CHEMICAL INVESTIGATIONS OF *LANTANA CAMARA* LINN.

Lantana camara, growing all over the waste lands throughout the country, is reported to be highly poisonous to live-stock. Lantadenes isolated from the South African species were found to cause photosensitization and severe icterus in animals.[1] Toxic symptoms of lantana poisoning from Indian species of lantana have been reported earlier from this laboratory[2] and the present paper deals with the chemical investigations.

Preliminary chemical examination of the air-dried leaves by successive extraction with various solvents in a soxhlet yielded extractives as follows : Petroleum ether (40-60°), 3·4 ; ether, 5·1 ; chloroform 0·8 ; and alcohol 13·6%. The alcoholic extract indicated the presence of reducing sugars, tannins, resinous substances and colouring matter. Alkaloids and glycosides were found to be absent. Steam distillation of the fresh leaves yielded 0·16% essential oil. Analysis of total ash (8·2%) showed the presence of chloride sulphate, phosphate, iron; calcium, magnesium, sodium and potassium. The acid-insoluble ash was 1·5%. Hot alcoholic extract of the powdered leaves was decolourised and concentrated. It was allowed to cool in a refrigerator and the granules deposited at the bottom of the container were separated and identified as potassium chloride. The filtrate was further concentrated and separated into chloroform soluble and insoluble fractions. Lantadenes were isolated from the chloroform residue.

The chloroform-insoluble fraction obtained above was dissolved in water, treated with lead acetate and basic lead acetate. The filtrate was freed from lead, concentrated to a low volume and precipitated with excess of alcohol. Residue from the decolourised filtrate was taken in boiling methyl alcohol and evaporated to dryness. It yielded an osazone derivative melting at 205° indicating the presence of glucose.

Assay of total lantadenes was carried out by exhausting powdered leaves with alcohol in a soxhlet apparatus. Residue from the decolourised extract was taken in solvent ether and further decolourised. This on evaporation yielded a white amorphous compound representing the total lantadenes. The yield varied from 0·2% during March and April to 1·4–1·7% during the months of July to September depending on the sample collected at different stages of growth.

Processing the plant material for reducing the toxic principles was attempted by preparing silage according to the usual procedure but under different conditions using pH-4 acetic acid 10% molasses or according to A.I.V. method.[3] The containers were opened after two or three months and the total lantadenes determined after drying the material. Fresh leaves were also subjected to steam heating for 10 hours or continuous boiling with water for the same period. The material was squeezed, dried and assay carried out. Powdered leaves were also treated with water, 20% aqueous KOH, 10% acetic acid, pH-4 hydrochloric acid and sulphuric acid separately. The yield was found to be the same in all these cases indicating that the toxic constituents are highly resistant and the plant material cannot be easily processed into cattle feed.

Our thanks are due to Dr. H. D. Srivastava, Director, for his keen interest in this work.

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August 28, 1962.

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PRELIMINARY STUDIES ON THE EFFECTS OF GLUTETHIMIDE ON CERTAIN ENZYME SYSTEMS

GLUTETHIMIDE (2-ethyl-2-phenyl glutarimide) is a non-barbiturate hypnotic synthesized by Tagman, Suny and Hoffman (1952). There are many studies indicating the favourable clinical results with this drug.

Rushbrooke *et al.* (1956), during a well-designed clinical trial, found that 0·5 gm. of glutethimide and 0·2 gm. of cyclobarbitone were certainly better than a placebo but did not significantly differ from each other in terms of patient's preference or post-drug drowsiness.

The effect of glutethimide on cellular metabolism and enzymes has been carried out in the present study as the neurophysiological experiments alone do not generally fully explain the mechanism of action of hypnotics.

The effects of the drug on cholinesterase, mono-amine oxidase, glutamic oxaloacetic acid transaminase, glutamic pyruvic transaminase, amino-acid oxidases, and catalase have been studied.

MATERIAL AND METHODS

The stock solution of the drug was prepared by dissolving it in a dilute solution of sodium

hydroxide and the pH was adjusted to 7·2 by adding dilute hydrochloric acid. The final strength was made up to 10 mgm./ml.

The enzymatic studies were carried out on tissues obtained from freshly killed healthy rats weighing between 120 and 150 gm. The tissue was immediately washed and homogenised in ice-cold phosphate buffer (pH 7·4) in case of experiments with catalase and amine oxidase. Phosphate buffer pH 8·4 in case of amino-acid oxidases and water in case of transaminase and cholinesterase were used. Suspensions of 100 mgm./ml. of tissues were prepared and enzyme activity in control and test experiments, containing graded doses of the drug, determined.

Catalase activity was determined by following the method of Euler and Josephson, as modified by Bonnichsen *et al* (1947). The enzyme was allowed to act on hydrogen peroxide under specific conditions and the peroxide, remaining after 3 and 6 minutes, determined by iodometric titration.

The activities of glutamic oxaloacetic acid transaminase and glutamic pyruvic transaminase were determined by the method of Cabaud (1956) and Wroblewski (1957) respectively.

Monoamine oxidase activity was measured from the rate of oxygen utilisation by the Warburg apparatus using tyramine as the substrate.

L and D amino-acid oxidase activities were measured from the utilisation of oxygen during the oxidation of L-leucine and DL-alanine, by Warburg technique.

Cholinesterase activity was estimated by Warburg method using acetylcholine as substrate and measuring the amount of carbon dioxide liberated.

Results

It was observed that the drug influenced the catalase activity as shown in Table I.

Table I

Effect of glutethimide on the catalase activity

(The figures, expressing the unit activity, are the averages of ten sets of observatoins in each case)

	Concentration μg./ml.				
	2	4	8	16	20
Activity:					
3 minutes	+10 ± 0·7*	+17 ± 1·2	+32 ± 4·2	+ 7 ± 2·5	−18 ± 1·9
6 „	+30 ± 2·5	+42 ± 3·1	+53 ± 2·9	+32 ± 4·1	−44 ± 3·7

* Standard deviation.

It will be observed that the drug up to a concentration of 8 μg./ml. increased the catalase activity but at higher concentrations, the enzyme activity was decreased.

It was also observed that the drug did not affect the transaminases, mono-amine oxidase, L and D amino-acid oxidases and the cholinesterase activities, in concentration up to 100 μg./ml.

Department of Pharmacology, M.G.M. Medical College, Indore, *September* 15, 1962.

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PLASTICIZERS BASED ON EPOXIDISED C.N.S.L. (CASHEW-NUT-SHELL LIQUID)

Cashew-nut-shell liquid, a phenolic group containing important indigenous raw material of this country, is showing immense potentiality both in the fields of coating compositions and polymers in general. Although a major part of it was exported to U.S.A. up till now, growing interest has been shown lately by the manufacturers and the investigators in this country to find better uses for it.

Out of the two major constituents of C.N.S.L. the phenolic portion contributes to its resin-forming property with methylene donors, and the ethylenic unsaturation plays an important part in the auto-oxidative polymerisation in the presence of driers. The unsaturated $C_{15}H_{27}$ side chain in the meta position bears resemblance to the drying oils except that the double bonds are less reactive; as such this may indicate clearly to its use as a plasticizer. The dark colour of the product due to inherent quinone formation limits its use in this field. By epoxidation under suitable conditions of the double bonds in the side chain products can be obtained which are useful as plasticizer-stabilizer for chlorine containing resins. Work to this effect has been carried out therefore, to etherify or esterify the hydroxyl groups of the material and epoxidise the ethylenic unsaturation present in the side chain. Methylation and acetylation have been accomplished by the use of di-methyl sulphate and acetic anhydride with peracetic acid, and oxirane contents approaching the theoretical value have been