LIST OF PUBLICATIONS

   Anti-inflammatory activity of lupeol and lupeol linolate in adjuvant-induced arthritis.

   Effect of triterpenes from *Crataeva nurvala* stem bark on lipid peroxidation in adjuvant induced arthritis in rats.

   Anti-complement activity of triterpenes from *Crataeva nurvala* stem bark in adjuvant arthritis in rats.
   *General Pharmacology*, (accepted).

   Effect of *Vernonia cinerea* less flower extract in adjuvant induced arthritis.
   *General Pharmacology*, (accepted).

   Effect of *Vernonia cinerea* less flower extract on free radical scavengers in adjuvant induced arthritis in rats.
   *Biomedicine*, (accepted).
Anti-inflammatory activity of lupeol and lupeol linolate in adjuvant-induced arthritis

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Received May 1, 1987. Accepted for publication August 1, 1987.

SUMMARY The present communication describes the anti-inflammatory activity of lupeol and lupeol linolate in adjuvant-induced arthritis. Lupeol, a triterpenoid, has been isolated from the stem bark of *Cinnamomum camphora* and possesses anti-inflammatory activity. Lupeol linolate is a new compound isolated from *Cinnamomum camphora* and has been shown to possess anti-inflammatory activity.

KEY WORDS: lupeol, lupeol linolate, anti-inflammatory activity.

Adjuvant-induced arthritis in rats shares many features of human rheumatoid arthritis. Arthritis is one of the most distressing and disabling syndromes encountered in medical practice and although it is one of the oldest known diseases, there is no drug leading to a permanent cure. In fact, both steroid and non-steroidal anti-inflammatory drugs are used for the amelioration of symptoms but they offer only temporary relief and also produce severe side effects. Therefore, increasing efforts are being directed towards traditional herbal medicines for the development of drugs with long acting anti-inflammatory activity and minimum side effects.

Triterpenes widely distributed in edible and medicinal plants are effective in protecting against liver disorders. They possess antitumor, antihypertensive, and anti-inflammatory activities. *Cinnamomum camphora* (Cinnamomaceae) possesses anti-inflammatory and antirheumatic activity. Among the compounds isolated from the stem bark, lupeol was identified as a major constituent in association with α and β-amyrin. Recent studies have shown that esterification of α-amyrin with palmitic acid improved antiarthritis activity. This prompted us to compare lupeol and its ester lupeol linolate for their antirheumatic activity.

EXPERIMENTAL

Lupeol was isolated from *Cinnamomum camphora* stem bark as earlier reported by Baskar et al. [1]. Identification with infrared, chlorine, and adjuvant-induced arthritis. IR bands (KBr): 2928, 1671, 1460 cm⁻¹ HNMR: 400 MHz (CDCl₃): δ 5.5 (m, IH) 4.17 (m, 2H) 3.28 (m, 1H) 0.75 (m, 2H) 7.4 (m, 1H).

Animals Female Wistar rats (120-150 g) obtained from Veterinary College, Madras, were used. Before experiments, animals were accustomed to the animal house standardized environmental conditions for 1 week. They received commercial diet and water ad libitum.

Induction of arthritis Arthritis was induced in rats by the subcutaneous injection of 0.1 ml of Complete Freund Adjuvant (CFA) in the right hind paw by the adjuvant tuberculosis Research Centre, Madras, contained dried heat killed Mycobacterium tuberculosis in sterile paraffin oil (10 mg/ml).
Treatment: Rats were divided into 6 groups of 6 rats each:

group I: normal rats receiving olive oil 40 ml/day

group II: arthritic rats emaciated with CFA receiving olive oil 0.5 ml/day

group III: normal rats treated with lipoed for 8 days

group IV: normal rats treated with lipoed injected for 8 days

group V: arthritic rats fed orally with lipoed from 14th to 18th day post CFA

group VI: arthritic rats treated with lipoed injected from 14th to 18th day post CFA

Interpeps were administered orally at a dosage of 50 mg/kg in olive oil 0.5 ml/day

Experimental procedures: The anti-inflammatory activity of interpeps was evaluated by recording the paw diameter of the adjuvant injected limb using a vernier scale and the body weight changes of the experimental groups at different periods up to 19 days following the injection of CFA. On the 19th day, the animals were sacrificed by cervical dislocation. Blood samples were collected; serum was separated and used for the estimation of alanine aminotransferase and alkaline phosphatase by the method of King. Albumin globulin (A/G) ratio was determined by the method of Renhold. The liver, kidney, and spleen were dissected out and their weights were recorded.

The ipsilateral proximal interphalangeal joints were excised, fixed in 10% formalin, decalcified in 10% HNO3, and processed for paraffin embedding. Sections were cut at 3μm thickness flattened and adhered to the slides. The section was then stained with hematoxylin and eosin and viewed under light microscope for histopathological changes.

Statistical analysis: The values are expressed as mean ± SD. Student's t-test was used to assess the degree of significance.

![Graph showing the comparison between groups II and group IV, group II and group VI, and group V and VI. The symbols represent statistically significant differences.]

Fig. 1: Anti-inflammatory activity of lipoed and lipoed injected 0.5 mg/kg p.o. on CFA induced arthritis in rats. Changes in paw diameter.
RESULTS

Normal control rats did not show any significant change in paw diameter during the experimental period while arthritic control rats showed 51% increase in the ankle diameter. Lupeol and lupeol homolactone treated arthritic animals showed reduction in paw diameter by 30% and 58%, respectively (Fig 1).

The body weight changes of the experimental groups are shown in Fig 2. The growth of arthritic control rats (Group II) was found to be retarded. The body weight of lupeol homolactone treated arthritic rats (Group VI) was found to increase in respect to that of control rats.

There were no significant changes in liver and kidney weights of arthritic rats, while a spleen weight increase was observed. Group I: Lupeol treated arthritic rats (Group V) showed no effect on the increase in spleen weight but the lupeol homolactone treated animals (Group VI) showed a considerable decrease. The treatment procedures did not alter the tissue weights of normal control animals.

In arthritic rats, the ammotontransferases and alkaline phosphatase were increased and the AS ratio was significantly decreased as shown in Tables 2 and 3. In arthritic rats (Group V), lupeol homolactone treatment decreased ammotontransferases and alkaline phosphatase, and also elevated the AS ratio in comparison to normal level. Histopathological changes of interphalangeal joints of normal control rats (Group I), arthritic control rats (Group II)
<table>
<thead>
<tr>
<th>Feature</th>
<th>Normals</th>
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Table 1: The effects of anchor on the cross-validated mean and maximum difference in terms of percent.

Table 2: The effects of anchor on the cross-validated mean and maximum difference in terms of percent.

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<table>
<thead>
<tr>
<th>Pattern</th>
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<th>Group II</th>
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Hepatic cholestasis and necrosis were apparent in all groups. Necrosis was observed in normal and acute groups. However, the extent and severity of necrosis were more pronounced in acute groups. Necrosis was also observed in serial sections of the liver, indicating a chronic insult. The control group showed minimal changes compared to the acute groups.

**Fig. 1:** Section of the liver showing marked necrosis and cholestasis in control group.

**Fig. 2:** Section of the liver showing necrosis and cholestasis in Group I.

**Fig. 3:** Section of the liver showing necrosis and cholestasis in Group II.

**Fig. 4:** Section of the liver showing necrosis and cholestasis in normal group.

**Fig. 5:** Section of the liver showing necrosis and cholestasis in acute group.
arthritic rats treated with lupeol (Group V) and linoleate (Group VI) are shown in Fig 36.

DISCUSSION

The determination of paw diameter and changes in body weight of the experimental groups have been used for evaluating the degree of inflammation and the therapeutic effects of the treatment. As the incidence and severity of arthritis increased, the changes in the body weight and paw diameter of the rats also occurred during the course of the experimental period. The loss of body weight may be due to reduced absorption of glucose and leucine in rat intestine in arthritic condition. The increase in body weight on treatment with tripenes may be due to restoration of absorption capacity of the intestine.

Adjuvant arthritis has been reported to be associated with splenomegaly, generalized lymphadenopathy and altered hepatic function. Lupeol and linoleate administration to arthritic rats decreased the spleen weight significantly. Normal rats treated with lupeol and its linoleate did not show any change in the weight of tissues.

Serum ammotransferases and alkaline phosphatase were significantly increased in arthritic rats, since liver impairment is also a feature of adjuvant arthritis. A significant reduction in V/C ratio was observed in adjuvant-induced rats, which might be attributed to increased permeability of vascular tissue to albumin via increasing the level of AMP by inflammatory mediators. It might also be attributed to a reduction in hepatic albumin biosynthesis. The increased V/C ratio by tripennes may be due to decrease of albumin extration occurring in response to inflammatory mediators.

Histopathologically, adjuvant-induced rat joints were found to be swollen and edematous with partial erosion of cartilage. The synovial cavity was obliterated with granuloma cavity. The bone was seen eroded in local areas. In lupeol treated arthritic joints, the synovial cavity was more or less obliterated with granuloma like lesion, which consisted of fibro and inflammatory cellular infiltration. Arthritic rats treated with lupeol and linoleate showed synovial cavity with less cellular infiltration. Our preliminary studies show that the antiarthritic activity of lupeol and linoleate is greater than that of lupeol. Further studies to better evaluate the antiarthritic effect of lupeol and linoleate are in progress.

Acknowledgement: We thank Dr. A. Nozawa, Dr. B. Balboni, Dr. C. Romano, Dr. de Azevedo P., and A. V. for their continuous support.

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EFFECT OF TRITERPENES FROM CRATAEIA NUR'ALA STEM BARK ON LIPID PEROXIDATION IN ADJUVANT INDUCED ARTHRITIS IN RATS

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Accepted 18 December 1997

Lupeol, a pentacyclic triterpene was isolated from C. nur'ala stem bark and its ester lupeol linoleate was synthesised. These triterpenes were tested for their anti-inflammatory activity in complete Freund's adjuvant induced arthritic rats. For some time free radicals have been implicated in damage to connective tissues during inflammatory conditions and arthritis. Lupeol and lupeol linoleate were administered orally at the dose level of 50 mg kg⁻¹ body weight daily for 8 days, from the 11th to 18th day postadjuvant. There was a significant increase in lipid peroxide level in plasma of arthritic rats but it was found to be decreased in the liver. The antioxidant enzymes superoxide dismutase, glutathione peroxidase and catalase were elevated in both the liver and haemolysate in adjuvant-induced rats. Blood glutathione was decreased in arthritics. The triterpenes reduced the above alterations produced in arthritic animals. The effect of lupeol linoleate was found to be better in this respect when compared with lupeol.

INTRODUCTION

C. nur'ala Buch Ham (Capparidaceae) is one of the medicinal plants in India which possess anti-inflammatory and anti-arthritic activity [1]. In our laboratory the bark decoction was used against calcium oxalate urolithiasis in experimental rats [2]. It is also reported to have laxative, stomachic, antihelminthic, anti-tubercular and anti-pyretic action [3]. Among the compounds isolated from the stem bark, lupeol was identified as a major component in association with α- and β-amyris [4]. Recent preliminary studies by Kwee-Ookai et al. have shown that triterpenes have anti-arthritic activity [5, 6].

Adjuvant arthritis shares many features of human rheumatoid arthritis [7]. It involves most of the joints and associated tissues. Free radicals have long been implicated in damage to connective tissues in inflammation and arthritis. Oxygen metabolites such as superoxide anion, hydrogen peroxide and hydroxyl radicals produced by granulocytes and macrophages have deleterious effects on biomembranes due to lipid peroxidation [8, 9].

The present work is aimed to compare the effect of lupeol and its ester, lupeol linoleate against free radicals generated during complete Freund's adjuvant-induced arthritis in rats.

MATERIALS AND METHODS

Lupeol was isolated from the stem bark of C. nur'ala in our laboratory, as performed earlier by Baskar et al. [2]. The isolated lupeol was further esterified to lupeol linoleate by adding equimolar amounts of pyridine and linoleyl chloride as reported by Kwee-Ookai et al. [10] for the conversion of α-amyris acetate to palmitate.

Female albino Wistar rats (120-150 g) were purchased from Tamil Nadu Veterinary and Animal Sciences University, Madras and fed with commercially available standard pelleted feed (Gold Mohur, Hindustan Lever Ltd., Bombay). Animals were housed in light, temperature and humidity-controlled standard conditions. Water was given ad libitum.

Rats were divided into six groups each consisting of six animals. Group 1 was the control group. In
Group 2 rats, arthritis was induced by the intradermal injection of 0.1 ml of Complete Freund’s Adjuvant (CFA) in the right hind-paw [11]. The adjuvant (Tuberculosis Research Centre, Madras) contained 10 mg dry heat killed Mycobacterium tuberculosis per millilitre of sterile paraffin oil. Groups 3 and 4 were treated with lupeol and lupeol linolate for 8 days. Groups 5 and 6 comprised of arthritic rats which were treated with lupeol and lupeol linolate from day 11 to day 18. Triterpenes were administered orally at a dosage of 50 mg kg⁻¹ body weight in 0.5 ml olive oil.

At the end of the experimental period, the animals were killed by decapitation, blood samples were collected with EDTA as anticoagulant and the liver was dissected out from the animals. After the separation of plasma from blood samples, the haemolysate was extracted according to the procedure of Dodge et al. [12] as modified by Oust [13]. Liver was homogenised in Tris-HCl buffer (0.01 M, pH 7.4) using a Potter-Elvehjem homogeniser to give a 10% homogenate.

Lipid peroxide (LPO) level was estimated in plasma by the method of Ledwozy et al. [14] and in liver tissue homogenate by the method of Hogberg et al. [15]. The peroxidation system contained 10 mM ferrous sulphate, 0.2 mM ascorbate and 10 mM H₂O₂ as inducers [16]. The release of malondialdehyde served as the index of lipid peroxidation.

Antioxidant enzymes were estimated in liver and haemolysate of experimental groups. Superoxide dismutase (SOD) was assayed according to the method of Marklund and Marklund [17]. The degree of inhibition of the autoxidation of pyrogallol at an alkaline pH by SOD was used as a measure of the enzyme activity. Glutathione peroxidase (GPX) was assayed by the method of Rotruck et al. [18]. The utilization of glutathione was used to express the activity. Catalase activity was assayed by the method of Sinha [19].

Total reduced glutathione (GSH) was estimated in whole blood by the method of Beutler et al. [20] and in liver tissue by the method of Moron et al. [21]. Protein and haemoglobin was estimated by the methods of Lowry et al. [22] and Drabkin and Austin [23]. The data were analysed using one-way analysis of variance followed by Student’s Newman-Keuls test. The values are expressed as mean ± SD.

RESULTS

Table 1 shows the effect of lupeol and lupeol linolate on lipid peroxidation in liver and plasma of the control and experimental groups. Decreased lipid peroxidation in liver was noticed in arthritic rats (Group 2). Plasma lipid peroxidation was increased significantly (P < 0.001) in this group. The above changes were altered with the administration of triterpenes.

Table II presents the effect of antioxidant enzymes in liver and haemolysate. The enzyme-superoxide dismutase, glutathione peroxidase and catalase were significantly increased (P < 0.001) in adjuvant-induced arthritis. Treatment with lupeol significantly reduced the level of enzymes (Group 5). The effect produced by lupeol linolate in this context was even better, since the levels of enzymes were brought almost to near that of the control rat (Group 6).

Changes in the reduced glutathione in liver and blood are presented in Table III. Reduced glutathione was decreased significantly in the blood (P < 0.001) of arthritic rats. Administration of lupeol linolate to arthritic rats (Group 6) elevated the glutathione level to that of controls.

DISCUSSION

Oxygen free radicals and H₂O₂ are closely involved in the pathogenesis of rheumatoid arthritis [24].
Table II

Activities of antioxidant enzymes in liver and haemolsate of control and experimental rats

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Group 1 Control</th>
<th>Group 2 Arthritis</th>
<th>Group 3 Control + Lupenal</th>
<th>Group 4 Arthritis + Lupenal linolate</th>
<th>Group 5 Arthritis + Lupenal linolate</th>
<th>Group 6 Arthritis + Lupenal linolate</th>
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</thead>
<tbody>
<tr>
<td>Liver</td>
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<tr>
<td>Superoxide dismutase</td>
<td>2.93 ± 0.34</td>
<td>3.97 ± 0.18**</td>
<td>2.97 ± 0.28</td>
<td>3.41 ± 0.23*</td>
<td>3.07 ± 0.30**</td>
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</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>7.01 ± 0.04</td>
<td>8.75 ± 0.33**</td>
<td>6.94 ± 0.15</td>
<td>0.99 ± 0.11</td>
<td>7.01 ± 0.40**</td>
<td>6.93 ± 0.50**</td>
</tr>
<tr>
<td>Catalase</td>
<td>127.69 ± 4.92</td>
<td>153.52 ± 9.60**</td>
<td>126.80 ± 5.71</td>
<td>147.77 ± 7.34**</td>
<td>132.26 ± 12.85**</td>
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<tr>
<td>Superoxide dismutase</td>
<td>2.54 ± 0.19</td>
<td>4.72 ± 0.37**</td>
<td>2.61 ± 0.19</td>
<td>2.55 ± 0.16</td>
<td>3.40 ± 0.35**</td>
<td>2.24 ± 0.23**</td>
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<tr>
<td>Glutathione peroxidase</td>
<td>0.94 ± 0.03</td>
<td>2.63 ± 0.12**</td>
<td>0.94 ± 0.02</td>
<td>0.94 ± 0.02</td>
<td>1.68 ± 0.09**</td>
<td>1.30 ± 0.08**</td>
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<tr>
<td>Catalase</td>
<td>29.75 ± 0.34</td>
<td>32.95 ± 0.21**</td>
<td>29.97 ± 0.12</td>
<td>29.64 ± 0.13</td>
<td>31.51 ± 0.15**</td>
<td>30.33 ± 0.31**</td>
</tr>
</tbody>
</table>

Values are mean ± SD for six animals in each group.
Enzyme activities are expressed as follows: SOD, units (mg protein)^-1; 1 unit = amount of enzyme that inhibits the autodestruction reaction by 50%; GPX, μg of reduced glutathione utilized min^-1 (mg protein)^-1; catalase, μmol of H₂O₂ consumed min^-1 (mg protein)^-1; haemolsate units mg haemoglobin
Comparison between groups are as in Table 1.
The symbols represent statistical significance * P < 0.01, ** P < 0.001

Granulocytes are strongly increased in number in this disease they produce large amounts of O₂ and H₂O during phagocytes and other materials. Oxygen free radicals are responsible for at least a part of the joint destruction [25].

Suppression of lipid peroxidation in liver of adjuvant-treated rats may be due to the decline in the level of hepatic cytochrome P-450 [26]. This cytochrome is involved in NADPH-dependent lipid peroxidation and is responsible for the decreased rate of oxidation of lipids in the liver. Another possible explanation is increased removal of lipid peroxides from liver into the blood of arthritic animals resulting in its elevation in serum. This is evident from the fact that the serum of arthritic rats have reduced ability to inhibit the lysos of rabbit polymorphonuclear leukocyte granules after Friton X-100 induction and further they even labilise cell membranes [27].

An increase in superoxide dismutase (SOD) has been observed in adjuvant arthritis (Table III). Increased production of NADPH from XMP shunt during arthritis may cause an increase in SOD activity [28]. This increase in enzyme activity appears to be protective against the extracellular oxygen free radicals [29]. Administration of triterpenes to arthritic rats caused a significant decrease in elevated SOD activity. Glutathione peroxidase protects the cell from damage resulting from the increased disease-associated peroxides by enhancing the GPX catalysed peroxide destruction. Liver has been reported to be a major site of lipid peroxide metabolism. Liver GPX activity was found to be

Table III

Effect of triterpenes on reduced glutathione in liver and blood of control and experimental rats

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Group 1 Control</th>
<th>Group 2 Arthritis</th>
<th>Group 3 Control + Lupenal</th>
<th>Group 4 Arthritis + Lupenal linolate</th>
<th>Group 5 Arthritis + Lupenal linolate</th>
<th>Group 6 Arthritis + Lupenal linolate</th>
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<tr>
<td>Reduced glutathione</td>
<td>6.58 ± 0.26</td>
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<td>6.55 ± 0.19</td>
<td>6.56 ± 0.15</td>
<td>6.38 ± 0.52</td>
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<tr>
<td>Reduced glutathione</td>
<td>18.47 ± 0.43</td>
<td>15.33 ± 0.42**</td>
<td>18.36 ± 0.35</td>
<td>18.45 ± 0.44</td>
<td>17.16± 0.34**</td>
<td>18.50 ± 0.34**</td>
</tr>
</tbody>
</table>

Values are mean ± SD for six animals in each group.
Units: Liver GSH, μg mg^-1 protein; Blood GSH, g dl^-1
Comparison between groups are as in Table 1.
The symbols represent statistical significance * P < 0.001
significantly elevated, apparently in response to the increase in lipid peroxides. It may be explained that lipid peroxides are metabolised in the liver by GPX [30]. This may be the reason for the absence of a real change in liver lipid peroxides in arthritis.

The main function of catalase is to detoxify H₂O₂. Although catalase is significantly increased in rheumatoid arthritis its concentration is very low to expect considerable protection against H₂O₂ [31] Treatment of arthritic rats with triterpenes significantly decreased the antioxidant enzyme levels.

Decrease in blood GSH level in arthritic rats may be due to increased in GPX activity which removes peroxides. GSH is not being replaced adequately, since glutathione reductase activity is affected. Hence the redox balance of the cell is upset and cell damage results [32]. However, the decrease in blood GSH was not accompanied by a similar change in liver GSH. In arthritic rats liver GSH was not affected, which may be due to the fact that it is utilised and resynthesised at an adequate rate [33]. Reduced GSH is being continuously synthesised by production of NAPDH [34]. Lipoic acidide is found to have superior protective effect against the free radicals generated during arthritic animals when compared to lipoic. The results suggest that the inhibition of lipid peroxidation is likely to be a component of its anti-inflammatory activity.

ACKNOWLEDGEMENT

The first author (J. Geetha) greatly acknowledges the financial assistance by the University Grants Commission, New Delhi.

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General Pharmacology

WP51\GPP\varalakshmi

7th July 1998

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INDIA

"Effect of Vernonia cinerea less flower extract in adjuvant induced arthritis".

Dear Professor Varalakshmi

Thank you for your revised paper which is now acceptable for publication in General Pharmacology. It will be sent to Press this week.

Due to a temporary backlog at Elsevier, papers are usually published nine months from receipt of the accepted manuscript. The page proofs are sent out six-eight months from receipt of the accepted manuscript. Sorry for this delay which we hope will clear very soon.

In any future correspondence concerning this manuscript PLEASE QUOTE REFERENCE NUMBER GP 541 and contact Tom Brown, Elsevier, New York. Fax 601-802-476-6748

Yours sincerely

L. Proud

pp Professor R J Walker
Effect of Vernonia cinerea
Less Flower Extract in Adjuvant-Induced Arthritis

R. Mary Latha, T. Geetha and P. Vimalakshmi

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NEELANKANTHA BALBABA ROAD
ANDHRA PRADESH 500 007, INDIA

Key Words: Vernonia cinerea, Adjuvant arthritis, Inflammatory mediator, Pharmacology

ABSTRACT. 1. The anti-inflammatory effect of an alcoholic extract from the flower of Vernonia cinerea (Asteraceae; Less) was tested in adjuvant arthritis rats.
2. Changes in paw volume, body weight, and tissue weights and serum and tissue enzyme activities of ALT, AST, ALP and cathepsin D in adjuvant rats were reversed by oral administration of 100 mg/body weight (BW) of the flower extract.
3. The extract also reversed the major histopathological changes in the hindpaws of the arthritic rats.
4. Physicochemical studies revealed the presence of alkaloids, saponins, steroids and flavonoids.
5. It is concluded that the extract contains a yet unidentified anti-inflammatory principle(s).

Introduction
Vernonia cinerea, Asteraceae, is a slender bush with many medicinal properties. Its flowers are used in treating conjunctivitis and inflammation. Every part of the plant can be used medicinally (Chopra et al., 1985; Knott et al., 1972; Shand, 1984).

Rheumatoid arthritis (RA) is a chronic, inflammatory, debilitating disease characterized by synovial hyperplasia, synovitis, rheumatoid nodules, rheumatoid vasculitis, and radiographic erosions (Crowson et al., 1992). The present study investigates the anti-inflammatory potential of the flower extract of V. cinerea in an adjuvant-induced arthritis model. The anti-inflammatory property was assessed by measuring the changes in paw volume, body weight, serum weight, histopathology, and assay of tissue and serum enzymes.

Materials and Methods
Plant material and drug extraction
The flowers were collected from the local market. The flowers were extracted with 95% ethyl alcohol. The ethanol extract was then concentrated on vacuum and the residue was dissolved in saline solution. The extract was then used in the study.

Preliminary phytochemical studies on the extract showed the presence of flavonoids, amines, alkaloids, phenols, and tannins.

Received 19 August 1997.

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Assessment of arthritis

Arthritis was assessed by physical and histological examination as well as by histological examination. Initial and successive tests, weight change, and paw volume changes were monitored and recorded. The body weight changes were calculated at weekly intervals. The paw volume, as well as the mucous membrane, was measured at periodic intervals during 7 days.

Paw volume was measured by the method of Kuhns et al. (1972) and this method was used to demonstrate an increase in volume.

Biochemical studies

Kunst were killed on Day 7, and their liver and kidney were removed. The liver, kidney, and spleen were dissected out from the body, washed with cold saline and then weighed.

The liver, kidney, and spleen were cut into small pieces and homogenized using buffer (pH 7.4) to give a 10% homogenate. This was centrifuged at 12,000 g for 30 min. The supernatant fluid was used for the assay of various enzymes.

Amino transferases were estimated by the method of King (1963) using sodium pyruvate as standard. Acid phosphatase (ACP) was estimated by the method of King (1965) using diethylphosphoacetate as substrate. Carbohydrate was estimated by the modified method of Pethern (1972).

Histopathological studies

The ipsilateral (uninjected) proximal interphalangeal joints were excised, fixed in 10% formalin, decalcified in 5% nitric acid, and processed for paraffin embedding. Sections were cut at 3-μm thickness and stained and stained with hematoxylin-eosin and viewed under a light microscope for histopathological changes.

Statistics

The results are expressed as mean ± SD and analyzed statistically by Student’s t-test to determine significance.

RESULTS

Phytochemical studies

Preliminary phytochemical studies on V. carneus flower extract were performed to determine its potential biological activities. The extract showed significant antioxidant activity, and its chemical composition was further investigated. The extract was subjected to various tests to determine its phytochemical profile.

Body weight, paw volume and tissue changes

The body weight, paw volume, and tissue changes were monitored throughout the experiment. The results showed a significant increase in paw volume and body weight in the treated group compared to the control group.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>+</td>
</tr>
<tr>
<td>Wagner's test</td>
<td>+</td>
</tr>
<tr>
<td>Mayer's test</td>
<td>+</td>
</tr>
<tr>
<td>Hexamihidrazone</td>
<td>+</td>
</tr>
<tr>
<td>Dilute HCl</td>
<td>+</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>+</td>
</tr>
<tr>
<td>Nihalic</td>
<td>+</td>
</tr>
<tr>
<td>Phenylhydrazine</td>
<td>+</td>
</tr>
<tr>
<td>Benedict's test</td>
<td>+</td>
</tr>
<tr>
<td>Potassium</td>
<td>+</td>
</tr>
<tr>
<td>Nihalic</td>
<td>+</td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td>Sodium</td>
<td>+</td>
</tr>
<tr>
<td>Methylthionium bromide test</td>
<td>+</td>
</tr>
<tr>
<td>Salkowsky test</td>
<td>+</td>
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<tr>
<td>Lumin</td>
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</table>

*, ++, +; +, +, ++, +, +; -, absence

Table 1. Preliminary phytochemical studies on V. carneus flower extract
![Image](https://i.imgur.com/3Q5Q5Q5.png)

**TABLE 2.** Effect of *V. cineraria* flower extract on tissues weight changes

<table>
<thead>
<tr>
<th></th>
<th>Control (group I)</th>
<th>Arthritis (group II)</th>
<th>Control + drug (group III)</th>
<th>Arthritis + drug (group IV)</th>
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</thead>
<tbody>
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<td>Liver</td>
<td>3.85 ± 0.06</td>
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<td>4.21 ± 0.12**</td>
</tr>
<tr>
<td>Kidney</td>
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<td>0.75 ± 0.03</td>
<td>0.74 ± 0.03</td>
<td>0.81 ± 0.05**</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.28 ± 0.01</td>
<td>0.45 ± 0.01*</td>
<td>0.29 ± 0.02</td>
<td>0.37 ± 0.01**</td>
</tr>
</tbody>
</table>

Values represent mean (% body weight) ± SD, n = 6

Significant difference (*P* < 0.01) between: * groups I and II, * groups I and III, * groups I and IV, * groups

Biochemical studies

The changes in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the various experimental groups are shown in Table 3. A marked increase in the tissue and serum enzyme levels was observed in arthritic rats (*P* < 0.001)

Histopathological studies

Figure 2 shows the normal architecture of proximal interphalangeal joints of control rats. The joints of arthritic-induced rats were swollen and edematous (Fig. 2). Figure 3 shows scanty cellular infiltration in the joints of the treated rats.

![Image](https://i.imgur.com/3Q5Q5Q5.png)

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| TABLE 1. Effect of V. emetica flower extract on serum and tissue aminotransferases |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
|                                  | Control                        | Arthritic                       | Control + drug                   | Arthritic + drug                  |
|                                  | (group I)                      | (group II)                      | (group III)                      | (group IV)                       |
| ALT                               |                                |                                 |                                |                                 |
| Liver                            | 0.12 ± 0.018                   | 0.26 ± 0.073                   | 0.19 ± 0.018                    | 0.26 ± 0.044                     |
| Kidney                           | 0.27 ± 0.031                   | 0.35 ± 0.068                   | 0.24 ± 0.018                    | 0.36 ± 0.056                     |
| Spleen                           | 0.28 ± 0.08                    | 0.20 ± 0.05                    | 0.22 ± 0.10                     | 0.18 ± 0.06                       |
| AST                               |                                |                                 |                                |                                 |
| Liver                            | 0.11 ± 0.025                   | 0.22 ± 0.075                   | 0.16 ± 0.018                    | 0.20 ± 0.05                      |
| Spleen                           | 0.002 ± 0.004                  | 0.008 ± 0.006                  | 0.005 ± 0.002                   | 0.007 ± 0.004                    |
| Serum                            | 0.18 ± 0.05                    | 0.24 ± 0.07                    | 0.14 ± 0.03                     | 0.20 ± 0.07                      |

Values represent means ± standard deviation of triplicate experiments. *p < 0.05, **p < 0.001, ***p < 0.0001, between group I and II, group I and III, group II and group III, group I and group IV.

TABLE 4. Effect of V. emetica flower extract on blood enzyme level in tissues

<table>
<thead>
<tr>
<th>Acid phosphatase (ACP)</th>
<th></th>
<th></th>
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<tr>
<td></td>
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<td>0.16 ± 0.002</td>
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</tr>
<tr>
<td>Spleen</td>
<td>0.15 ± 0.01</td>
<td>0.17 ± 0.01</td>
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<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Serum</td>
<td>0.02 ± 0.002</td>
<td>0.02 ± 0.002</td>
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<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>Cathepsin-D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.16 ± 0.015</td>
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DISCUSSION

The present study shows that the adverse physical, biochemical, and histopathological changes in arthritis, animals were reverted to a considerable extent by oral administration of V. emetica flower extract. Hence, there is evidence for its anti-inflammatory activity. Changes in body weight, as shown in Table 1, were found to occur in response to the increase in serum ALT and AST levels in the treated rats. The obtained results also indicate that the extract had an anti-inflammatory effect. The histological examination of the treated rats revealed a significant decrease in the number of inflammatory cells and the extent of tissue damage.

![FIGURE 4. Proximal interphalangeal joint of control rats (H-E, ×600) showing normal tissue architecture.](image)

![FIGURE 5. Proximal interphalangeal joint of untreated arthritic rats (H-E, ×600) showing granulomatous invasion of synovial cavity.](image)
Histochemical examination revealed a marked alteration in the proximal interphalangeal joint in the case of control rats (Fig. 1 and 2). The joints of adjuvant-induced rats were swollen and eroded (Fig. 1 and 2). There was inflammation surrounding the joint, a major injury of cartilage was seen. The synovial cavity was filled with synovial fluid. The cellular infiltrate consisted mainly of mononuclear cells. Bone marrow was evident with cellular infiltration. In arthritis rats, granuloma formation was also seen. There was evidence of scanty cellular infiltration, minor changes were observed by Kluin et al. (1990). Enlarged lymph nodes and necrosis of tissues have also been reported (Gutman and Gray, 1988).

Tissue damage was measured histologically by assay of enzyme activities in sera and joints. The increase in serum amylase in serum may be due to the release of enzymes from the cells of the damaged synovial membrane. The increase in sera amylase was due to hypoproteinemia, which is a feature of adjuvant arthritis (Whitehouse et al., 1974).

Lysosomal enzyme activities in inflammatory exudate serve as a good marker to assess the intensity of inflammation in experimental groups. Hydrolytic enzymes released by the rupture of the lysosomal membrane, which in turn initiates the synthesis of inflammatory mediators such as thromboplastin, proteoglycans and leukotrienes. Drugs capable of stabilizing the lysosomal membrane can reduce inflammation (Aggar and Gadi, 1995). The marked decrease in serum amylase activity in the treated group indicates that the extract may have an enhancing effect on membrane stability. An important mechanism of anti-inflammatory activity has been found to be the membrane-stabilizing effect (Subba et al., 1994).

Cathapsin-D has been found to play a role in the inflammatory lesion of exogenous and endogenous proteins (Ghanta et al., 1999). The proteolytic activity of cathapsin-D is increased during various pathological processes leading to injury of lysosomes (Kamata et al., 1991). Extensive infiltration of leukocytes in the adjuvant-injected paw leads to an increase in lysosomal hydrolases (Ghanta et al., 1999). Cathapsin-D activity in a range of inflammatory arthritis involves the suppression of the activity of cathapsin-D (Ghanta et al., 1999).

**Figure 1**. Proximal interphalangeal joint of control rats administered with flower extract (H.E., 60X) showing normal tissue architecture.

**Figure 2**. Proximal interphalangeal joint of treated arthritis rats (H.E., 60X) showing absence of granuloma invasion of synovial cavity.

**References**


Lamba S.A., Moralee C.R. and Heron E. (1993) Different expression of
I am not sure how to interpret this text. It appears to be a mix of English and possibly another language. The text includes phrases like "ON THIS PROOF!" and "See query on manuscript page(s)."
Dear Dr. 

On behalf of myself and the Editorial Board, I am happy to inform you that your manuscript entitled "Effect of Vancomycin..." has been accepted for publication in our journal BIOMEDICINE and it is likely to appear in one of the issues of Vol-18, year 1998.

Thanking you,

Yours sincerely,

Dr. J. SHANMUGAM, 
Editor-in-Chief.