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Research Article

Immunomodulatory Activity of Fruit Rinds of *Garcinia indica* (Family Guttiferae) on Swiss Albino Mouse Model

*Khan S[1], Sonar P K[2], Saraf S A [3], Saraf S K [2], Javed S A[1]

[1]*College of Pharmacy, Jazan University, Jazan, Kingdom of Saudi Arabia.*
[2]*Faculty of Pharmacy, Northern India Engineering College, Lucknow, India*
[3]*Faculty of Pharmacy, Babu Banarasi Das National Institute of Technology and Management, Lucknow, India*

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ABSTRACT
Garcinia indica (Guttiferae) is a plant widely used in the traditional medicine in India as antiscorbutic, cholaguage etc. It is prescribed in certain immune disorders like asthmatic affections, chronic catarrah and cough. The study was aimed at scientifically evaluating the immunomodulatory activity of *Garcinia indica* L. (Guttiferae), on Swiss albino mice against sheep RBC (SRBC) antigen challenge, thereby verifying the traditional usage. The major constituent of the extract was ascertained to be garcinol, by chromatographic and spectroscopic studies. Oral administration of hexane extract (HE) (50 – 300 mg/kg) elicited a dose-dependent increase in Haemagglutination antibody (HA) titers, delayed-type hypersensitivity response and phagocytosis. At the selected maximum dose, HE demonstrated higher immunostimulatory activities in comparison with control and positive standard (levamisole).

Key words*: Garcinia indica*; SRBC; Phagocytosis; HA titer; DTH

INTRODUCTION

Natural products including plants, animals and minerals have been the basis for treatment of human diseases. Herbal drugs possess negligible side effects, are less expensive and not only eliminate the disease from the patient's body but also enhance the immunity, besides playing an appreciable role towards suppressing untoward immune reactions[1, 2].

Garcinia indica is a slow-growing slender tree of moderate size; it bears berries with arillate seeds, consists of 180 species, out of which ~30 are found in India[3].

Several studies have demonstrated that garcinol derived from the fruit rinds of *Garcinia indica* exhibit significant antibiotic[4], antileukemic[5], antioxidant [6], antiulcer, antioxidative and anti-glycation activities[7]. Garcinol also inhibits nitroquinoline 1-oxide-induced tongue carcinogenesis in rats[8]. *G. indica* is an ingredient of Amlavetasa- which is prescribed in chronic catarrh, cough and in various immune disorders like asthmatic affections[9], bronchitis[10] etc. in the south, IMPCOPS, Chennai[9, 11]. These findings prompted the present investigators to explore the immunomodulatory effect of hexane extract of fruit rinds of *G. indica.*

MATERIAL AND METHODS

Collection of plant material: Fruit rinds (semi-dried) of the plant *Garcinia indica* were collected in the month of March from Udupi (Karnataka), India. Identification and authentication of the crude drug was carried out at National Botanical Research Institute, Lucknow (India).

Extraction and Isolation: Dried fruit rinds of *Garcinia indica* (500gm) were cut into irregular pieces and extracted with water. The extract was filtered, and the squeezed residue was dried in shade and powdered by grinding. The powder (200gm) was extracted with hexane (500ml) by hot percolation method using Soxhlet apparatus. The extract obtained was filtered, solvent evaporated under vacuum, and further dried in a desiccator under reduced pressure to complete drying (yield - 9.8gm w/w). The extract was subjected to preliminary qualitative tests to identify the various phyto-constituents like alkaloids, phytosteroids, flavanoids, phenolics, saponins, carbohydrates and amino acids[12, 13, 14].

Dried extract (1gm) was impregnated with 2 gm of silica gel and loaded onto silica gel column. The column was eluted with hexane and mixture of hexane: ethyl acetate in increasing order of polarity. The pure compound (A) was isolated by monitoring TLC with hexane: ethyl acetate (60:40) and the solvents from the eluates were evaporated under vacuum. Yield of recrystallized compound (A) was ~250 mg [15].

Animals: The present study was conducted on either sex of Swiss albino mice weighing between 20-25gm. The animals were maintained in polyacrylic cages under standard laboratory conditions with temperature maintained between 22 - 27 C°, with a 12-h light and dark cycle. Commercial diet and water were given ad libitum. All procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Faculty

*Author for correspondence

Table 1: *In vivo* effect of HE on weight changes and humoral immune responses (primary and secondary HA titer) in Swiss Albino mice.

		Weight change in animals	Antibody response HA titer (mean ± SEM)	
Treatment	Dose (mg/kg)	Percentage (w/w)	Primary HA titer	Secondary HA titer
Control	Ground nut oil (vehicle)	07.66 ± 0.66	4.66 ± 0.33	4.5 ± 0.22
Livamisole	2.5	10.16 ± 0.60* (32.63%↑)	6.50 ± 0.42** (39.48 % ↑)	6.16 ± 0.40** (36.88 % ↑)
HE-1	50	7.83± 0.30 (2.22% ↑)	4.66 ± 0.33 (no change)	4.33 ± 0.33 (3.7% ↓)
HE-2	100	8.5 ± 0.42 (10.96% ↑)	6.16 ± 0.30* (32 % ↑)	5.50 ± 0.22 (22.22 % ↑)
HE-3	300	14.16 ± 0.79*** (84.86%↑)	8.0 ± 0.36*** (71.67 % ↑)	7.83 ± 0.30*** (74 % ↑)

*Six mice were used in each group. (↑) indicates stimulation and (↓) indicates suppression. Data are expressed as mean ±SEM. The comparison of control Vs. treated group was performed by one-way ANOVA (Turkey's Multiple comparison test). * P < 0.05, ** P < 0.01 and *** P < 0.001*

Table 2: *In vivo* effect of HE on Cell Mediated Immune Response (CMI): Hypersensitivity response (Early and

		DTH response Paw oedema (mm) Mean ± SEM		Phagocytosis
Treatment	Dose (mg/kg)	24 h	48 h	Percentage (%)
Control	Ground nut oil (vehicle)	1.85 ± 0.04	1.75 ± 0.04	53.66 ± 2.44
Livamisole	2.5	2.63 ± 0.02*** (42.16 % ↑)	2.35 ± 0.04*** (34.28 % ↑)	79.00 ± 3.66*** (47.22 % ↑)
HE-1	50	2.11 ± 0.04*** (14.05 % ↑)	1.95 ± 0.04* (11.42 % ↑)	53.50 ± 3.35 (0.29 % ↓)
HE-2	100	2.35 ± 0.04*** (27.02 % ↑)	2.11 ± 0.03*** (20.57 % ↑)	63.33 ± 1.97* (18.02 % ↑)
HE-3	300	2.81 ± 0.03*** (51.89 % ↑)	2.58 ± 0.04*** (47.42 % ↑)	88.83 ± 2.41*** (65.54 % ↑)

*Six mice were used in each group. (↑) indicates stimulation and (↓) indicates suppression. Data are expressed as mean ±SEM. The comparison of control vs. treated group was performed by one-way ANOVA (Turkey's Multiple comparison test). * P < 0.05, ** P < 0.01 and *** P < 0.001*

of Pharmacy, Northern India Engineering College, Lucknow, which is registered with CPCSEA.

Treatment: SRBC were collected in Alsever's solution and were suspended in phosphate buffer saline (PBS) [pH-7.2 (0.9%)] for further use. They were adjusted to different concentrations [0.1ml (20% approx.) or 20µl (1% approx.)] for immunization and challenge via i.p. injection. The experimental groups were divided into five sub-groups of six animals each. GROUP–1, Control, received only vehicle (ground nut oil), GROUP–2, Positive control, received levamisole (2.5mg/kg body weight), GROUP-3, HE (50 mg/kg body weight), GROUP–4, HE (100 mg/kg body weight), GROUP–5, HE (300 mg/kg body weight). The hexane extract (HE) was suspended in groundnut oil (vehicle) to prepare a suitable dosage form and administered once daily (orally) for 14 days. The dose volume was 0.1ml. The body weight of all animals was recorded weekly.

HA Titer or Antibody Titer (Humoral Immune Response): On the 7th and 15th day, the blood was withdrawn from retro-orbital plexus of all antigenically challenged mice and antibody levels were determined by Mungantiwar's techniques [16] of haemagglutination with slight modification: Two-fold serial dilutions of serum samples were made in 100µl of PBS in 96-well microtiter plates to get the antibody concentrations half of the previous dilution. 100µl (1%) SRBC suspension in PBS was mixed in each well, shaken and was allowed to stand at room temperature for 2h. The reciprocal of the highest dilution of the test serum showing agglutination formation was taken as the **Antibody titer** or HA titer, which is defined as the "reciprocal" of the last dilution with positive reaction.

DTH reaction or Footpad reaction (Cell-mediated immune response): After 2h of SRBC injection, HE was administered once daily for successive days. Six days later, the thickness of the left hind footpad was measured with the help of screw guage and was recorded as the control. Immunization was then performed by injecting 20µl (1%) of SRBC, intradermally into the left hind

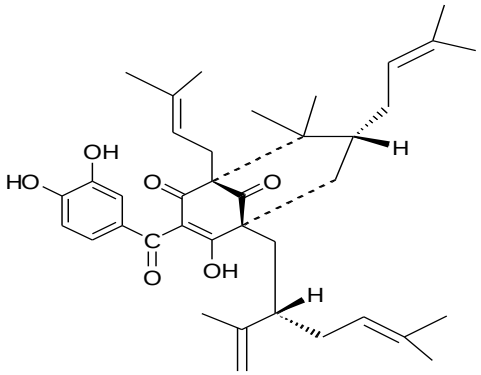

Fig. 1: Garcinol (A)

footpad. The DTH response was measured, in terms of increase in footpad thickness due to swelling caused by the hypersensitivity reaction, 24 and 48h later. The footpad reaction was expressed as the difference in thickness (mm) of footpad [17, 18, 19].

Phagocytosis: The phagocytic function of peritoneal macrophages was assessed by the method of phagocytosis. The peritoneal macrophages of HE treated mouse were harvested by flushing the peritoneal cavity with 3.0ml HBSS containing 10% peptone. The macrophages (3×10^5/100µl), present in the aliquots, were incubated on glass slides at 37ºC for 30 min. in a humidified chamber. The glass slides were washed thoroughly to remove non-adherent cells. The adhered cells were incubated with 100µl of heat killed (boiled for 30 min in normal saline) and opsonized (20% analogous serum or autologous plasma) *Candida albicans* cells (2×10^6), at 37ºC for 30 min. Finally, the cells on the slides were stained with Wright's dye after thorough washing with HBSS, to determine the adherent cells containing yeast cells microscopically. Three hundred cells were counted and expressed as percentage phagocytosis [18, 20, 21].

Statistical Analysis: Data were expressed as mean ± SEM. All treated-groups were compared with control and positive control (Levamisole) by one way-Analysis of Variance with Turkey's Multiple comparison test. The significance level was set at $P < 0.05$ with the help of GraphPad Instat ® software.

RESULTS AND DISCUSSION

During pharmacological screening, animals treated with different doses of the extract showed an increase in the Haemagglutination antibody titers, DTH-response and phagocytosis in a dose dependent manner. Per oral administration of HE (50, 100 and 300 mg/kg body weight) resulted in dose related increase in the primary and secondary antibody syntheses (Table-1). Hemagglutination titer did not show any significant change with the minimum dose taken as 50mg/kg body weight of HE. However, the HE showed an increase in the hemagglutination titer at a dose of 100mg/kg body weight, while significant ($P<0.001$) increase was observed at a dose of 300mg/kg body weight, which is an almost two fold increase compared to the control untreated group. The agglutination of the humoral responses as evidenced by an enhancement of antibody responsiveness to SRBC indicates the enhanced responsiveness of macrophages and B-Lymphocytes that are closely associated with antibody production.

The DTH-response, which is the direct correlate of cell-mediated immunity (CMI), showed a significant dose-related increase in footpad thickness found at 24 and 48h as compared with the control and Levamisole group. In-vivo animal studies showed a significant increase in the early and delayed type hypersensitivity reactions to SRBC at doses of 50, 100 and 300mg/kg body weight. However, the delayed response at a dose of 50mg/kg body weight was slightly less significant ($P<0.05$) when compared with the control group. This indicated the overall stimulatory effect of HE on cellular immunity when compared with the control group. Also, the stimulatory effect was significantly higher than that observed for Levamisole (a positive standard) at a dose of 300mg/kg body weight.

These results are expected to be mediated through sensitized T-lymphocytes, which when challenged by the antigens are converted to lymphoblasts and secrete lymphokines, attracting more scavenger cells to the site of reaction [22]. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction [23].

The results of the *in-vitro* polymorphonuclear (PMN) function test (phagocytosis) showed a significant increase in the percentage phagocytosis. This indicates that HE enhanced the phagocytic efficacy of the macrophages by causing greater engulfment of the *Candida* cells in comparison with the control, thereby stimulating a non-specific immune response.

Thus, the HE exhibited maximum significant activity at a dose of 300 mg/kg body weight (65.5%), and a decline in activity was observed at a dose of 50mg/kg body weight (0.29%) when activity was compared with the control group as well as the Levamisole group. Thus, the crude extract of *G. indica* showed significant immunostimulant activity in a dose dependent manner. Hence, the hexane extract was loaded on to silica gel column and the major compound (A), m.p. 121–122˚C was isolated in pure state.

The spectral characteristics of compound (A) are as follows, UV- absorption bands at λ_{max} (EtOH) – at 257.6, 232.4 and 353.6nm; FT-IR ν_{max} (KBr, cm^{-1}) 3365, 1731, 1635 and 1600 cm^{-1}, ^{1}H NMR ($CDCl_3$), 7.26 ($CDCl_3$), δ 1.03 (3H, s, Me), 1.18 (3H, s, M+e), 1.27-2.76 (31H, m, Methylene and Methyne), 4.41 (1H, d, J = 12Hz), 4.94 (1H, s), 5.07 (1H, d, J = 6Hz), 6.65 (1H, d, J = 9Hz) and 6.99 (1H, d, J = 9Hz). FAB-MS spectra of compound (A) showed fragments at m/z 603 ([M+H]), 604, 534, 465, 341, 231, and 137.

From the above spectral data, compound (A) was identified as garcinol (Fig.1). The chemical shifts were identical with reported values[24, 25].

CONCLUSION

The hexane extract of fruit rinds of *Garcinia indica* exhibit significant increase in humoral and cell-mediated immunity. The major active constituent was isolated and characterized to be garcinol, which could be one of the

constituents responsible for the activity. Thus, this study confirms and authenticates the traditional claim and usage of *Garcinia indica* as an immunomodulatory agent.

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