Immunomodulatory and Anti-inflammatory Effects of Semecarpus anacardium Linn. Nut Milk Extract in Experimental Inflammatory Conditions

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Immunomodulatory effects of Semecarpus anacardium Linn. nut milk extract (SA) were investigated in adjuvant induced arthritis by studying the alterations in humoral and cell mediated immune responses and also the anti-inflammatory effects by evaluating the changes in paw edema, tumour necrosis factor (TNF-α), nitric oxide and myeloperoxidase activities. Pharmacological studies were also conducted with SA and indomethacin on experimental animals for evaluating the anti-inflammatory, analgesic, antipyretic and ulcerogenic activities. The alterations in the humoral and cell mediated immunity were significantly reverted back to near normal levels on treatment with SA. The drug significantly reduced the elevation in the paw edema, TNF-α, nitric oxide and myeloperoxidase levels when compared with adjuvant induced arthritic animals, which shows the anti-inflammatory activity of the drug. SA showed strong anti-inflammatory effects in xylene-induced ear edema and formalin-induced inflammation. In analgesic test, the extract elicited a potential activity on both acetic acid-induced writhing response as well as hot plate test showing its central and peripheral mediated action. The drug also elicited antipyretic action in yeast-induced hyperemia in rats. In addition, the extract did not produce any ulceration on gastric mucosa during ulcerogenic test and did not produce any serious adverse effects. All these effects are nearly similar to the activities of indomethacin except the ulceration where indomethacin produced significant ulceration. From this study, the protective immunological and pharmacological role of SA is demonstrated.

Key words Semecarpus anacardium; immunomodulatory; anti-inflammatory; analgesic; antipyretic

In autoimmune diseases, on one hand pathogenic self-reactivity of T-cells plays an important role, while on the other hand self-reactivity is needed to regulate auto-aggressive responses. Modified immuno-inflammatory reactivity in rats with adjuvant arthritis has been reported by several groups.1—3) The pathogenesis of adjuvant disease in the rats results from a cell mediated immune response to disseminated mycobacterial antigen.4) Delayed type hypersensitivity can be elicited in rodents against a variety of antigens such as bacteria, sheep red blood corpuscles (SRBC) and histocompatibility antigen and is a T lymphocyte dependent phenomenon. The arthritogenic T cells likely migrate to the joints5) and initiate inflammation in the synovium by recruiting other lymphocytes monocyte/macrophages and polymorphonuclear leukocytes (PMN). These may release cytokines and other products, which contribute to resorption of bone and destruction of cartilage. Thus, pharmacological inhibition of this leukocyte migration and accumulation in arthritis may have beneficial effects for joint preservation.6)

Intracutaneous injection of killed mycobacteria in oil results in a systemic disease and severe arthritis. This arthritis is an immunologic response to an antigen present in the capsule of the mycobacteria. Direct comparisons between finding in adjuvant arthritis in rats and rheumatoid arthritis (RA) in men are not justified because significant difference does exist. However, similarities occur where the cellular involvement in adjuvant arthritis are provocative in attempting to understand the role of lymphocytes in RA.7)

The most challenging question for the study of RA concerns the specificity of immune reactions, which indicate and perpetuate the autoimmune pathology. Those reactions are most likely dependent on activated autoreactive T-cells but do also involve certain autoreactive B-cells and such immune specific lymphocytes can be anticipated to be involved in both delayed type hypersensitivity (DTH) and immune complex mediated pathogenic inflammation.8) Inflammation is an essential protective process preserving the integrity of organisms against physical, chemical and infective insults. However, it is frequent that the inflammatory response to several insults erroneously leads to the damaging of normal tissues.9)

One of the early cellular events in inflammation is the margination of leukocytes, primarily neutrophils. This response can be measured by using the neutrophil specific enzyme myeloperoxidase (MPO), an indicator of neutrophil accumulation.10) In addition, nitric oxide (NO) plays an important role in inflammation and NO synthase inhibitors can reverse several classic inflammatory symptoms.11) TNF-α a cytokine plays an important role in inflammation. TNF-α stimulates neutrophils to transcribe and release cytokines and chemokines biosynthesis.12) Semecarpus anacardium is a deciduous tree distributed in the sub-Himalayan tract and in tropical parts of India13); (Family: Anacardiaceae). It has high priority and applicability in indigenous system of medicine. Semecarpus anacardium Linn. also called as “marking nuts” contains many compounds like biflavanoids, which include semicarpufllavanone, jeediflavonone, gallufllavanone and also some phenolic compounds like bhilawanols, sterols and glycosides. The bhilawanol, a catechol derivative and monohydroxy phenol called ‘semecarpol’ was also isolated from the nuts.14) Further analysis revealed the presence of iron, copper, sodium,
calcium and aluminium in traces.

The phytochemical studies of the milk extract has shown in our laboratory to contain flavonoids, phenols and carbohydrates and the drug was found to be effective against adjuvant arthritis. The milk extract of the nut was found to inhibit acute tuberculin reaction in sensitized rats and also the primary phase of adjuvant arthritis. Earlier studies have shown that the chloroform extract of the nut possesses the antitumour activity and effective against various experimental tumours. Our previous studies have shown SA as a potent antioxidant agent in adjuvant arthritis condition. The present study was undertaken to investigate the immunomodulatory, anti-inflammatory, analgesic, antipyretic and ulcerogenic activities of the drug.

MATERIALS AND METHODS

Animals Male albino rats of Wistar strain weighing about 120—30 g and male Swiss albino mice (25—30 g) procured from “Tamil Nadu Veterinary and Animal Sciences University,” Chennai, Tamil Nadu, India were employed. The animals were housed in well ventilated spacious cages and given food and water ad libitum during the course of the experiment. The temperature remained around 27°C. All animals were acclimatized for at least 1 week before the commencement of the experiments.

Formulation of the Drug The drug SA is a Siddha preparation, which was prepared according to the method described in the Formulary of Siddha Medicine by boiling the nuts (200 g) with 500 ml milk. Decanting the decoction, 500 ml of milk was added to the boiling nuts and again boiled for some time. The decoction was recovered and the process was repeated again with the milk (500). All the three portions of milk nut decoction were mixed with ghee (1.5 kg) and boiled till dehydration. Then it was filtered and stored. Olive oil was used as a vehicle for the drug.

Immunomodulatory and Anti-inflammatory Activities of SA in Adjuvant Arthritis Animals and Experimental Design: Adult male Wistar rats, weighing 120—130 g were divided into 4 groups of 6 animals each. Group I: Control animals, Group II: Arthritis induced [Freund’s complete adjuvant containing 10 mg of heat killed mycobacterium tuberculosis in 1 ml paraffin oil (0.1 ml) was injected into the left hind paw of the rat intradermally]. Group III: Drug treated [The drug (150 mg/kg body weight dissolved in 0.5 ml olive oil) was administered after 14 d from the day of adjuvant injection for 14 d by intubation]. Group IV: Drug treated [The drug (150 mg/kg body weight) was administered to normal animals for 14 d by intubation].

Humoral Immunity Organ cell count was carried out according to the method of Cross et al. 21

Estimation of Immunoglobulins: IgG was quantitatively measured in rat serum by the coagulation of gammaglobulin by gluteraldehyde reagent and the procedure was described by Tennant et al. 22 as modified by Satpathy et al. 23 The IgG content is expressed as mg/dl. IgM and IgA were quantitatively measured in rat serum by turbidimetric immunoassay. Turbidity is the measurement of light scattered by particulate matter in solution. It can be applied to the measurement of antigen, since in the presence of appropriate antibody; immune complexes are formed, which can be related to the concentration of antigen. The turbidity is photometrically measured at a wavelength of 340 nm. The IgM and IgA contents in serum are expressed as mg/dl.

Haemagglutination Assay: Antibody titre was estimated by the haemagglutination technique according to the method of Puri et al. 24 The value of the highest dilution of the test serum giving agglutination was expressed as the haemagglutination titre.

Plaque forming cells were evaluated by the method of Smith et al. 25 Plaques were enumerated and evaluated for approximate size manually via low-powered microscopy with a 10× ocular strength.

Cell Mediated Immunity Delayed Type Hypersensitivity: Delayed type hypersensitivity reactions, an in vivo indicator of specific cell-mediated immune responsiveness were measured in control and arthritic rats. 3 A soluble extract of M. tuberculosis was prepared by sonication of a saline suspension (10 mg/ml) followed by centrifugation to remove particulate material. The concentration of M. tuberculosis material was determined by weighing the residue after drying 1 ml of solution and this was appropriately diluted for i.d. injection. Rats were lightly anaesthetized with ether and injected i.d. into two sites of the shaved dorsal skin with 0.4 mg mycobacterial antigen and 0.1 ml saline. In a time course study rats were injected on days 0, 7, 14, 21 and 30 following complete Freunds adjuvant injection. At 24 h after i.d. injection the DTH response was measured using skin calipers and expressed as the increase in skin fold thickness (mm) relative to the adjacent saline-injected site.

Leucocyte Migration Inhibition: LMI test was carried out by the method of Tewari et al. 26 Using planimeter, the area of migration in each instant was calculated. The average of 4 to 6 capillaries was taken as the mean area of migration. The migration index was calculated as follows:

\[
\text{the migration index} = \frac{\text{mean area of migration in presence of antigen (test)}}{\text{mean area of migration in absence of antigen (control)}}
\]

Measurement of Serum Soluble Immune Complex: The level of Serum soluble immune complex was measured by the method of Seth and Srinivas. 27 A simplified turbidimetric assay based on the precipitation of immune complex by low concentration of polyeethylene glycol (PEG).

Evaluation of Anti-inflammatory Activity of SA in Adjuvant Arthritis The paw edema produced was determined by measuring the difference of the paw diameter using an analogic pakimeter (vernier). The thickness of the injected foot was measured initially and daily. Changes in thickness over the course were employed as a measure of degree of inflammation. The levels of nitric oxide was measured in terms of nitrate and nitrite28 in serum and urine spectrophotometrically at 540 nm. The values are expressed as mmol/dl, for serum and µmol/24 h for urine samples. The level of TNF-α was measured by using enzyme linked immunosorbent assay (ELISA). The protocol was a modified version of Sharma and Singh. 29 The level of TNF-α is expressed as ng/mg protein using a calibration curve obtained from standard TNF-α. Myeloperoxidase (MPO) activity was assayed as an index of neutrophil infiltration in the joints. MPO in synovial tissue and hind paw tissue were measured by a specific assay as
mentioned earlier with some modifications.\textsuperscript{30} MPO activity has been defined as the concentration of enzyme degrading 1 μmol of peroxide/min and was expressed as Units/mg of protein.

Xylene-Induced Ear Edema: Mice were divided into three groups of six animals each. The drug SA at a dose of 150 mg/kg in olive oil was administered to the animals orally by gastric intubation. Indomethacin at a dose of 10 mg/kg body weight in olive oil served as reference drug. Thirty minutes after dosage of the extract and indomethacin, 0.03 ml of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. Two hours after xylene application, mice were killed and both ears were removed. Circular sections were taken, using a cork borer with a diameter of 7 mm, and weighed. The increase in weight caused by the irritant was measured by subtracting the weight of the untreated left ear section from that of the treated right ear sections.\textsuperscript{31}

Formalin Induced Inflammation: Rats were divided into three groups of six animals each. The inflammation was produced by subaponeurotic injection of 0.1 ml of 2% formaldehyde in the right hind paw of the rats on the first and third days. The animals were treated daily with the extract (150 mg/kg body weight) or indomethacin (10 mg/kg body weight) for 10 d orally. The daily changes in paw size were measured by vernier calipers.\textsuperscript{32}

Analgesic Activity This was investigated by monitoring test animals exposed to noxious chemical and thermal stimuli.

Acetic Acid (Chemical Induced) Writhing Method: The method was essentially as described by Nakamura et al.,\textsuperscript{33} in mice. The writhing response was elicited by an intraperitoneal injection of 0.75% acetic acid at the dose of 0.1 ml/10 g body weight. The drug SA at a dose of 150 mg/kg in olive oil was administered orally 30 min before acetic acid injection. Indomethacin at a dose of 10 mg/kg body weight in olive oil served as reference drug and control animals were given the vehicle alone. The number of writhing was noted for 15 min beginning 5 min after acetic acid injection. Reduction in the number of abdominal contraction compared to the control was considered as analgesic response.

Hot Plate (Thermal) Test: The test was performed according to Jacob and Ramabhadran.\textsuperscript{34} Albino rats were administered orally with the extract (150 mg/kg) and immediately placed on a hot plate maintained at 50±1 °C. The time taken by the animals to lick the fore or hind paw or jump out of the plate was taken as the reaction time, which was noted at 0, 30, 60, 90 and 120 min after the administration of the test samples. Indomethacin was used as reference drug. Control groups received the vehicle alone.

Antipyretic Activity: The test was performed according to the method of Mukerjee et al.\textsuperscript{35} Rats were subcutaneously injected with 20% suspension of brewer’s yeast (10 ml/kg). Initial rectal temperature was recorded. After 18 h, animals that showed an increase of 0.3—0.5 °C in rectal temperature were selected. SA was administered orally to a group of animals. Control groups received the vehicle. Indomethacin (10 mg/kg) dissolved in olive oil was used a reference drug. Rectal temperature was recorded by Hick’s clinial thermometer, before and every hour for 5 h after the administration of the test samples.

Ulcerogenic Activity: The method was based on the method of Cashin et al.\textsuperscript{36} Rats were fasted for 16 h and \textit{Se-mecarpus anacardium} extract (150 mg/kg) was administered orally, 3 h later, the animals were sacrificed and the stomachs were removed, cut along the lesser curvature and washed with saline. The ulceration of the gastric mucosa was examined under microscope and scored according to the scale.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Arthritis</th>
<th>Arthritis + SA treatment</th>
<th>Drug control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque forming cells (PFC)</td>
<td>472.51±43.18</td>
<td>728.82±68.59*</td>
<td>539.63±51.04*</td>
<td>471.92±41.92NS(c)</td>
</tr>
<tr>
<td>Antibody titre</td>
<td>83.45±8.1</td>
<td>177.12±17.06*</td>
<td>104.3±9.52*</td>
<td>82.09±7.7NS(c)</td>
</tr>
<tr>
<td>Leukocyte migration inhibition (migration index)</td>
<td>0.84±0.06</td>
<td>1.55±0.1*</td>
<td>0.98±0.08*</td>
<td>0.82±0.07NS(c)</td>
</tr>
</tbody>
</table>

Values represented as mean±S.D of six animals. Comparisons were made between: a) control and arthritic animals; b) arthritic and SA treated arthritic animals; c) control and drug control animals. * Denotes significance at the level of \(p<0.05\). NS denotes the non-significance at the level of \(p<0.05\). PFC: expressed as \(10^8\) spleenocytes. Antibody titre: the value of the highest dilution of the test serum giving agglutination was expressed as the haemagglutination titre.
of lymphoid organs in control and arthritic animals. The weight andcellularity of spleen as well as the weights of po-pleital lymph nodes were found to be increased but the thymic weight and cellularity were noticed to be decreased significantly in arthritic animals. These changes were re-verted to near normal upon treatment with SA. The effect of SA on the extent of leukocyte migration in control and arthritic animals was depicted in Table 1. In group II arthritic animals, the migration of leukocytes was found to be significantly ($p<0.05$) elevated. Whereas, in drug administered arthritic (group III) animals, these changes were brought back significantly ($p<0.05$) to near normal levels. No significant changes were observed in sole drug administered (group IV) animals although there were some alterations but not statistically significant when compared with that of control animals. Figure 1 demonstrates a time course alterations in the DTH response of the control and experimen-tal animals. The rats induced with adjuvant depicted a significant elevation in the DTH response and reached a peak at 14 d. Thereafter, it was found to decrease to some extent during the rest of the experimental period. SA treated (group III) animals showed a significant ($p<0.05$) decrease in the DTH response at 21 and 28 d of the experimental period when compared with the arthritic animals which shows the efficacy of the drug. Group IV drug control animals although showed altered levels, did not show any statistical significance when compared with that of control animals.

**Anti-inflammatory Effects of SA in Adjuvant Arthritis**

Swelling and redness developed over a 24 h period in the foot injected with adjuvant and reached maximum intensity on day 5. This inflammatory reaction subsides slightly during the next 8 to 10 days and then increased (Table 4) at that time when disseminated arthritis appeared which was greater than primary phase swelling. During the second swelling phase, the non-injected hind paw also began to swell. The drug treatment started after 14 d from the day of adjuvant injection suppress the secondary increase in swelling of the injected foot, which occurs at the appearance of polyarthritis.

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**Table 2. Effect of SA on the Levels of Immunoglobulins and Immune Complex in Control and Arthritic Animals**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Arthritis</th>
<th>Arthritis + SA treatment</th>
<th>Drug control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (mg/dl)</td>
<td>274.16 ± 26.51</td>
<td>345.72 ± 30.83$^{*\dagger}$</td>
<td>297.36 ± 27.15$^{*\dagger}$</td>
<td>281.28 ± 26.94NS$^{\dagger}$</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>207.44 ± 19.53</td>
<td>298.81 ± 28.46$^{*\dagger}$</td>
<td>229.83 ± 21.73$^{*\dagger}$</td>
<td>214.92 ± 20.67NS$^{\dagger}$</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>82.73 ± 8.0</td>
<td>171.94 ± 16.43$^{*\dagger}$</td>
<td>106.41 ± 9.57$^{*\dagger}$</td>
<td>85.36 ± 8.26NS$^{\dagger}$</td>
</tr>
<tr>
<td>Serum soluble immune complex (PEG indices)</td>
<td>17.62 ± 1.5</td>
<td>41.57 ± 4.03$^{*\dagger}$</td>
<td>25.27 ± 2.3$^{*\dagger}$</td>
<td>17.25 ± 1.4NS$^{\dagger}$</td>
</tr>
</tbody>
</table>

Values represented as mean±SD of six animals. Comparisons were made between: a) control and arthritic animals; b) arthritic and SA treated arthritic animals; c) control and drug control animals. $^\dagger$ Denotes significance at the level of $p<0.05$. NS denotes the non-significance at the level of $p=0.05$.

**Table 3. Effect of SA on the Changes in Weights and Cellularity of Lymphoid Organs in Control and Arthritic Animals**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Arthritis</th>
<th>Arthritis + SA treatment</th>
<th>Drug control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen weight (g/100 g body weight)</td>
<td>0.34 ± 0.03</td>
<td>0.52 ± 0.02$^{*\dagger}$</td>
<td>0.38 ± 0.03$^{*\dagger}$</td>
<td>0.35 ± 0.02NS$^{\dagger}$</td>
</tr>
<tr>
<td>Thymus weight (g/100 g body weight)</td>
<td>0.112 ± 0.01</td>
<td>0.051 ± 0.004$^{*\dagger}$</td>
<td>0.101 ± 0.01$^{*\dagger}$</td>
<td>0.110 ± 0.01NS$^{\dagger}$</td>
</tr>
<tr>
<td>Popliteal lymph node weight (g/100 g body weight)</td>
<td>8.3 ± 0.72</td>
<td>21.94 ± 1.5$^{*\dagger}$</td>
<td>13.04 ± 0.86$^{*\dagger}$</td>
<td>8.3 ± 0.61NS$^{\dagger}$</td>
</tr>
<tr>
<td>Spleen cell count (×10⁸ cells)</td>
<td>4.4 ± 0.41</td>
<td>7.2 ± 0.68$^{*\dagger}$</td>
<td>5.27 ± 0.41$^{*\dagger}$</td>
<td>4.5 ± 0.40NS$^{\dagger}$</td>
</tr>
<tr>
<td>Thymus cell count (×10⁸ cells)</td>
<td>4.6 ± 0.38</td>
<td>2.96 ± 0.23$^{*\dagger}$</td>
<td>4.1 ± 0.29$^{*\dagger}$</td>
<td>4.5 ± 0.41NS$^{\dagger}$</td>
</tr>
</tbody>
</table>

Values represented as mean±SD of six animals. Comparisons were made between: a) control and arthritic animals; b) arthritic and SA treated arthritic animals; c) control and drug control animals. $^\dagger$ Denotes significance at the level of $p<0.05$. NS denotes the non-significance at the level of $p=0.05$.

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**Fig. 1. Effect of SA on the Changes in DTH of Control and Experimental Animals**

Values represented as mean±S.D. of six animals. Comparisons were made between: a) control and arthritic animals; b) arthritic and SA treated arthritic animals; c) control and drug control animals. $^\dagger$ Denotes significance at the level of $p<0.05$. NS denotes the non-significance at the level of $p=0.05$.

Table 5 depicts the level of RNS in terms of nitrate+nitrite in the serum and urine; TNF-α in mononuclear cells and neutrophils; MPO levels in joint and paw tissues of control and experimental animals. Significant elevation in the levels of RNS in serum and urine were observed in arthritic animals, which on treatment with SA got decreased to near normal levels. The significantly elevated levels of TNF-α found in arthritic rats were found to be significantly restored back to near normal levels when compared with that of arthritic animals, which reveals the protective effect of SA. Treatment with SA significantly inhibited the accumulation of neutrophils at the site of inflammation, which is evident from the decreased levels of MPO in SA treated animals when compared with that of the joints of adjuvant induced arthritic rats. No significant alterations were observed in the sole drug
Models treated rats when compared with that of control animals.

**Anti-inflammatory Effects in Various Experimental Models** In the xylene induced ear edema, SA showed significant anti-inflammatory activity by reducing the edema about 50% (Fig. 2). In the chronic inflammation (formalin test), SA exhibited a significant ($p<0.05$) anti-inflammatory activity (Fig. 3). In indomethacin treated (10 mg/kg) groups; the hind paw edema of rat disappeared after 6 d.

**Analgesic Activity** Acetic Acid Induced Writhing Test:

The effects of SA and the reference drug indomethacin on the writhing response in mice are shown in Fig. 4. It was found that the extract as well as indomethacin caused a significant inhibition on the writhing response induced by acetic acid.

**Hot Plate Test** The hot plate analgesic activities showed an increase at 60 min after the administration of the drug. Maximal effect was observed after 2 h (Fig. 5). Indomethacin treated rats also showed an increase in reaction time more than the drug treated animals.

**Antipyretic Activity** The basal temperature of rats was 37.0 ± 0.1°C. After 18 h of yeast administration, the mean...
Antigen. No selective competition for antigen or antibody vant arthritis but also capable of responding to the second injected with adjuvant and SRBC not only developed adjuvant arthritis but also capable of demonstrating response to SRBC. As stated already, increased Cellularity in the spleen of adjuvant injected rats engendered interest as to the potential for concomitant classical antibody formation. Increased antibody titre in arthritic animals further supports the hyperimmune status by humoral immunity.

IgA has been implicated in the pathogenesis of RA. As a matter of fact, a high rate serum IgA has been described in RA patients. A persistent high serum level of IgA could be a marker of a subgroup of patients with RA, characterized by a significant increase in the incidence of distal interphalangeal arthritis, unilateral sacroiliitis and microscopic haematuria. Abnormal elevation of IgG, A and M are reported in RA and routine measurement of IgG, A and M, rheumatoid factor (RF) by ELISA have been shown to provide a useful information in the differential diagnosis of patients with RA. High IgG and A (hyper gamaglobulinemia) was shown in arthritic condition. These elevated serum gama globulins are associated with increased activity in the inflammatory process. It was shown that levels of both CMI and HI rose during arthritis.

The reversion of these pathological changes upon drug treatment shows the profound immune mediated antiarthritic effect of SA. This immunomodulatory effect of SA may be due to the presence of flavonoids as one of their components. Reports revealed that administration of flavonoids shows a significant inhibition of SRBC specific haemagglutination titre and suppresses the production of circulating antibodies.

During CMI response, sensitized T-lymphocytes challenged by the antigens are converted to lymphoblasts, and secrete lymphokines; attracting more scavenger cells to the site of reaction. Early studies suggested a DTH response to disseminated mycobacterial Ag. Increased DTH was reported during adjuvant arthritis. DTH reaction is a form of cellular immune reaction mediated by T

DISCUSSION

Several traditional remedies claim the usefulness of *Semecarpus anacardium* against inflammations and rheumatoid arthritis. Therefore, our main aim of the investigation was to consider the symptoms of rheumatoid arthritis, like immunomodulation, inflammation, pain and pyrexia so as to give adequate scientific backing and explanations to the usage in folklore medicine of *Semecarpus anacardium* in rheumatoid arthritis and its symptoms.

Arthritis is an immune response to an antigen present on the capsule of the mycobacterium. It has been shown that rats injected with adjuvant and SRBC not only developed adjuvant arthritis but also capable of responding to the second antigen. No selective competition for antigen or antibody producing cells were observed since both PFC response and development of adjuvant arthritis occurred when either treatment was used alone. This tempts to theorize that this evidence may indicate a worsening of the disease by the second bombardment of an already hyperimmune system.

PFC response in arthritic rats was shown to be increased enormously when compared to control rats received SRBC only. Challenge with SRBC was shown to accelerate the onset and intensified subsequent joint inflammation. These data support that the suggestions of other investigators that adjuvant arthritis mirror a hyperimmune state. The spleen provided a readily available source of cells known to be involved in adjuvant arthritis as well as cells capable of showing a useful information in the differential diagnosis of patients with RA. IgA has been implicated in the pathogenesis of RA. As a matter of fact, a high rate serum IgA has been described in RA patients. A persistent high serum level of IgA could be a marker of a subgroup of patients with RA, characterized by a significant increase in the incidence of distal interphalangeal arthritis, unilateral sacroiliitis and microscopic haematuria. Abnormal elevation of IgG, A and M are reported in RA and routine measurement of IgG, A and M, rheumatoid factor (RF) by ELISA have been shown to provide a useful information in the differential diagnosis of patients with RA. High IgG and A (hyper gamaglobulinemia) was shown in arthritic condition. These elevated serum gama globulins are associated with increased activity in the inflammatory process. It was shown that levels of both CMI and HI rose during arthritis.

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Values are expressed as mean±S.D. of six animals. a, denotes significance at a level of \( p<0.05 \) when compared with control group respectively.

Values are expressed as mean±S.D. of six animals. a, denotes significance at a level of \( p<0.05 \) control vs. indomethacin treatment. NS denotes nonsignificance at a level of \( p<0.05 \) control vs. SA treatment.
the inflamed joints. T-lymphocytes from this lymphoid pool recirculate and can be persistently recruited to arthritic joints, leading to subsequent accumulation of other inflammatory cells, such as monocyte/macrophages, plasma cells and PMNs, and generation of cytokines which contribute to resorption of bone and destruction of cartilage.

In the present study, administration of SA produced a significant reduction in DTH indicating thereby a decrease in lymphokine production or release. This finding is supported by the observation that the drug produced a decrease in leukocyte migration. SA has been shown to significantly inhibit acute tuberculin reaction. It has also been reported that the flavonoids significantly inhibit DTH in a dose dependent manner. The reversion of the weights and cellularity of the lymphoid organs during the administration of the drug further supports the above evidences.

In the investigation of paw edema, arthritic rats showed the soft tissue swelling that was noticeable around ankle joints and was considered to be due to edema of peri-articular tissues such as ligament and joint capsule. The initial reduction of edema and soft tissue thickening at the depot site is probably due to the effect of adjuvant, whereas the late occurring disseminated arthritis and flare in the injected foot are presumably immunological event. Arthus has postulated that polymorphonuclear leukocytes could contribute to the edema. The ability of the drug to reduce edema formation may thus be related to its inhibitory action on prostaglandin synthesis. The findings of the present study demonstrated that SA reduced MPO activity in the joints and paw tissue of arthritic animals. This could be on the basis of inhibition of neutrophil infiltration. Inflammation is characterized by the infiltration of PMNs such as neutrophils. The primary function of neutrophils is the release of MPO into phagosome containing the ingested microorganism leading to a rapid microbial effect. MPO can be released outside to the cell inducing damage to adjacent tissue and thus, contribute to the pathogenesis of inflammation.

NO is a highly fat soluble free radical, having numerous promiscuous roles. NO synthesis is greatly amplified during inflammation. Several studies have demonstrated that inflammation correlates with the level of NO. In the study SA have shown remarkable reduction in nitrate/nitrite level, which can be attributed to the antioxidant property of SA. TNF-α is a pleiotropic cytokine, which plays a critical role in both acute and chronic inflammation. Several inflammasomes have the ability of inducing the synthesis of TNF-α. The formation of a number of small molecular mediators of inflammation is linked with TNF-α and thus contributes to the range of mediators that critically control inflammation. TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells. When TNF-α is specifically blocked, the severity of inflammation is reduced.

In xylene induced (acute) inflammation and formalin induced (chronic) inflammation, SA exhibited a strong anti-inflammatory activities. As preliminary phytochemical results indicated, it could be suggested that the antinociceptive and anti-inflammatory effects of SA may be due to various components present in SA such as flavonoids, tannins and anthocyanins. Other studies have demonstrated that various flavonoids such as rutin, quercetin, luteolin, hesperidin and biflavonoids produced significant antinociceptive and/or anti-inflammatory activities. There are few reports on the role of tannins in antinociceptive and anti-inflammatory activities.

The thermal stimuli in hotplate test and the writhing response of the animals to an intra-peritoneal injection of noxious chemical are used to screen both peripherally and centrally acting analgesic activity. Acetic acid causes algesia by liberating endogenous substances and many others that excite painner endings. From the results it is evident that the nut extract showed a significant analgesic effect in hot plate test and writhing response, which are slightly lesser than indomethacin. NSAIDs can inhibit cyclo-oxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors. The mechanisms of analgesic action of the nut extract could be due to the presence of flavonoids and mediated through central and peripheral mechanisms, thus providing some pharmacological rationale for the traditional use of the plant as analgesic.

Since antipyretic activities commonly mentioned as a characteristic feature of drug or compounds, which have an inhibitory effect on prostaglandin synthesis, the yeast induced hyperemia in rat model was employed to investigate the antipyretic activity of the extract. It was found that the nut extract caused a significant decrease in rectal temperature comparable to that of indomethacin. This result seems to support the view that the nut extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature.

Gastraic discomfort and ulcers are generally the major side effects related to the currently employed non steroidal anti-inflammatory agents, which is evident from the data of the present study. But no significant ulceration was found in the animals administered with the nut extract.

This study has indicated that Semecarpus anacardium Linn. nut extract exhibits a potential protective immunomodulatory effect by humoral as well as cell mediated immune mechanisms. Analgesic effect of SA was observed to be acting through both peripheral and central mechanisms and also exerted strong anti-inflammatory and antipyretic effects. These activities are probably attributed to the presence of flavonoids in the drug. Moreover, the drug did not produce any serious adverse effects, which is evident from the results of ulcerogenic index. All these results thus predict that the drug provide pharmacological rationale for the traditional use of the drug against inflammatory disorders.

REFERENCES

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