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Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent*

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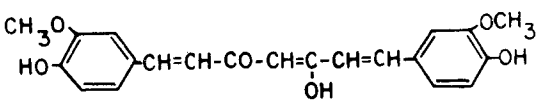
Some pharmacological actions of curcumin (diferuloyl methane) have been examined in rats, mice and cats. The compound possesses significant anti-inflammatory activity in acute as well as in chronic models of inflammation. It is as potent as phenylbutazone in the carrageenan oedema test but only half as potent in chronic tests. Curcumin possesses a much lower ulcerogenic index than phenylbutazone. It prevents the inflammation induced increase in SGOT and SGPT levels. It lacks analgesic and antipyretic activity. It has no other significant pharmacological effects. The oral LD50 in mice is more than 2·0 g kg^{-1}.

Curcumin (diferuloyl methane, I) is an important constituent of rhizomes of *Curcuma longa* Linn and gives the characteristic yellow colour to the rhizome. It has been widely used in indigenous medicine (Nadkarni, 1954). The present communication describes a significant anti-inflammatory activity in curcumin. Part of this work has been briefly reported by Srimal, Khanna & Dhawan (1971).



(I)

METHODS

Male albino rats (90–110 g) and albino mice (15–22 g) of either sex of the CDRI colony were used at 26 ± 2°. Curcumin was isolated from the rhizome and its purity established by melting point determination (180–182°) and nmr data. It was administered orally suspended in 2·0% gum acacia.

Acute toxicity. Graded doses of curcumin were injected intraperitoneally or given orally to groups of 10 mice and the animals were observed for 4 h for any apparent change in the behaviour due to the compound and again 24 h later to assess mortality.

Anti-inflammatory testing. Groups of 5 rats or mice were used. In acute tests the compound was administered orally 1 h before the injection of irritant. In chronic tests curcumin was fed once a day for the duration of the experiment. In every test a control group was fed the vehicle only and another group was administered cortisone acetate or phenylbutazone sodium as a standard drug. In most of the experiments graded doses of curcumin were given and the 50% effective dose (ED50) calculated graphically wherever possible.

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Carrageenan-induced oedema in mice. Oedema in mice was induced by injecting 0·025 ml of 1·0% carrageenan solution into the subplantar region of left paw, the other paw acting as control (Srimal & Dhawan, 1971). The animals were killed with ether 4 h later and the difference between the weight of the paws of treated and untreated animals was a measure of the suppressive action of the compounds tested.

Carrageenan-induced oedema in rats. Oedema in rats was induced in the left paw according to Winter, Risley & Nuss (1962). The volume of the paw was measured plethysmographically (Harris & Spencer, 1962) immediately and 4 h after the subplanter injection of 0·1 ml of 1·0% carrageenan solution in normal saline. The percentage inhibition of the oedema effected by the compounds was then calculated.

Oedema was also induced in rats two days after bilateral adrenalectomy performed under ether according to Schultzer (1935). Animals were given normal saline freely in place of water. The percentage inhibition in each group was obtained as for normal rats.

Formaldehyde-induced arthritis. Arthritis in the left paw of the rat was induced by injecting 0·1 ml of 2·0% formaldehyde in normal saline in the subplantar region on the 1st and the 3rd day of the experiment. The volume of the paw was measured daily for 10 days. The mean increase in the paw volume for each group was calculated to find out the percentage inhibition compared with the control group.

Granuloma pouch test. The technique of Selye (1953) was used to prepare a pouch in the subcutaneous tissue of the back of rat. Injection of 20 ml air was followed by 1·0 ml of 1·0% croton oil in olive oil. The rats were killed on the 14th day and pouches were dissected and dried at 60° to a constant weight.

Cotton pellet test. Two pellets of 50 $\pm$ 1 mg were implanted in the back of each rat according to Winter & Porter (1957). Animals were killed on the 7th day and pellets dissected and dried to a constant weight. The mean weight of the granulation tissue formed around each pellet of the group was calculated.

The effect on adrenal function. The compounds were administered orally to rats daily for 7 days before they were killed and total and differential white blood corpuscles counts made. Both adrenal glands were removed, freed from fat and weighed. The ascorbic acid and cholesterol contents were estimated by the methods of Roe & Kuether (1943) and Zlatkis, Zak & Boyle (1953) respectively.

Effect on serum transaminases and liver ATPase. Formaldehyde arthritis was induced in rats as previously described. The compounds were fed daily for 7 days and the rats were killed on the 8th day. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels were determined (Hanson, 1959). One unit of the enzyme activity was equivalent to the formation of 0·047 μg pyruvate min^{-1} ml^{-1}.

ATPase activity was assayed in 10% (w/v) homogenate of pooled livers prepared in 0·25 M sucrose. The reaction mixture consisted of 0·05M tris at pH 8·0, 1mM ATP and 0·1 ml of 10% tissue homogenate in a final volume of 2 ml. Release of inorganic phosphorus (Pi) from ATP was measured according to Fiske & Subbarow (1925). The hydrolysis of 1 μM of Pi per 100 mg of tissue in 15 min at 37° was considered equivalent to one unit of enzyme activity.

Ulcerogenic index in rats. This was based on the method of Thuillier, Bessin & others (1968). Rats were fed the compounds by stomach tube daily for 6 days, the

food was withdrawn from 2 h before to 2 h after the treatment. Tap water was given freely. Rats were killed on the 7th day, stomachs were removed, cut along the lesser curvature and examined under a low power (20 x) microscope. The degree of single ulceration was determined for each stomach examined and scored according to a scale.

The average degree of single ulceration (ADU) for each group was determined by adding together the degree of single ulceration for the batch and dividing this by the number of animals in the lot. On the basis of the percentage of rats with ulcerations (% RU) the Ulceration Index (UI) was calculated:

$$(\text{ADU} \times \% \text{ RU}) / 100 = \text{UI}$$

Antagonism of phenylquinone writhing in mice

Writhing was induced in groups of 10 mice by phenylquinone (2·0 mg kg^{-1} i.p.) dissolved in 5·0% aqueous ethanol. Curcumin was fed 30 min before the injection of phenylquinone and animals were observed for the appearance of writhing for the next 30 min.

Antipyretic activity. Pyrexia was induced in rats by yeast (2·0 ml of 15% suspension in 2% gum acacia). Curcumin was administered after 6 h at peak temperature. Hourly temperatures were recorded per rectum.

Effect on cardiovascular system and respiration of cats. Cats (2–3 kg) were anaesthetised with pentobarbitone sodium (35·0 mg kg^{-1}, i.p.). Carotid blood pressure and respiration was recorded on a kymograph. A polythene tube was passed into a femoral vein for injections. Contractions of the nictitating membrane due to electrical stimulation (2–5 V, 1 ms, 10-s for 5 s) of the preganglionic sympathetic fibres were also recorded on the kymograph.

RESULTS

Acute toxicity

There was no mortality of mice in any of the groups up to a dose of 2 g kg^{-1}. It was not possible to administer a higher dose. Curcumin was not absorbed intraperitoneally.

Anti-inflammatory tests

Carrageenan-induced oedema in mice. Curcumin inhibited the oedema in mice in dose range of 50·0 to 200·0 mg kg^{-1}. Cortisone in 25·0 to 100·0 mg kg^{-1} (ED50 78·0 mg kg^{-1}, Table 1) inhibited the oedema. The regression lines for curcumin and cortisone are not parallel, but at the ED50 cortisone is about 1·25 times as active as curcumin in this test.

Carrageenan-induced oedema in rats. Curcumin as well as cortisone and phenylbutazone inhibited the oedema in normal rats in doses of 20·0 to 80·0 mg kg^{-1}. The dose of curcumin inhibiting oedema by 50% (ED50) was 48·0 mg kg^{-1} whereas the doses of cortisone and phenylbutazone were 45·0 and 48·0 mg kg^{-1} respectively. Thus they were nearly equiactive in this test (see Table 2) at the ED50 level.

In adrenalectomized rats the effect of curcumin was much reduced. At a dose of 80·0 mg kg^{-1} it produced an inhibition of only 4·9% compared to inhibition of 66·6% in normal rats. It the same experiment cortisone was as effective as in normal rats.

Table 1. *Carrageenan-induced oedema in groups of 5 mice.*

Pretreatment	Dose (mg kg^{-1})	Oedema (mg) ± s.c.	Inhibition %	ED50 (mg kg^{-1})
Control	—	69·8 ± 7·40		
Curcumin	50	47·8 ± 6·16	31·5	
	100	35·2 ± 4·10	49·8	
	200	23·4 ± 4·35	66·2	100·2
Control	—	53·4 ± 4·03		
Cortisone	25	35·6 ± 4·21	33·3	
	50	31·8 ± 4·15	40·4	
	100	22·4 ± 4·50	58·0	78·0

Formaldehyde-induced arthritis. Curcumin as well as phenylbutazone inhibited the formaldehyde-induced arthritis in rats at a dose of 40·0 mg kg^{-1} (see Fig. 1).

Granuloma pouch test. The compound inhibited granuloma formation at doses of 80·0 and 160·0 mg kg^{-1}. Phenylbutazone was effective at 40·0 mg kg^{-1} (see Table 3) and was more active than 80·0 mg kg^{-1} curcumin.

Cotton pellet test. Curcumin inhibited the formation of granulation tissue over dose range of 40·0 to 160·0 mg kg^{-1} in the cotton pellet test. Phenylbutazone (80·0

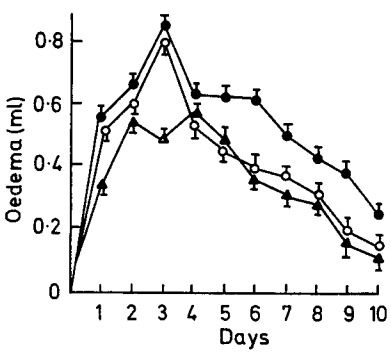

FIG. 1. Graph showing mean oedema (ml) along with the standard error of mean (vertical bars) following subplantar injection (0·1 ml) of 2% formaldehyde on 1st and 3rd day of the experiment. Note the reduced oedema in groups receiving curcumin, 40·0 mg kg^{-1} (○) and phenylbutazone, 40·0 mg kg^{-1} (▲) compared to the control group (●).

Table 2. *Carrageenan-induced oedema in groups of 5 rats.*

Pretreatment	Dose (mg kg^{-1})	Oedema (ml)	Inhibition %	ED50 (mg kg^{-1})
Control	—	0·84 ± 0·012		
Curcumin	20	0·57 ± 0·012	20·2	
	40	0·44 ± 0·033	47·6	
	80	0·28 ± 0·033	66·6	48·0
Control	—	1·15 ± 0·05		
Cortisone	20	0·73 ± 0·08	36·5	
	40	0·61 ± 0·08	47·0	
	80	0·40 ± 0·01	65·2	45·0
Control	—	0·58 ± 0·05		
Phenylbutazone	20	0·44 ± 0·04	24·1	
	40	0·33 ± 0·04	48·2	
	80	0 20 ± 0·03	65·5	48·0

Table 3. *Granuloma test in groups of 5 rats.*

Pretreatment	Dose (mg kg^{-1})	Granuloma pouch test: Dry granuloma weight (g) ± s.e.	Granuloma pouch test: Inhibition %	Granuloma pellet test: Dry granuloma weight (g) ± s.e.	Granuloma pellet test: Inhibition %
Control	—	1·291 ± 0·121		0·186 ± 0·002	
Curcumin	40	1·302 ± 0·090	0	0·156 ± 0·042	16·1
	80	1·106 ± 0·179	14·3	0·146 ± 0·006	21·5
	160	0·910 ± 0·096	29·5	0·145 ± 0·007	22·0
Phenylbutazone	40	1·011 ± 0·180	21·6	—	—
	80			0·151 ± 0·015	19·0

mg kg^{-1}) produced approximately the same degree of inhibition as an equivalent dose of curcumin (see Table 3).

Effect on adrenal function. Curcumin (40·0 mg kg^{-1}) did not produce any significant change in the total or differential leucocytic counts. It also did not cause any change in the adrenal cholesterol and ascorbic acid values. Phenylbutazone (20 mg kg^{-1}), however, caused a significant $P < 0.05$ reduction in the total leucocytic count as well as absolute lymphocytic count (6310 ± 212 and 4275 ± 154 mm^{-3} compared to 10710 ± 607 and 7242 ± 659 respectively in untreated controls).

Effect on serum transaminases and liver ATPase

SGOT and SGPT values were raised to 19·8 and 17·4 units in rats with inflammation (control group) compared to 7·3 and 3·0 units respectively in normal rats. Curcumin (80·0 mg kg^{-1}) treated rats had values similar to those of normal rats. Phenylbutazone (20 mg kg^{-1}) also had a similar effect.

Liver ATPase activity was increased by curcumin (40·0 mg kg^{-1}) as well as phenylbutazone (20·0 mg kg^{-1}) by 23% compared to the control group.

Other effects

Curcumin was found to have a lower ulcerogenic index (0·60) than a nearly equiactive dose of phenylbutazone (1·70). The ulcerogenic index of the control group was 0·08. It failed to prevent phenylquinone-induced writhing in mice up to a dose of 160·0 mg kg^{-1}. Up to a dose of 80·0 mg kg^{-1} it failed to lower the temperature of pyretic rats. Blood pressure and respiration of anaesthetized cats were not affected by curcumin up to a dose of 10·0 mg kg^{-1} (i.v.). There was also no significant change in blood pressure responses to adrenaline, histamine, acetylcholine and contraction of the nictitating membrane following preganglionic sympathetic stimulation.

DISCUSSION

The present study demonstrates the anti-inflammatory activity of curcumin, a major constituent of *Curcuma longa*. The compound is effective in acute as well as chronic models of inflammation and has comparable activity in some models with phenylbutazone, a commonly used anti-inflammatory agent. Further, it returns the SGOT and SGPT levels to normal after they have been raised by inflammation. The specificity of this action is proved by the absence of any change in liver ATPase

activity during inflammation. Both curcumin as well as phenylbutazone stimulate ATPase activity by 23%. Many other anti-inflammatory drugs are known to uncouple oxidative phosphorylation (Whitehouse & Haslam, 1962; Falcone & Madison, 1959) and to increase the activity of ATPase in liver mitochondria.

The potency of curcumin is approximately equal to phenylbutazone in the carrageenan-induced oedema test but it is only half as active in the chronic tests. The acute toxicity of curcumin is, however, much less than phenylbutazone. With curcumin there was no mortality up to a dose of 2·0 g kg^{-1} (cf. 418 mg kg^{-1} oral LD50 of phenylbutazone, Srimal, Sharma & others, 1973). It was not possible to administer a higher dose. Further, curcumin has a lower ulcerogenic index (0·6) than phenylbutazone (1·7). Finally, whereas phenylbutazone has produced a significant leucopenia and lymphocytopenia, curcumin did not do so.

The mechanism of action of curcumin as an anti-inflammatory agent is not clear. It is much less effective in adrenalectomized animals suggesting an indirect action through the adrenal cortex. It does not produce any significant change in the adrenal ascorbic acid and cholesterol levels which should have been lowered if increased adrenocortical activity is involved in the anti-inflammatory action. Further, curcumin does not produce any lowering of eosinophils and lymphocytes as might be expected with increased secretion of cortisone and related hormones from adrenal cortex. It is possible that it may sensitize tissues to the action of adrenal cortical hormones rather than affecting their secretion or release. Further work to elucidate the mechanism is in progress.

Curcumin unlike phenylbutazone lacks antipyretic as well as analgesic activity. There is no effect on the gross behaviour of mice and rats. It does not produce any change in the blood pressure level or in responses to adrenaline, histamine and acetylcholine on the blood pressure, showing lack of autonomic effects.

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