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SKIN AND LIVER TOXICITY IN EXPERIMENTAL *LANTANA CAMARA* POISONING IN ALBINO RATS

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Abstract : Dried alcoholic extract of fresh *Lantana camara* leaves (LE), on oral administration to albino rats of both sexes, induced photodermatitis during exposure to clear sunlight for 1 hr. Its severity was related to the dose of LE and was maximal in rats exposed to sunlight from 4 to 14 hr after feeding LE and gradually declined over 40 hr. Wavelengths of light about 540 to 570 mμ only were effective. In control study, the alcoholic extract of edible spinach leaves was only $^1/_3$ in potency and its effect lasted for less than 20 hr.

LE did not raise serum bilirubin, SGOT, SGPT or cause liver injury as assessed by light microscopy. However, like CCL_4 but unlike spinach extract, LE impaired excretion of BSP by liver, proportionate to the dose and also maximal at 5.5 hr declining thereafter over 40 hr.

Key words : Lantana toxicity rats

INTRODUCTION

Lantana camara (Var. aculeata Linn. Moldonke) an ornamental exotic plant from tropical America, has become naturalized in India. Ingestion of its leaves by grazing animals produces photodermatitis, jaundice, liver damage and death(1). We report here some details of skin and liver toxicity of its leaf extract in albino rats.

METHODS

Preparation of lantana extract (LE): Fresh green leaves of locally growing lantana plants which bear red flowers were put in rectified spirit (95% v/v ethanol in water) at room temperature (50° to 100°F) for 1 to 2 weeks. The extract LE was air dried to a black viscous mass (mean yield, 3.9 g/100 g fresh leaves: water content 17% w/w), stored at 8°C and emulsified in water with polysorbate 80(0.4 ml/g LE) for oral feeding. Spinach leaf extract emulsion prepared similarly or polysorbate 80 solution in water served as controls.

Adult albino rats (Wistar strain, either sex, 80 to 300 g) maintained on dry pellet standard diet were exposed at varying time intervals to sunlight or other specific lighting conditions. In summer, sunlight exposure experiments were carried out early in the morning. Exposed animals were observed for upto 2 weeks.

Photodermatitis study : Photodermatitis in albino rats had 4 mainifestations-redness of ears(pinna) and paws and edema of ears and snout. The serverity of each was graded as 0 (no response), 1 (just perceptible), 2 (mild), 3 (moderate) and 4, (servere response). The grades for an animal were totalled and the average response of a group at a particular time was calculated as the mean ± SEM.

To ascertain the wavelength of sunlight responsible for photodermatitis, rats were immobilized with sodium pentobarbitone (35 mg/kg, ip) 12 hr after oral administration of LE. They were then exposed to sunlight for 1 hr with one ear covered with one of the optical filters of a colorimeter (450 to 670 mμ). These optical filters have surface area enough to cover the ear of a rat. The other ear served as a control.

Liver function tests: Rats were fed 3 g/kg LE. Control animals received a comparable volume of vehicle. After different intervals, they were killed with ether and blood collected from heart. Serum was used for estimating bilirubin(2) and glutamic oxaloacetic transaminase

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(SGOT) and glutamic pyruvic transaminase (SGPT,3). The liver was promptly removed, sliced, rinsed clean in saline and preserved in 10% formalin for histological study, (5mμ thick sections, stained with haematoxylin-eosin).

Bromsulphalein (BSP) serum retention : BSP (10 mg/kg in 1.0 ml saline) was injected into tail vein of pentobarbitone anaesthetized rats (120 - 200 g) which had orally received earlier LE, polysorbate 80, spinach extract or carbon tetrachloride (CCl_4, 0.05 to 1.0 ml/kg and diluted in equal volume of liquid paraffin). 5 min later, the chest was opened and blood collected from heart. From serum BSP level (4) BSP retention value was calculated assuming that a rat contains 6 ml blood/100 g body weight and that serum represents 55% volume of blood(5); it was then expressed as % of total BSP injected.

RESULTS

Rats fed LE appeared normal when kept in the dark or under the laboratory lighting conditions, but developed photodermatitis when exposed to clear sunlight at any hour, between sunrise and sunset, through all the seasons. Within 3 min of exposure, there was a perceptible redness of ears and paws which became maximal in about 10 min. Ear redness was often accompanied by cyanosis at its margin; as edema of ears progressed, redness or cyanosis were replaced by a pale waxy swollen appearance which became maximal in about 45 min and disappeared in about 6 hr with the return of the normal pink colour. Edema of the snout appeared after about 10 min reaching a maximum within 45 min. The swollen upper lip protruded and everted outwards and the eyes closed due to swollen eyelids. Snout edema disappeared in 12 to 24 hr. However, the animals did not manifest excessive sneezing, scratching, grooming or licking of the skin and all recovered completely. Thirst, appetite, urine, stools, behaviour, general appearance, fir and sclera remained normal over 2 weeks.

Photodermatitis developed fully when the rats were kept in clear sunlight for 45 min. Therefore 1 hr exposure was considered adequate.

Male and female rats were found about equally sensitive to photodermatitis (mean scores; male, 8.7 ± 1.04, n = 10 and female 9.5 ± 1.03, n = 10). Therefore, rats of both sexes were used in this study.

Severity of photodermatitis was related to the dose of LE and to the interval between its feeding and sunlight exposure (Table I and II). Acute mortality preceded by collapse was seen with 6 and 12 g/kg. After feeding LE, rats required 4 to 5.5 hr to develop full susceptibility to sunlight; which persisted for upto 14 hr and then gradually declined over 40 hr.

TABLE I: Sunlight-induced photodermatitis 5.5 hr after feeding different doses of LE to rats.

Dose g/kg LE	*No of rats*	*Mean photodermatitis grade ± SE*
1	7	0.7±0.29(a)
2	6	8.1±0.76(b)
3	27	12.3±0.29(c)
6	*7	13.7±0.36(d)
12	4	All died before exposure

P values (by t-test) between a and b, b and c, c and d are < 0.001, < 0.001, < 0.01 respectively. *2 rats died.

TABLE II: Temporal study of photodermatitis in rats exposed to sunlight at different intervals after 3 g/kg LE.

Interval between LE feeding and sunlight exposure, hr	*No of rats*	*Mean photodermatitis grade ± SE (P by t-test)*
1	6	0.5±0.22 (∠ 0.001)
4	6	11.5±0.62 (NS)
5.5	18	12.1*±0.36
12	9	12.6±0.47(NS)
14	8	12.1±0.64(NS)
21	10	9.4±0.54(∠0.001)
30	6	6.8±0.70(∠0.001)
40	6	2.4±0.76(∠0.0001)

* Compared with other groups for statistical significance of difference by t-test. NS = not significant.

Severity of photodermatitis was also proportional to the intensity of sunlight; dust haze or clouds in the sky reduced the effect form 12.3 (Table I) to 6.4 (n = 5) and 1.3 (n = 3) respectively. Artificial lighting and ultraviolet or infrared rays were not effective. Daylight scattered through window panes (n = 12) was ineffective like total darkness (n = 6).

It was noted that sunlight passing through the double-walled glass cylinder with 1 cm thick layer water between the walls (to cut off solar ultraviolet radiation) was as effective (scores, 12.4, n = 5) as direct sunlight (Table I).

Optical filters allowing wavelength 540 and 570 mμ produced redness and edema of the ear (mean scores 2.1/4 and 1.6/4, n = 9 and 2.4/4 and 2.0/4, n = 8). No effect was seen with other filters (450 to 520 mμ and 600 and 670 mμ).

In 5 black-hooded rats, LE (3 g/kg) produced, on exposure to sunlight, oedema of ears and face. Redness of ear, if any, could not be discerned because, ears (and face) had intense black hair and skin.

The spinach extract was about one-third potent than LE in inducing photodermatitis (Table III) and the effect of even its higher dose was more short-lived (<20 hr compared to the 40 hr after LE, Table II). Orally given polysorbate 80 in water did not produce photodermatitis.

TABLE III : Photodermatitis after feeding spinach extract and 1 hr sunlight exposure.

Dose of spinach extract g/kg	*Interval between spinach extract feeding and sunlight exposure (hr)*	*No of rats*	*Mean photodermatitis grade ± SE*
3	5.5	11	0.18±0.12
3	12.0	8	1.1±0.29
4	5.5	3	2.6
6	5.5	8	7.6±0.6
6	12.0	3	8.0
6	20.0	5	0.8±0.38

Groups of 6 to 8 rats were killed under ether anaesthesia 0.5, 1.5 or 10 days after LE feeding and sunlight exposure. The liver sections of rats in all these groups appeared normal under light microscopy. SGOT and SGPT levels and serum bilirubin levels were not different from those of the vehicle-fed control animals (SGOT-56 to 58 IU/1/min. SGPT - 23 to 27 IU/1/min and bilirubin levels less than 1 mg/dl serum).

5 min after BSP injection about 5% of the injected dye remained in the serum of the normal animals. Table IV indicates that feeding the vehicle or spinach extract (3-6 g/kg) did not increase this value. Feeding LE in the dose range of 1.0 to 5.0 g/kg proportionately increased serum BSP retention indicating a dose-related impairment of liver function. With 3 g/kg dose, maximum serum BSP retention occurred 5.5 hr after LE feeding; values obtained at intervals upto 40 hr gradually declined to control levels.

CCl_4 increased the serum BSP retention in rats which effect was dose-dependent (Table IV).

TABLE IV: BSP retention in the sera of rats pretreated with polysorbate 80, LE, spinach extract or CCl4. 10 mg/kg BSP iv; blood collected 5 min later.

Drug	*Dose/kg*	*Interval between drug feeding and BSP test (hr)*	*No. of rats*	*% serum BSP retention Mean + S.E.*
Nil	—	—	12	+5.0+0.82
Polysorbate 80 solution	1.2 ml	12	4	3.4
LE	1.0 g	12	6	13.4**±3.95
	2 g	12	6	12.0*±2.18
	3 g	2.75	5	13.2*±1.67
	3 g	5.5	6	30.2*±2.63
	3 g	12	6	25.4*±4.10
	3 g	30	3	16.0
	3 g	40	3	4.0
	5 g	12	6	31.8*±2.19
Spinach extract	3 g	5.5	2	2.3
	3 g	12	6	2.2***±0.39
	6 g	12	6	3.3±0.99(NS)
Carbon tetra-chloride	0.05 ml	18	3	13.9
	0.15 ml	18	5	19.3*±1.4
	0.5 ml	18	5	36.1*±2.31

+ compared with other mean values for statistical significance of difference by t-test. NS = not significant. P values* <0.005, **<0.01 and ***<0.05.

DISCUSSION

The toxic principles in lantana leaves known as lantadenes retain their activity even under severe procedures like solvents extraction, air drying, prolonged boiling, long storage at room temperature, ultraviolet light exposure and prolonged microbiological fermentation in the silage pits. Experimental procedures in the present work are not likely to significantly reduce their toxicity.

One toxic component of lantana, known as reduced lantadene A, induces liver injury in the female rats but not in the males(6). On the other hand LE which contains mixture of various toxic components, was equally effective in inducing photodermatitis and in reducing hepatic BSP excretion in both male and female rats. This aspect needs further work.

The present results indicate that only the wavelengths of around 540 to 570 mμ in the visible spectrum of sunlight are responsible for photodermatitis in lantana poisoning. This suggests presence of a phototoxic biochemical reaction that selectively needs the above-mentioned energy quanta.

Non-toxic green leafy plants when eaten in large quantities induce photodermatitis even in herbivorous animals (1). Extract of spinach (a non-toxic leafy edible vegetable) also produced photodermatitis in rats, which was milder and of a shorter duration than that induced by LE. Also, while the former had no effect on the hepatic function of BSP excretion, the latter clearly impaired it.

Photodermatitis and impaired liver function are the common features of lantana poisoning in the rat and ruminants; only the liver damage in the latter is for more serious. The dose of LE usually used in this work in rats (3 g/kg), represents about 75 g fresh leaves or 20 g dried leaves. This dose is comparable to that given in ruminants to induce poisoning (7).

The present work in LE-fed black-hooded rats shows inability of melanin to prevent photodermatitis. This finding agrees with the observation that dark-skinned buffalo calves get photodermatitis after orally receiving lantana leaves(7). On the other hand, it does not support the opinion that unpigmented skin and hair are necessary for photodermatitis (8), nor the suggestion(1) that the dark pigmented animals should be reared on such pastures which have plants capable of causing photosensitization.

Serum BSP excretion by the liver into bile (a very sensitive liver function test) was clearly impaired by the LE and by CCl_4, but was unaffected by spinach extract (a control). Like the photosensitizing effect (Table I), the impairment of hepatic BSP excretion by LE was proportional to its dose and its time course was remarkably parallel to that of the photosensitizing effect of LE.

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REFERENCES

1. Blood DC, Henderson JA. Diseases of the skin In "Veterinary Medicine." Bailliere, Tindall and Cox, London, 2nd edition, 1963, p. 326.
2. Lathe GH, Ruthven CRJ. Factors affecting the rate of coupling of bilirubin and conjugated bilirubin in van den Bergh reaction. *J Clin Path* 1958, 11:155.
3. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; 28:56-63.
4. Gaebler OH. Determination of bromsulphalein in normal, turbid, haemolyzed or icteric serum. *Am J Clin Pathol* 1945; 15:452-55.
5. Prosser CL. Circulation of body fluids. In "Comparative Animal Physiology" by Prosser, CL, Brown FA. Jr., Saunders, Philadelphia 1961, 2nd edition, p. 388, Reprinted 1969
6. Pass MA, Pollitt S. Goosem MW, McSweeney CS. The pathogenesis of lantana poisoning. In "Plant toxicology" by Seawright, A.A., H.P. Hegarty, L.F. James and R.F. Keeler, Queensland poisonous Plants Committee, Yeerongpilly, 1985; pp 487-94.
7. Lal M, Kalra DB. Lantana poisoning in domesticated animals. *Ind Vet J* 1960; 37:263.
8. Muenscher WC. Plants poisonous or injurious under special conditions - plants causing photosensitization. In "Poisonous plants of the United States," MacMillan Company, New York, Revised edition, 1964; pp 10 & 11.