OBSERVATIONS ON THE PHARMACOLOGY OF CURCUMA LONGA, LINN.

(N. O. Scitaminaceae)

Pharmacodynamic and toxicological studies of sodium curcuminate

By

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Curcuma longa is a reputed medicinal plant in the indigenous system of medicine, considered to be efficacious in liver disorders and certain pyogenic infections (Nadkarni, 1954). Earlier studies have shown that the soluble sodium salt of the pigment, sodium curcuminate is a potent inhibitor of staphylococcus aureus 'in vitro' (Ramaprasad and Sirsi, 1956) and is a choleretic, stimulating the flow of bile to a considerable extent (Ramaprasad and Sirsi, 1956). The present communication presents the results of some pharmacodynamic and toxicity studies of this compound.

METERIALS AND METHODS

The method of preparation of sodium curcuminate, in brief, was as follows:

Curcumin was prepared from the alcoholic extract by the method of Rao and Shintre (1928). The finely powdered rhizome was extracted with 98 percent alcohol, with frequent shaking of the mixture to facilitate complete extraction. After 48 hrs. the alcohol was separated out by filtration. This extraction procedure was repeated till all the coloring matter was removed. The alcoholic solution was fractionally precipitated with saturated solution of lead acetate, the precipitate washed with alcohol and decomposed by dilute sulphuric acid (2N). The precipitate containing lead sulfate and curcumin was dried and the dried mixture exhausted with hot alcohol, which on cooling deposited the coloring matter in prisms. The coloring matter was recrystallized, from methyl alcohol from which curcumin was obtained as well formed orange prisms.

The sodium salt of curcumin was prepared by suspending curcumin in water and adding 2 N. sodium hydroxide till the curcumin just dissolved. The pH of the solution was adjusted between 8.5 and 9.5 and the solution concentrated on a water bath (70°—80°C). The residue was cooled and the brownish red salt of sodium curcuminate obtained.

Healthy, mongrel dogs kept under observation for a week in the laboratory were used for haemodynamic, respiratory and intestinal movement studies.

Seconal sodium 30 mg./kg. given intraperitoneally was the anaesthesia used. Blood pressure records were taken through a mercury manometer connected to the carotid artery. Respiration was recorded by cannulating the trachea and connecting it through Gaddum's apparatus. Jackson's enterograph was used to record the intestinal movements. Myocardiogram of heart in situ was obtained for both the auricles and the ventricles separately. The drug was administered through a cannula in the femoral vein. The isolated organ studies were made on the guinea-pig ileum and rat uterus by the conventional Schultz-Dale technique using the oxygenated Tyrode solution as the perfusion medium in a 15 ml. bath. The cholagouge effect was studied by suspending the isolated gall bladder of guinea pigs and dogs in the bath and using the same Tyrode solution for perfusion. The toxicity studies were carried out on rats and mice. These included the effects on liver and kidney functions and haematological findings.

Observations

Studies in Anaesthetised Dog.

Blood pressure. A transient fall in blood pressure was associated with a slight increase in bile secretion with 5.0 mg./kg. dose of sodium salt; the effect is enhanced with 10 mg./kg. and 25 mg./kg. doses as seen in Figure 1. The records in Figures 2 and 3 also indicate the same effect on blood pressure.

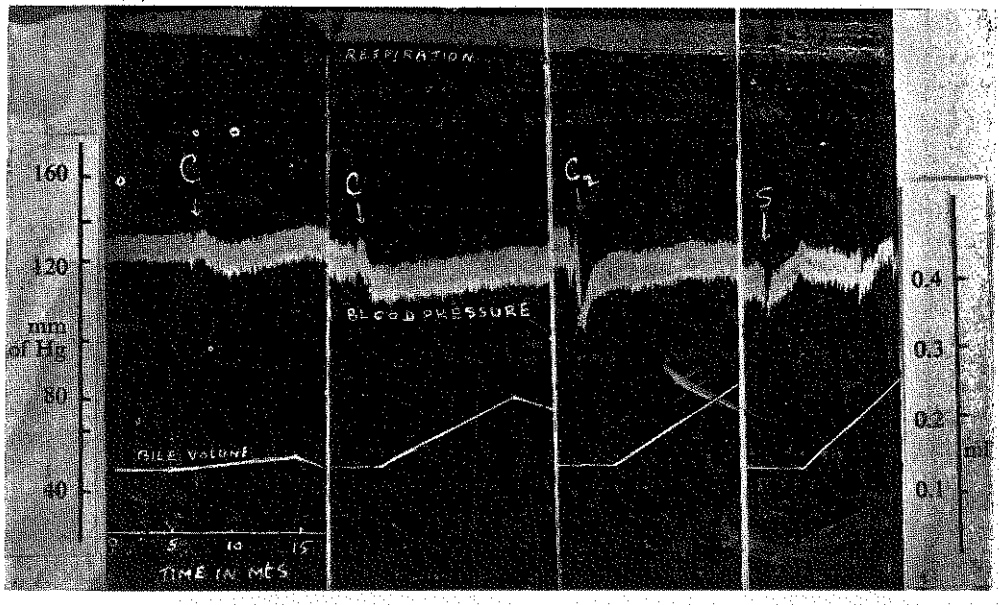


Fig. 1 The effect of sodium curcuminate on blood pressure respiration and bile secretion of dog.
C — 5 mg./kg., C_1 — 10 mg./kg., C_2 — 25 mg./kg.
S — Sodium deoxycholate 5 mg./kg.

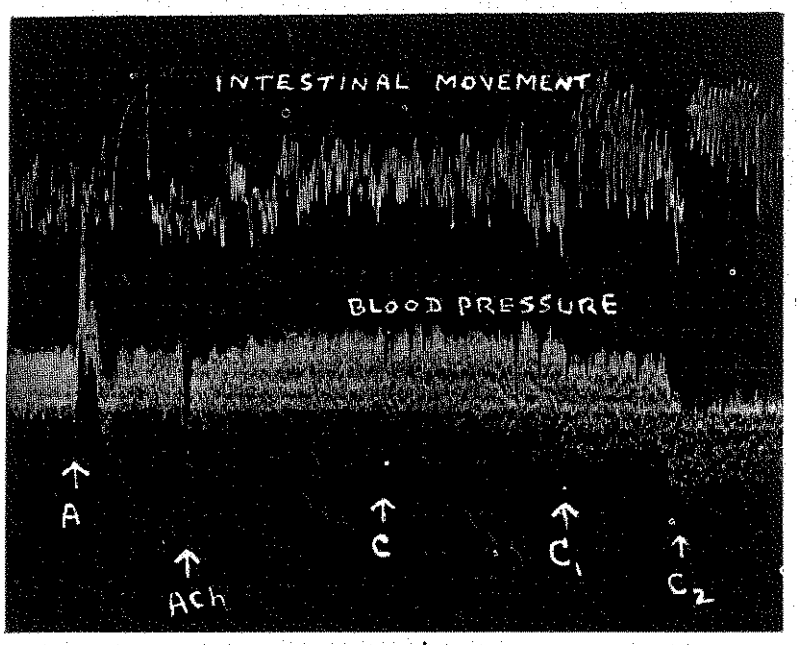


Fig. 2 Effect of Sod. Cuc. on blood pressure and intestinal movements of dog.
A — Adrenaline tartrate 3 γ /kg. Ach — acetylcholine 3 γ /kg.
C, C_1, C_2—Sod. Cuc. 1 mg./kg., 5 mg./kg., 10 mg./kg.

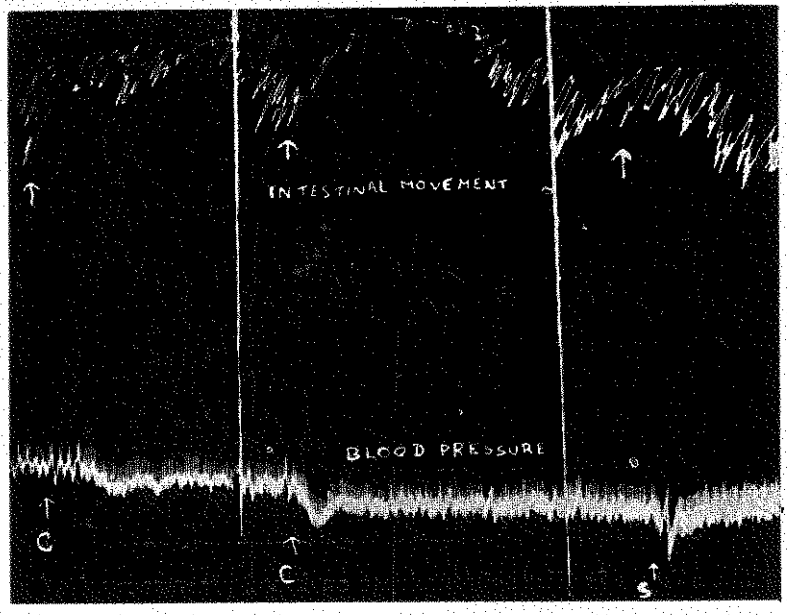


Fig. 3 Effect of Sod. Cuc. on blood pressure & intestinal movement of dog.
C, C_1—Sod. Cuc. 5 mg./kg., 10 mg./kg. S — Sodium deoxycholate 5 mg./kg.

Respiration. Sodium curcuminate in doses of 5.0 mg. and 10.0 mg./kg. caused no alteration either in the rate or in the depth of respiration. Associated with the fall in blood pressure with 25 mg./kg. of the drug, rapid shallow breathing was noticed for a short period (Figures 1,2 and 3).

Myocardiogram. The records of the arterial and ventricular contractions and the carotid blood pressure showed that sodium curcuminate (5.0 mg./kg.) caused a transient fall in blood pressure but had no effect on the atria or ventricles. With 10.0 mg./kg. of the drug a slight reduction in the amplitude of atrial contraction for a few minutes was observed, complete recovery occurred within a few minutes.

Intestinal movements. The effect of varying doses of the drug on the intestinal movements of the ileum in the dog is shown in figures 2 and 3. While no appreciable effect was observed with 1.0 mg./kg., the relaxation of the ileum was seen with 5.0 mg. and 10.0 mg./kg. Unlike the adrenaline relaxation of gut, the spontaneous movements continued even in this relaxed state (Figure 2). This feature is brought forth more vividly in figure 3. Sodium deoxycholate in 5.0 mg./kg. dose caused less relaxation as compared to sodium curcuminate. Since it is known that different parts of the intestinal tract do not respond to the same extent, the effect of sodium salt of curcumin on the doudenum and the colon was investigated. Though relaxation was noticed in these segments also, the effect on the duodenum was very slight, and the effect on the colon was much less in comparison to the ileum.

Isolated Organ Studies.

Excised gall bladder of dog. The effect of adding 1 γ to 50 γ per ml. of the sodium salt of curcumin to the perfusion bath containing the excised dog's gall bladder is shown in figure 4 (a). In 1 in 1000,000 dilution, the drug caused a contraction of the gall bladder musculature. A proportionate increase in the degree of contraction was seen with increasing doses.

In the right half of figure 4 (a) is shown the effect of histamine on gall bladder and the additive effect of histamine superimposed on the contraction caused by sodium salt of curcumin.

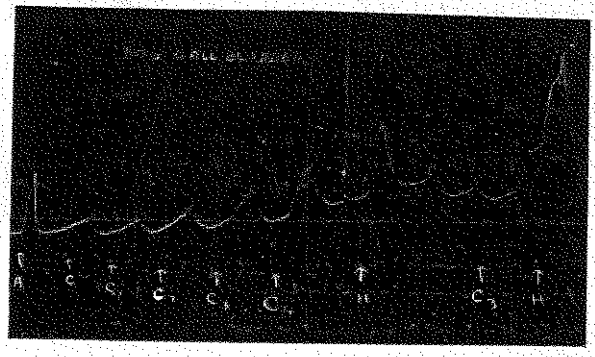
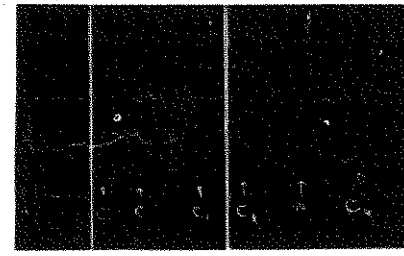


Fig. 4 (a) Effect of Sod. Cuc. on isolated gall bladder of the guinea pig.
A — acetylcholine C—C_4 Sodium Curcuminate (1 γ/ml., 10 γ/ml., 20 γ/ml., 50 γ/ml. 100 γ/ml.)

Excised gall bladder of the guinea-pig. The same type of result as in dogs was obtained in this species also (figure 4 b). The only difference was that the gall bladder tended to remain contracted for a longer period even after washing out the drug. The spasmogenic effect of acetylcholine on the contracted bladder from addition of 50 γ /ml. of sodium salt of curcumin is seen in the right half of the figure 4 (b).



(Fig. 4 (b) Effect of Sodium curcuminate on isolated dog gall bladder.
A — Acetylcholine Chloride ·15 γ / ml.; C — C_4, Sod. Cuc. 1 γ /ml., 5 γ /ml., 10 γ /ml., 20 γ /ml., 50 γ /ml.; H Histamine ·1 γ /ml.

Guinea-pig ileum. The effect of varying doses of sodium salt of curcumin from 10 to 5.0 mg./bath, on the ileum strip and its influence on the spasms induced by histamine is seen in figure 5. The drug caused contraction and the effect increased with increasing dosage. No antihistaminic action was seen even in high concentrations. Atropine slightly lessened the spasmogenic action of the drug.

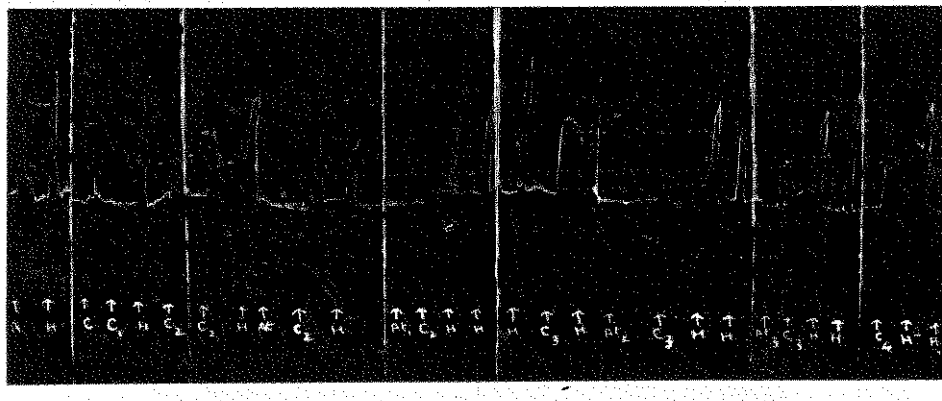


Fig. 5 Effect of Sodium curcuminate on isolated guinea pig ileum (15 ml. bath).

A — acetylcholine chloride	1 γ
H — histamine hy drochloride	0·2 γ
At— atropine sulphate	0·2 γ, AT_1- 0·4 γ AT_2- 1 γ, AT_3-10 γ
Sodium curcuminate	C — 10 γ, C_1 - 100 γ, C_2- 1mg. C_3- 2mg. C_4- 5 mg.

Rat intestine. This behaves in a manner similar to guinea-pig ileum.

Rat uterus. No effect either on the spontaneous movements or the tone of the musculature was produced by the pigment. There was a slight indication for its potentiating action, in higher doses, on the acetylcholine induced spasm.

Toxicity Studies

Acute toxicity. The effect of a single dose of sodium curcuminate given to rats in 0.25 ml. of water by various routes (Table 1) exhibited no toxic effect.

TABLE 1

Acute toxicity studies with sodium salt of curcumin in rats

No. of animals	Dose	Route of administration	Mortality in 7 days
6	500 mg./kg.	Oral	0
6	500 mg./kg.	Subcutaneous	0
6	500 mg./kg.	Intraperitoneal	0

The results of intravenous injection in mice are shown in Table 2.

TABLE 2

Intravenous toxicity in mice

No. of animals	Dose	Mortality	Remarks
6	500 mg./kg.	6	Death occured within 5 minutes. This was preceded by dyspnoea and convulsions.
6	250 mg /kg.	3	
6	100 mg./kg.	0	

Cumulative toxicity. A group of 12 albino rats of either sex weighing between 100 to 110 gm. were taken and divided into 2 groups each of six, three animals being housed in each cage. They were kept on the standard stock diet of the following composition :

Starch	..	61 per cent
Fat (groundnut oil)	..	10 ,,
Casein	..	15 ,,
Salt mixture*	..	4 ,,
Sugar	..	5 per cent
Yeast	..	5 ,,
Shark Liver Oil	..	0.5 ,,

* Sodium chloride 28.9 per cent ; potassium chloride 12.23 per cent ; potassium bicarbonate 19.82 per cent ; calcium carbonate 24.55 per cent ; magnesium sulphate 9.37 per cent ; ferric citrate 2.20 per cent ; copper sulphate 0 32 per cent ; manganese sulphate 0.1 per cent ; zinc sulphate 0.50 per cent ; potassium iodide 0.02 per cent.

Sodium salt of curcumin solution was given intraperitoneally daily for a period of 7 days. The controls were given the same volume of saline in a similar manner. The general behaviour of the animals and food and water intake were observed daily and weights recorded every alternate day.

No mortality was observed during the 7 day period and no appreciable difference in weight loss was seen.

The effect of sodium curcuminate on the haemopoietic system was observed by red blood cell and leucocytic counts and haemoglobin estimations in the rats prior to and at regular intervals of four days during treatment. The results are recorded in table 3.

TABLE 3

Effect of sodium salt of curcumin on blood picture in rats (average values)

	Initial	After 4 days	After 8 days
R.B.C. (in millions/mm.)	7.8±0.52	7.5±0.61	7.5±0.58
W.B.C. (in thousands/mm.)	10.2±1.12	10.0±1.2	10.1±0,94
Hb (gm./100 ml.)	14.4±1.23	141.±1.1	14.0±1.41

Effect on liver function. The urobilinogen excreted in the urine was used as an index of the liver function. It was estimated by the method of Sparkman (1939). No significant change in the urobilinogen content of the urine in control and treated animals was observed.

Kidney functions. The protein excreted in urine was used as an index of kidney function. The urinary protein was estimated by the method of Hiller et al. (1947). No significant difference in the urinary protein level was found in control and drug treated rats.

Histopathological studies. Immediately after the termination of experiments, all the animals were sacrificed and kidney, liver and spleen removed for any macroscopic and microscopic changes. No changes were observed in the drug treated rats as compared with the controls.

DISCUSSION

The preliminary studies on the biliary secretion in the dogs indicate the potential therapeutic usefulness of the pigment from *Curcuma longa*. The sodium salt of curcumin stimulates the flow of bile, the degree and duration of activity depending upon the dose administered. It is an hydrocholagogue in the sense that in the increased flow of bile the percentage of solids are decreased (Ramaprasad and Sirsi, 1956). Hence in conditions where the hydrocholagogic effect is desired this may be found useful.

In spite of a decrease in the concentration of solids in the bile, the total amount of solids excreted during the entire period of activity of the drug are found to be increased, particularly when higher doses of sodium curcuminate are used. The increased bile salt excretion favours the use of curcumin in

digestive disorders of fat metabolism. The increased cholesterol excretion may clinically be found useful in atherosclerosis and other conditions involving cholesterol metabolism and the bilirubin excretion in hastening the recovery from jaundiced conditions (Ramaprasad and Sirsi 1956).

Unlike other bile stimulants in clinical use, curcumin has, by its stimulating effect on the musculature of the gall bladder, an additional advantage as a therapeutic agent in biliary stasis, tendency for cholelithiasis and other affections needing an emptying of the gall bladder contents.

Its powerful antibacterial action on *Staphylococcus aureus* needs particular attention. Many infections of the gastro-intestinal tract, the biliary system and gall bladder are attributed to staphylococcal infection. Drugs like urotropine are now combined with choleretics in therapy of such conditions. Curcumin seems to combine the choleretic and hydrocholagogic action with the antiseptic property and probably would be an ideal therapeutic agent in conditions due to suspected staphylococcal infection.

The low toxicity and absence of adverse pharmacodynamic action of curcumin also favours its clinical use. The relaxation of the intestines while maintaining the spontaneous contractions would probably assist in thorough digestion of the food and complete absorption of the digested material.

The experiments carried out on curcumin so far indicate its therapeutic possibilities only. A detailed investigation in other species of animals and in varying physiological conditions and finally, controlled clinical trials are essential for a proper assessment of its therapeutic place in medical armamentarium.

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