit it decreased then increased.

On the bases of (24) study, the decline in body mass could be preliminarily attributed to a decrease in food intake which is related to the release and absorption of toxins in the gastrointestinal tract. Loss of body weight, general weakness and death, before death depresses body temperature (24). Clinical, signs that appear in animals depend on quantity of toxic substance in leaves, the physiological conditions of animals, and duration of exposure to plant (19). Clinical signs include increase body temperature, pulse and respiration.

Sharma et al (24) attribute the increase in respiratory rate to blood capillary congestion in pulmonary tissues and alveoli and occurrence of pulmonary emphysema. The results of the study revealed that bleeding and clotting times prolonged in both groups exposed to fruit and leaf.

Increase bleeding and clotting time in rabbits this agree with (19, 25-27). Others (28) attribute it to decrease in prothrombin and protein synthesis and fibrinogen due to hepatic damage. (27, 29), the cause due to decrease absorption of Vit K. Others (20, 33) refer to the disturbances of clotting and occurrence of bleeding due to decrease in platelets count which has active role in clotting process due to liver damage and effect on bone marrow.

(30, 24, 26) referred to prolongation in bleeding and clotting times during toxicity; (28) add that the increase in clotting in sheep start in day 3 reach the longest time in day 7 (7,68- 9,5 minute clotting time, and he concluded that the increase in clotting time depend on dose and duration of poisoning. They attributed this increase to decrease in synthesis of prothrombin with decrease in protein and fibrinogen synthesis which occur as a result of liver damage.
Hematologically the study revealed that RBC count, Hb concentration, PCV % decreased during the study in both groups. While MCV MCH MCHC decreased then increased during the study.(33)Hari et al 1973 showed decrease PCV in end of toxicity.

In rabbits increase in mean PCV (19, 28, 35, 31).Hematological changes(30,24 and 28) referred to increased PCV in sheep, cows and buffalo.(32)showed mild increased in PCV.(24) attribute this increase in PCV to dehydration and animal loss of appetite. (28) Referred it to increase to hemconcentration due to dehydration. The results showed decrease in RBC and Hb (31, 32 and 25).(33, 19) a attributed this decrease to RBC destruction and hemolysis. Decrease of RBC due to increase its fragility.

TLC decreased in group exposed to leaf, and increased in group exposed to fruit. Lymphocytes none significantly increased in group exposed to leaf, decreased in group exposed to fruit. Monocytes none significantly decreased. Heterophils decreased in both groups. In rabbits increase in WBC and heterophils with decrease lymphocytes (36,31,32,25,26). (33) attribute this increase in WBC to increase in neutrophils as results of reflex systemic response of animal body which exposed to any foreign body.(34) O’S.J.; Sidebottom, P.J.; et al. (1998). Isolation of polymorphic crystal forms of lantana toxins on icterogenic action in guinea pigs. Toxicol. Lett; 42(1): 29-37.

References


