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Comparitive Analgesic and Anti-inflammatory Activity Study of Three Herbal Medicines

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ABSTRACT

Achyranthus aspera Linn, *Moringa oleifera* Lam and *Scopharia dulcis* Linn are used in the traditional system of medicine for the treatment of many diseases. *Achyranthus aspera (AA)* the whole plant and especially the roots, characterized by their uterine stimulant activity, are prescribed in the therapy of rheumatism, lumbago, osteodynia, dysuria, post-partum haematomata and dysmenorrhoea. There is no doubt that the pure *Moringa oleifera* (MO) Tree leaf is the source of incredible health benefits. It's the ultimate, natural, organic, energy and endurance health supplement. *Scopharia dulcis (SD)* plant has long held a place in herbal medicine in almost every tropical country where it grows, and it is much used by indigenous people. In this present work the flavonoid and phenolic content of the three plants were estimated. Then the anti inflammatory activity of the plants were evaluated by Carrageenan induced paw oedema method with alcoholic and aqueous extracts of the plants and the Analgesic activity was evaluated by Acetic Acid Induced Writhing Test Method. The plants are having very good significant effect in the Anti-inflammatory and Analgesic activity and it may be due to the presence of flavonoids .

Keywords: anti-inflammatory, paw oedema, Carregeenan, Writhing.

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INTRODUCTION

The drugs which are used in the treatment of inflammatory conditions are steroids and non steroids. Both are having adverse effects. Because of such adverse effects, the search for natural constituents effective in treating inflammatory conditions has been increased. Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries[1] most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperanalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required[2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease[3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions[4]. However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding,and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes5-7. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates[8,9]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs[9]. Based on the ethno survey Extracts of AA, MO AND SD was evaluated for its analgesic and anti-inflammatory effects on experimental induced pain and inflammation.

MATERIALS AND METHODS

Approval for the Project

Approval for the experiment was obtained from the Institutional Animal Ethical Committee (IAEC), K.M. College of Pharmacy, Madurai. vide letter No. KMCP /IAEC/Ph.D/60

Plant material

The leaf of *Moringa oleifera* Lam.and *Achyranthus aspera* L are available locally were collected in and around Coimbatore and *Scopario dulcis* L is available and collected from Palakad district in Kerala. The botanical identity has been confirmed and authenticated by the Director, Botanical survey of India, Coimbatore, No: BSI/SRC/5/23/2012-13/Tech/496. The voucher specimen has

been submitted and preserved in herbarium for future reference.

Processing of Plant material

The plant materials were collected and shade dried at room temperature and was subjected to size reduction to get coarse powder of desired particle size. This powdered material was subjected to successive extraction. Each (1kg) powdered drugs were extracted with methanol and water separately by cold maceration method for 7 days. Then the extracts were filtered and the last traces of the solvent were evaporated under reduced pressure in a rotary evaporator to get the dry extract. The yield of the dry extracts were calculated and stored in desiccators and used for further experiments.

Estimation of total flavonoids

The flavonoid content was determined by the use of a slightly modified colorimetry method[11]. A 0.5ml aliquot of appropriately (2mg/2ml) diluted sample solution was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% NaNO2 solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent. Values are means of three independent analyses ± standard deviation (n = 3) RE – Rutin equivalent

Estimation of Total Phenols

The total phenolic content was determined by the following method[12]. Ten microlitre aliquots of the extracts (2mg/2ml) were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents. Values are means of three independent analyses ± standard deviation (n = 3) TAE – Tannic acid equivalent

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Acute Toxicity Test of Plant Extract.

Acute toxicity studies

The acute oral toxicity study was carried out as per the OECD guidelines (No:423). For acute toxicity studies, Wistar albino rat of either sex weighing between 180 and 220 g were selected. One-tenth of the median lethal dose (LD50) was taken as an effective dose[13]. Aqueous and methanolic extract of AA, MO and SD was found to have lethal effect (LD_{50}) at the dose of 2000 mg/kg .

Anti-Inflammatory Activity

Carrageenan Induced Paw Oedema Method

Anti-Inflammatory activity of the three plants extracts were estimated by Carrageenan- induced paw inflammation method[14]. One hour after oral administration of the Aqueous and Methanolic extract of AA,MO and SD at a dose of 200 mg/kg), reference drug Diclofenac sodium 50 mg/kg) or vehicle (Normal saline 5%), an injection of 0.1ML of carrageenan (1% suspended in 0.9% NaCl) was made into the right hind limb of each rat under the subplantar aponeurosis. Measurement of paw volume was done by means of volume displacement technique using plethysmometer (Ugo Basile Italy) immediately after carrageenan injection and after 6th hr.Percentages of inhibition were obtained using the following ratio:

(Vt-Vo) control – (Vt –Vo) treated/ (Vt-Vo) control X 100 (1)

Vt is the average volume for each group after treatment, and

Vo is the average volume for each group before any treatment.

Analgesic Activity

Acetic Acid Induced Writhing Test Method.

The Analgesic activity of the three drugs were evaluated by Acetic acid Induced Writhing method[15]. The total number of writhings following intraperitoneal administration of acetic acid solution (1%, 10mL/kg) was recorded over a period of 10min, starting 5min after acetic acid injection. The mice were treated with the Aqueous and methanolic Extract of AA,MO and SD at a dose of 200 mg/kg),or vehicle (Normal saline (5%)) or standard drug (diclofenac sodium, 50mg/kg), 30 min before administration of acetic acid. The number of writhing and stretching was recorded and permitted to express the percentage of protection.

Statistical Analysis.

All values were expressed as mean ± SEM, and data was analyzed by one way analysis of variance (ANOVA) followed by Newmann Keuls multiple range tests using Graph Pad Instat.

RESULTS AND DISCUSSION

Carrageenan-Induced Paw Edema in Rats.

In this method, the paw edema of each animals were measured by the displacement of mercury was measured before and after treatment. After 3-6 hours of the treatment of drugs extract it was started to reduce the volume. It shows the significant effect of these drugs towards Anti-inflammatory activity. The percentage of volume inhibition by the Aqueous and Methanolic extract of AA, MO and SD and standard drugs were calculated and shown in diagram 1. Aqueous and methanolic extract of AA, MO and SD at a dose 200mg/kh showed a significant inhibition of paw oedema at six hour as compared to reference drug (Table1 & Diagram 1). Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorph nuclear cells, and prostaglandins produced by tissue macrophages[16]. Since the extract/ fractions significantly inhibited paw oedema induced by carrageenan in the second phase, this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by nonsteroidal anti-inflammatory drugs such as diclofenac sodium, whose mechanism of action is inhibition of the cyclooxygenase enzyme. The inflammatory granuloma is a typical feature of an established chronic inflammatory process[17].The brain and spinal cord plays a major role in central pain mechanisms. The dorsal horn of the spinal cord is endowed with several neurotransmitters and receptors including substance P, somatostatin, neuropeptide Y, inhibitory amino acid, nitric oxide, endogenous opioids, and the monoamines which are the major targets for pain and inflammation[18].The extract inhibits pain pathways mechanisms, suggesting that the plant extract may act as a narcotic analgesic. On the other hand, acetic leads to the release of free arachidonic acid from the tissue phospholipid[19]. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels[20], and the prostaglandin pathways. Flavonoids may increase the amount of endogenous serotonin or may interact with 5 acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus -HT2A and 5- HT3 receptors may be involved in the mechanism of central analgesic activity The aqueous and methanolic extract AA, MO and SD (200 mg/kg) dose significantly and dependently reduced the number of abdominal constriction induced in mice by a solution of acetic acid 1%. This dose-dependent protective effect reached a maximum inhibition at the dose of 200 mg/kg. Diclofenac sodium (reference drug) exerted a significant protective effect, with percentage of protection of 83.24% (Diagram2 & Table 2).Moreover,

aqueous and methanolic extract Of AA, MO and SD extract showed highest analgesic activity in all the experimental model which may be due to its high flavonoid contents which are responsible for free radical scavenging activity, as these free radicals are involved during pain stimulation, and antioxidants showed reduction in such pain[21] .The results of the present study have shown that the crude extracts of the investigated plant exhibited very high anti-inflammatory and analgesic activities. These activities may be linked with the presence of polyphenolic compounds present in the extracts .Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation.The ability of flavonoids to inhibit eicosanoid biosynthesis has been documented. Eicosanoids, such as prostaglandins, are involved in various immunological responses and are the end products of the cyclooxygenase and lipoxygenase pathways . Further, flavonoids are able to inhibit neutrophils degranulation and thereby decrease the release of arachidonic acid[22]. Thus, the presence of flavonoids in the extract/fractions of Aqueous and methanolic extract of AA, MO and SD might be responsible for the anti-inflammatory and analgesic activity in Swiss albino mice and rats.

RESULTS AND DISCUSSION

Anti-Inflammatory Activity:

Table 1: Protective effect of Aqueous and Methanolic Extract of AA, MO and SD on paw oedema induced by carrageenan in rats.

Treatment	Dose (mg/kg)	Percentage % inhibition of paw edema
Group I Normal saline	10ml/kg orally	-
Group II Std	50mg/kg I.P.Diclofenac sodium	68.25%*a
Group III AEAA	200mg/kg through orally.	62.12%*a
Group IV MEAA	200mg/kg Through orally.	63.06%*a
Group V AEMO	200mg/kg through orally	60.70%*a
Group VI MEMO	200mg/kg through orally	62.41%*a
Group VII AESD	200mg/kg through orally	62.76%*a
Group VIII MESD	200mg/kg through orally	64.54%*a

* Data are expressed as Mean ± S.E.M.

*Data were analyzed by one way ANOVA followed by Newman's keul's multiple range tests, to determine the significance of the difference between the control group and rats treated with the test compounds.

*a Values were significantly different from normal control at P< 0.01.

Table 2A: analgesic activity of aqueous and methanolic extract of AA, MO and SD by acetic acid induced writhing reflux in mice.

Treatment	Dose (mg/kg)	% reduction in reaction time
Group I Normal saline	Inject 1%v/v acetic acid 1ml/100g of body weight	-
Group II Std	50mg/kg I.P.Diclofenac sodium	86.24%**
Group III AEAA	200mg/kg through orally.	81.67%*a
Group IV MEAA	200mg/kg Through orally.	82.15%*a
Group V AEMO	200mg/kg through orally	80.62%*a
Group VI MEMO	200mg/kg through orally	81.93%*a
Group VII AESD	200mg/kg through orally	82.41%*a
Group VIII MESD	200mg/kg through orally	83.28%*a

AEAA-Aqueous extract of *Achyranthus aspera*, **MEAA**-methanolic extract of AA

AEMO- Aqueous extract of *Moringa oleifera*, **MEMO**- Methanolic extract of MO

AESD- Aqueous extract of *Scoparia dulcis*, **MESD**- Methanolic extract of SD

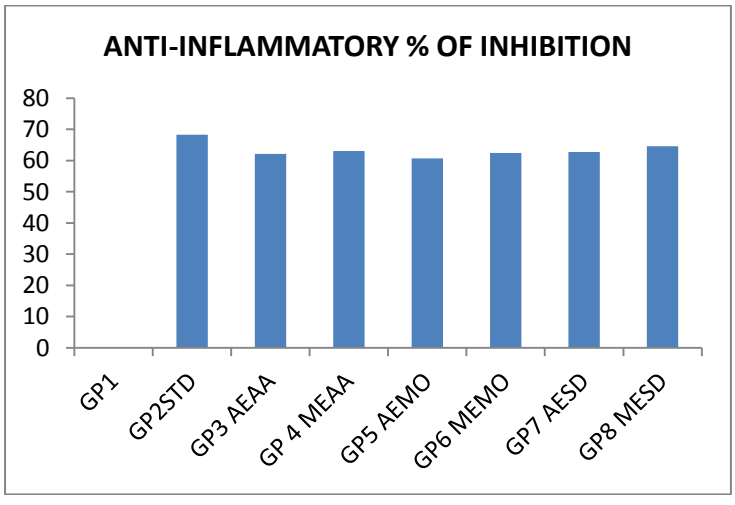

Diagram.1Shows the percentage of inhibition in inflammation

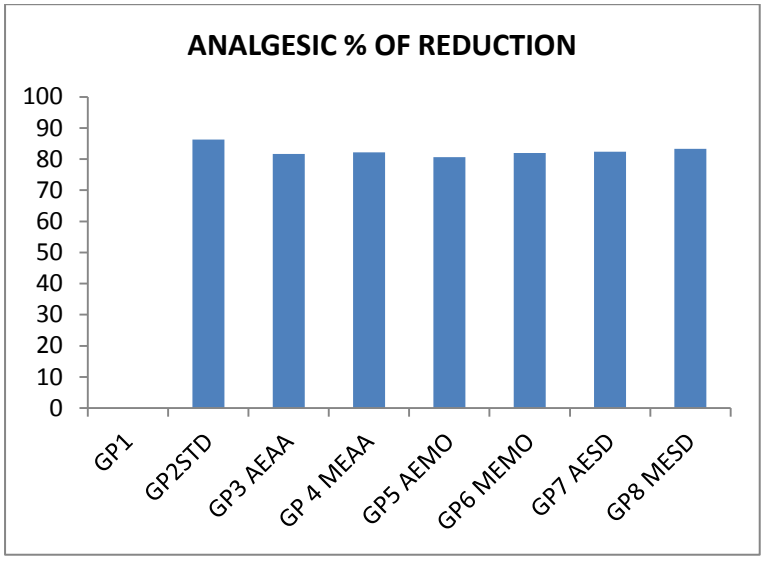

Diagram.2 Shows the percentage of reduction in analgesic activity

Table 3: Quantitative estimation of Total phenols and Flavonoids

Sample name	Total Phenolics (mg TAE/g extract)	Total Flavonoid (mg RE/g extract)
AA	23.57 ± 0.48	1.06 ± 0.100
MO	61.56 ± 2.50	1.95 ± 0.030
SD	70.16 ± 2.52	3.62 ± 0.020

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

CONCLUSION:

These plants were selected based on their traditional uses. The phenolic and flavonoid content of the three plants were identified and estimated. Three plants are showing significant effect related to the analgesic and anti-inflammatory activity when comparing with the standard drugs. In all drugs methanolic extract is better than aqueous extract and the *Scoparia sulcis* plant is more active than the other two comparatively. This present work suggest that the isolation of phytoconstituent for these activities will be useful for the new herbal formulation.

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