

## Effect of Mangostin, a Xanthone from *Garcinia mangostana* Linn. in Immunopathological & Inflammatory Reactions

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Mangostin from the rinds of the fruits of *G. mangostana* inhibited systemic anaphylaxis, Schultz-Dale's reaction and immunocytotoxicity in guineapigs and rats. It also significantly inhibited the primary and secondary responses of adjuvant-induced arthritis in rats. The results have been correlated with the previous findings on the pharmacological effects of mangostin and the probable mode of action is discussed.

**R**IND of the fruits of *Garcinia mangostana* Linn. is an astringent and is useful in the treatment of diarrhoea and dysentery<sup>1</sup>. Xanthone mangostin is isolated<sup>2</sup> and its structure conclusively established<sup>3</sup>. Not many investigations have been made on the pharmacology of these glycosides<sup>4</sup>. Except for a few reports on the schistosomicidal activity of certain synthetic xanthones<sup>5</sup>, CNS stimulant effect<sup>6</sup>, tuberculostatic effect<sup>7</sup>, cardiotoxic, anti-depressant, and hydrocholeretic effect for mangiferin, a xanthone-C-glucoside<sup>8</sup>, antiinflammatory and antihepatotoxic effect for magniferin<sup>9,10</sup>. In view of this it is felt worthwhile to investigate the complete pharmacological profile of the xanthone mangostin and its derivatives. Previous studies have revealed CNS depressant, antiinflammatory, cardiotoxic, antimicrobial and antihepatotoxic effects of mangostin and its derivatives<sup>11-15</sup>. In view of the finding that mangostin exhibits a pronounced antiinflammatory activity as tested by various parameters, at a dose level of 50 mg/kg (i.p), it is of interest to elucidate the effects of mangostin in various immunopathological and inflammatory reactions, since a number of non-steroidal antiinflammatory agents have been reported to interfere with one or more discrete phases of immunopathological and inflammatory events<sup>16</sup>.

### Materials and Methods

**Drug preparation and administration** — Mangostin was isolated according to the method of Kalyanaraman<sup>17</sup> from the dried, powdered rinds of *G. mangostana*, collected from the hills of Courtallam. Since mangostin was insoluble in water, a fine suspension of the compound was prepared with 2% gum acacia using a "Remi" homogeniser at 3000 rpm. This was administered (i.p) to rats and guineapigs at a dose level of 50 mg/kg. The control animals received (i.p) 2% gum acacia at a constant vol. of 1 ml/kg.

**Animals** — Inbred strains of Wistar albino rats were obtained from Madras Veterinary College and

guineapigs from the King's Institute, Guindy, Madras, for the immunological experiments.

**Immunological studies** — To evaluate the effect of mangostin on humoral and cellular hypersensitivity reactions, the following four parameters have been studied:

(1) **Systemic anaphylaxis** — This was done according to the method of Harnath and Shyamalakumari<sup>18</sup>, using guineapigs of either sex (200-300g). The animals were sensitised with an ip injection of egg albumin in saline (4g/kg). The animals were divided into 3 groups of 10 each and were administered with gum acacia, mangostin (50 mg/kg) and dexamethasone (1 mg/kg) respectively, 2 days before the commencement of the experiment and subsequently for 6 days after the injection of the antigen. A challenging dose of egg albumin (4g/kg) was administered (i.p), after 10 days. Death of animals up to 24 hr following the challenging dose was taken as lack of protection.

(2) **Schultz-Dale's reaction** — Two groups of 5 guineapigs each, were sensitised with egg albumin as described earlier. The control animals were administered with gum acacia and the test group with mangostin (50 mg/kg), 2 days before the commencement of the experiment and subsequently continued for 6 days. Ten days later all the animals were sacrificed, pieces of ileum were removed from the test and the control groups, mounted in communicating organ bath containing Tyrode solution at 37°C. The tissues were exposed to the antigen-egg albumin and histamine at various concentrations, for a fixed time and the responses were recorded on a rotating smoked drum kymographically.

(3) **Immunocytotoxicity or "resette formation"** — This method is based on the specific binding of an antigen with the surface of the cells containing the corresponding antibody and the experimental procedures have been described by Sriram and Srinivasarao<sup>19</sup>. The heterologous cells adhere strongly *in vitro*

to the lymphoid cells containing the corresponding antibody on their surface and give rise to characteristic "rosettes", defined as any cell with more than half its circumference covered with red cells. Any decrease in the number of rosettes in comparison to control samples is taken as a positive response indicating possible interference in immune-reactions.

**Sensitisation:** Freshly collected sheep blood in 'Alsever's' solution was centrifuged, processed according to the method of Sriram and Srinivasarao<sup>19</sup>, the red blood cells were separated, standardised by counting in a haemo-cytometer, so as to give a concentration of  $1 \times 10^9$  cells/ml and this was used for further studies. Three groups of albino rats (10 in each) were sensitised with sheep red blood cells (SRBC) at a dose level of 0.5 ml/100g i.p, and the animals were administered with gum acacia, mangostin (50 mg/kg) and dexamethasone (1 mg/kg), respectively for 7 days. The animals were sacrificed on the 8th day, the spleen was removed, washed free of clots and cut into small bits. The cells were separated by pressing the fragments with a glass rod in a test tube, washed with cold buffered saline and counted in a haemocytometer. The lymphoid cell suspension thus obtained was then treated with SRBC and the percentage of rosettes was counted using a haemocytometer and the results were interpreted according to the method mentioned above<sup>19</sup>.

(4) **Adjuvant-induced arthritis** — This was induced in 3 groups of albinorats (10 in each) by injecting killed *Mycobacterium tuberculosis* cells suspended in paraffin oil (10 mg ml), into the plantar region of the animals at a concentration of 0.1 ml/100 g, according to the method described by Edordo Arrigoni-Martelli<sup>16</sup>. The animals were administered with gum-acacia, mangostin (50 mg/kg) and dexamethasone (1 mg/kg), respectively, 24hr before the injection of the adjuvant and subsequently continued for 10 days. The primary reaction, characterised by pronounced swelling of the injected paw, was observed within 4 hr after the injection of the adjuvant and persisted for several weeks. The oedema volume was measured plethysmographically on the 1st day, 5 hr after the induction of oedema and subsequently on the 15th day. The paw volume of the contralateral limb was also measured on the 1st and 15th days. The delayed systemic response or the secondary response, was characterized by the swelling of the front paws, the contralateral

paw and the appearance of arthritic nodules on the ear and tail. The ability of the test drug to inhibit the arthritic-syndrome was assessed and compared with the control and positive-control animals. The course of adjuvant-induced arthritis and the activity of the test compounds were also assessed by the grip-strength method in which the ability of the animals to balance on a rotating rod (8 rpm) was tested, as per the procedure of Bhide<sup>20</sup>.

**Results**

(1) **Systematic anaphylaxis** — Pretreatment of mangostin at 50 mg/kg, for 2 days prior to the sensitisation of the animals with egg albumin and subsequently for 6 days thereafter, afforded 60% protection against the mortality induced by the challenging dose of egg albumin on the 10th day after sensitisation. Pretreatment of animals with dexamethasone afforded 50% protection.

(2) **Schultz-Dale's reaction** — The isolated ileal tissue of guineapigs pretreated with gum acacia responded powerfully when the antigen-egg albumin was added into the bath (1 : 100). The tissues also responded well to histamine (10 ng/ml). However, the ileal strips removed from the animals pretreated with mangostin responded only very weakly to the administration of the antigen. The response to histamine (10 ng/ml) was not altered to any significant extent (Fig. 1).

(3) **Immunocytoadherence** — While addition of SRBC suspension to the lymphoid cell suspension of

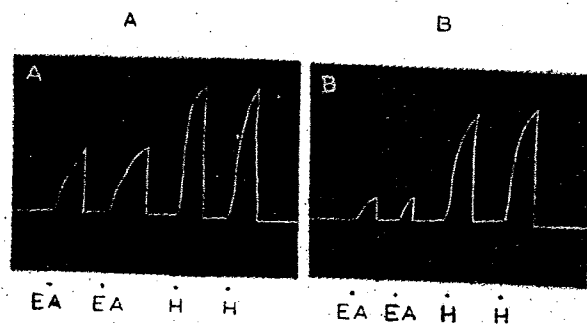


Fig. 1 — Effect of mangostin in Schultz-Dale's reaction. Effect of antigen egg albumin (EA, 1:100) and histamine (H, 10 ng/ml) on the isolated ileum of sensitized guineapigs treated with gum acacia (A) and mangostin (B)

TABLE I — EFFECT OF MANGOSTIN ON ADJUVANT INDUCED ARTHRITIS IN RATS

[Values are mean ± SD]

|               | Primary response<br>Injected paw vol. (ml) |             |             |             |             | Secondary response<br>Contralateral paw vol. (ml) |             |             |
|---------------|--|-------------|-------------|-------------|-------------|---|-------------|-------------|
|               | Initial                                    | 5th hr      | % reduction | 15th day    | % reduction | Initial   | 15th day    | % reduction |
| Control       | 1.20 ± 0.15                                | 3.20 ± 0.20 | —           | 2.40 ± 0.25 | —           | 1.20 ± 0.15                                       | 1.95 ± 0.20 | —           |
| Mangostin     | 1.30 ± 0.15                                | 2.40 ± 0.20 | 25*         | 1.60 ± 0.20 | 33.33*      | 1.30 ± 0.10                                       | 1.45 ± 0.05 | 25.64*      |
| Dexamethasone | 1.25 ± 0.15                                | 2.35 ± 0.05 | 26.5*       | 1.55 ± 0.10 | 35.42*      | 1.25 ± 0.15                                       | 1.35 ± 0.05 | 30.77*      |

\*P < 0.01

TABLE 2 — EFFECT OF MANGOSTIN ON ADJUVANT INDUCED ARTHRITIS AS TESTED BY THE GRIP STRENGTH METHOD

 [Method : Rota-rod (8 rpm); animal : Wistar rats. Values are mean  $\pm$  SD]

| Group                                      | Balancing time in sec |       |
|--|-----------------------|-------|
| Control                                    | 126.8                 | 13.9  |
| Sensitized control                         | 55.0                  | 11.8* |
| Sensitized rats-treated with mangostin     | 117.7                 | 20.1  |
| Sensitized rats-treated with dexamethasone | 120.5                 | 14.5  |

 \* $P < 0.001$ 

the unsensitised animals did not result in the formation of 'rosettes', addition of SRBC to the sensitised lymphoid cell suspension obtained from the gum acacia treated rats resulted in the formation of  $38.5 \pm 8.5\%$  rosettes. Addition of SRBC to the sensitised lymphoid cell suspension obtained from mangostin treated animals resulted in the formation of only  $14 \pm 6.5\%$  rosettes, thereby showing an inhibition of immunocytoadherence ( $P < 0.01$ ). Similarly, in the dexamethasone treated animals there was only  $12.5 \pm 5.5\%$  rosettes ( $P < 0.01$ ).

(4) *Adjuvant-induced arthritis* — The results of the effects of mangostin on adjuvant-induced arthritis in rats are given in Table 1. Mangostin is highly active in inhibiting the primary as well as the secondary responses of the adjuvant-induced arthritic in rats. The additional parameter (grip strength method) to assess the course of adjuvant-induced arthritis and the activity of mangostin in inhibiting the arthritic-syndrome also confirms the activity of mangostin. The results of the effect of mangostin on adjuvant-induced arthritis as tested by the grip-strength method are presented in Table 2.

## Discussion

Mangostin, a major xanthone of *G. mangostana*, interferes with one or more discrete phases in the sequence of events leading to immunopathological and inflammatory responses as evidenced by the ability of the xanthone to inhibit systemic anaphylaxis, Schultz-Dale's reaction, immunocytoadherence and adjuvant-induced arthritis in various experimental animals. From previous studies<sup>11</sup>, it has been found that mangostin is active in suppressing the 'acute' as well as the 'chronic' inflammations as evidenced by the ability of the xanthone to inhibit carrageenin-induced hind paw oedema, granuloma pouch and cotton-pellet granuloma in albino rats. Additional evidence has been obtained from the present study of the ability of the xanthone to inhibit the primary and secondary response in adjuvant-induced arthritis in rats. Little evidence exists, in fact, that correlates clinical and experimental antiinflammatory activity with immunosuppression. Among the several mechanisms of actions proposed are (i) interference with the synthesis of enzymes necessary for the release of inflammatory mediator, (ii) interference with the pharmacological mediators of the inflammatory responses, (iii) reduction of complement levels and

(iv) inhibition of mononuclear cell exudation. Though mangostin has been shown to inhibit prostaglandin synthetase<sup>15</sup> — a group of enzymes which play a key role in the pathogenesis of inflammatory and immunopathological reactions, further studies are required to elucidate the activity of mangostin with respect to levels of complement, mononuclear cell exudation, etc. in order to understand the mechanism by which mangostin interferes with various inflammatory and immunopathological reactions.

Antiinflammatory as distinct from immunosuppressive activity, has been described in several models of experimental inflammation for many immunosuppressive agents,<sup>16</sup> such as 6-mercaptopurine, methotrexate, cyclophosphamide, chlorambucil and actinomycin-D. These drugs mainly act by preventing the participation of macrophages in delayed hypersensitivity and their infiltration into an inflammatory site. Similarly, a number of antiinflammatory agents such as steroids, aspirin like compounds, gold salts and pharmacological doses of oestrogen, etc. have been shown to interfere with the immunopathological and inflammatory reactions such as adjuvant-induced arthritis. In view of the above findings, it would be interesting to extend such studies on mangostin as an immunosuppressive agent and elucidate its role in various immunological reactions as well as in experimental tumors in animal models.

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