**INTRODUCTION**

Inflammation typically characterized by redness, swelling and pain. It is one of the most important host defense mechanisms against invading pathogens. Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting the synovial joints that leads to joint destruction, which is responsible for the deformity and disability (Buch and Emery, 2002; Katz and Piliero, 1969). During the response to inflammation, the pro-inflammatory (e.g., TNF-α) stimuli activates the cellular responses that increase production of many cytokines, including prostaglandins (PGs) and NO. The cyclooxygenase 2 (COX-2) is responsible for the increase in the levels of PGs in inflammatory conditions (Sims and Smith, 2010; Vodovotz et al., 2004). Numerous studies have indicated that NO and PGs participate in inflammatory and nociceptive events (Holthusen and Arndt, 1994). Inhibition of NO and PGs production via the inhibition of iNOS and COX-2 expression is beneficial in treating inflammatory diseases (Bogdan, 2001). Currently, steroids, non-steroidal anti-inflammatory drugs (NSAIDS) and immunosuppressant drugs are used in the relief of inflammation and are often associated with severe adverse effects (Corley, 2003).

Matricaria recutita (Asteraceae) is also known as German chamomile and it is one of the most useful and well documented medicinal plants in the world (Salaman, 1992). The plant is rich in many active constituents such as several phenolic compounds primarily the flavonoids apigenin, quercetin, patuletin and luteolin. It also contains terpenoids, chamazulene and sequiterpenes. It is used externally for wounds, ulcers, eczema, gout, skin irritations, neuralgia, sciatica, hemorrhoids, leg ulcers, rheumatic pain (Newall, Anderson and Phillipson, 1996) and treatment of inflammation (Motavalizadeh et al., 2012). It is traditionally used to treat anxiety, hysteria, nightmares, insomnia and sleep problems, convulsions and even delirium tremens (Martens, 1995). In the present study we evaluated the anti-inflammatory activity of *Matricaria recutita* against acute and chronic inflammatory models.

**MATERIALS AND METHODS**

**Plant Material**

In the present study, capitula of *Matricaria recutita* were collected from Horticulture University, Bagalkot, Karnataka, India during the month of June 2012.
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Herbarium was prepared and the specimen was further identified and authenticated in Department of Botany, Basaveswara Science College, Bagalkot, Karnataka and voucher specimen (B.sc./Bot./14/2012) was deposited in the herbarium of the same college. The dried samples were reduced to a fine powder of 444 and stored in amber colored air tight glass bottles. The powdered capitula were subjected to successive extraction with methanol (64-65.5°C). After solvent was distilled off from the residue of extraction and excess solvent was completely removed by using a rotatory flash evaporator to get concentrated, then completely dried by lipolysis (Mini Lyotrap, LTE Scientific Ltd. Great Britain), and stored in air tight container under refrigeration. The dried extract (81g, percentage yield - 12.05%) was used for anti-inflammatory activity.

Phytochemical Screening

Phytochemical screening of methanol extract was carried out by employing the standard procedure and tests (Trease and Evans, 1989).

Animals

Female Sprague-Dawley rats (200-250g) and Swiss Albino mice (20-25g) were obtained from animal house of H.S.K. College of Pharmacy and Research Centre, Bagalkot. The animals were housed under standard conditions (temperature 25±2°C, relative humidity 50-55%) for 12h dark and 12h light cycle respectively. They were given standard laboratory feed (Pranava Agro Industries Ltd, Sangli, Maharashtra) and water ad libitum. The study was conducted after obtaining clearance from the Institutional Animal Ethical Committee as per the CPCSEA guidelines (F. No. HS/K COP/IAEC, Clear/2011-12/1-14).

Acute Toxicity Study

Acute toxicity studies were carried out as per the OCED guideline No. 425, a method was adopted. The anti-inflammatory activity was performed at three dose levels 100, 200 and 300 mg/kg of body weight (Chandrashekar, 2010).

Carrageenan-induced Hind Paw Edema in Rats

The acute anti-inflammatory effect was evaluated by carrageenan-induced rat paw edema according to the method described by Prabhakar et al. (2006) and Prashith Kekuda et al. (2013). Edema was induced by injecting carrageenan (1% w/v, 0.1 ml/paw) in the right hind paw of rats. The extract of Chamomile (100, 200 and 300 mg/kg), Diclofenac (10 mg/kg), or vehicle was administered orally 1 h before the carrageenan administration. Paw volume was measured with digital Plethysmograph (Model No. 7140, Ugo Basile Srl, Comerio, Italy). The percentage inhibition of paw oedema volume of each treated groups is calculated as above.

Chronic Inflammatory Model

Complete Freund's Adjuvant FCA-induced Arthritis in Rats

Female Sprague Dawley rats (200-260 gm) were divided into five groups consists of FCA Control, Diclofenac (10 mg/kg, p.o.), Matricaria recutita L. (100, 200 and 300 mg/kg, p.o.) with 6 animals each. On day 1, the induction of arthritis is done by injecting 0.1 ml of FCA (each ml contains 1 mg of Mycobacterium tuberculosis, heat killed and dried, 0.85 ml of paraffin oil and 0.15 ml of Mannide monoooleate, Sigma Aldrich, Saint Louis, USA) into the sub-plantar region of right hind paw of rat (Kalia, Rao and Kutty, 2007). The rats are carefully observed for 28 days.

Evaluation of inflammatory activity was performed at three dose levels 100, 200 and 300 mg/kg of body weight (Chandrashekar, 2010).

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Effect of Matricaria recutita on Paw Volume of FCA-induced Arthritis in Rats

Paw volumes were measured on 0 day before injection and on the 7th, 14th, 21st and 28th day after the injection with digital Plethysmograph (Model No. 7140, Ugo Basile Srl, Comerio, Italy). The percentage inhibition of paw oedema volume of each treated groups is calculated above.

Effect of Chamomile Methanol Extract on Open Field Test

Animals were subjected to open field test on 0, 7th, 14th, 21st and 28th day. Rat was placed in an open field in the sound-attenuated room. The flooring was white polyvinyl with a black grid dividing open field into 100 squares (10x10). Illumination was provided by a bulb (60 W) while the rest of the room was darkened and observations were made between 6pm and 10pm. Observation were done for 5 minutes for all behavioural parameters, which include latency (in sec), time to start exploring the open field, ambulatory movements (horizontal locomotor activity or grid line crossed) (Costa, Sutter and Gybel, 1981), rearing (vertical locomotor activity), grooming (rubbing of nose with its forepaws and preening) and defecation (number of boluses) (Tomita, Yoshimi and Philip, 2006).

Effect of Methanol Extract of Matricaria recutita on Development of Arthritis in Rats

Development of arthritis was assessed after every seventh day up to 28 days. i.e. on 0, 7th, 14th, 21st and 28th day after the injection of FCA for all groups. The scoring was done by using a three point scale for each paw: 0 = normal joint, 1 = slight inflammation and redness; 2 = severe erythema and swelling affecting the entire paw with inhibition of use; and 3 = deformed paw or joint with...
Effect of Methanol Extract of *Matricaria recutita* on Vascular Permeability

The rats are anaesthetized with ketamine hydrochloride (i.p.) 45mg/kg body weight. Evan’s blue (50 mg/kg of B.W.) was administered via the jugular vein into the anaesthetized rat. After 4 hr, the rats were sacrificed and their anterior and posterior synovial capsules and fat pad were dissected from each knee joint and paw. The amount of evans blue in the sample was estimated by extracting the dye. The knee joints capsule and paw joints are cut into smaller pieces and mixed with acetone in 1% NaSO4 in the ratio of 7:3. The test samples were shaken at room temperature. Then each sample preparation was centrifuged for 10 min at 2000 rpm and 2 ml of the supernatant was separated for measurement of absorbance at 620 nm using UV-spectrophotometer (UV- 1601, Shimadzu Corporation, Kyoto, Japan). The amount of dye retained in joint, by releasing by disrupting the cells with perchloric acid and ammonium oxalate and residual histamine in cells was calculated.

The severity of the lesions was given scores: 0 = no change, 1 = mild change, 2 = moderate change and 4 = marked change (Shore, Burkhalter and Cohn, 1959).

Assessment of Morphological Changes

The rats were anaesthetized and exsanguinated and their knee joints are dissected, freed from muscles and fixed in 10% formalin. The joints are decalcified, embedded in wax, sectioned and stained with haematoxylin and eosin. Histological analysis was carried out by a single observer, focusing on polymorpho-nuclear cell infiltration, tissue proliferation and cartilage erosions. The severity of the lesions was given scores: 0 = no change, 1 = mild change, 2 = moderate change and 4 = marked change (Shore, Burkhalter and Cohn, 1959).

Statistical Analysis

All the data are presented as mean±S.E.M. The significance of difference in means between control and treated animals for different parameters was determined by using one way analysis of variance (ANOVA) followed by multiple comparisons Dunnett’s test. A value of *P*<0.05 was considered statistically significant.

RESULTS

Phytochemical Screening

Preliminary phytochemical screening showed the presence of flavonoids, saponins, tannins and volatile oils in methanol extract.

Carrageenan-induced Hind Paw Edema in Rats

The extract and Diclofenac inhibited carrageenan-induced paw edema by 44.78%, 67.35% and 54.70% at the dose of 100, 200 and 300 mg/kg and 56.23% respectively, in the late phase at 5rh hr after induction. Chamomile extract exhibited significant anti-inflammatory activity (Table 1).

Arachidonic Acid Induced Rat Paw Edema

Matricaria recutita (100, 200 and 300 mg/kg) and dual blocker (Nimesulide and Cyproheptadine) were found to inhibit arachidonic acid induced paw edema significantly (*P*<0.05--<0.001) as compared to control (Table 1).

Acetic Acid Induced Peritoneal Inflammation in Mice

In acetic acid induced writhing in mice, the methanol extract (100, 200 and 300 mg/kg) and Diclofenac decreased protein content as compared to control treated group (Table 2).

**Table 1**: Effect of *Matricaria recutita* on carrageenan induced paw edema and acetic acid induced peritoneal inflammation.

| Groups  | Carrageenan induced paw edema | Acetic acid induced inflammation |
|---------|-------------------------------|---------------------------------|
|         | ½ hr                          | 1st hr                          | 2nd hr                          | 3rd hr                          | 5th hr                          | No. of writhing | Total protein (mg/ml) |
| Normal  | ---                           | ---                             | ---                             | ---                             | ---                             | 0.00±0.00        | 14.31±0.90             |
| Control | 0.361±0.03                    | 0.426±0.03                      | 0.488±0.04                      | 0.526±0.05                      | 0.585±0.06                      | 44.83±2.48       | 34.89±2.01a            |
| M.R.    | 0.155±0.05                    | 0.251±0.02**                    | 0.333±0.03                      | 0.393±0.02                      | 0.323±0.02**                    | 33.50±3.67       | 20.79±0.84***          |
| 100mg/kg| (57.06%)                      | (41.07%)                        | (37.76%)                        | (25.28%)                        | (44.78%)                        |                  |                      |
| M.R.    | 0.146±0.03                    | 0.230±0.02**                    | 0.490±0.05                      | 0.381±0.05                      | 0.191±0.04**                    | 32.83±2.94       | 17.54±1.14***          |
| 200mg/kg| (59.5%)                       | (46.0%)                         | (0.0%)                          | (27.56%)                        | (67.35%)                        |                  |                      |
| M.R.    | 0.151±0.03                    | 0.245±0.05**                    | 0.273±0.05                      | 0.238±0.03**                    | 0.265±0.03**                    | 20.33±2.44       | 17.80±0.63***          |
| 300mg/kg| (58.17%)                      | (42.48%)                        | (44.05%)                        | (54.75%)                        | (54.70%)                        |                  |                      |

All the values are expressed as mean ± SEM, n=6, *P*<0.05, **P*<0.01, ***P*<0.001 as compared to control group. One way Analysis of Variance (ANOVA) followed by multiple comparisons Dunnett’s multiple comparison test.
Effect on Vascular Permeability

In vascular permeability test, the Diclofenac (10 mg/kg), chamomile extract 100, 200 and 300 mg/kg treated groups showed significant ($P<0.001$) inhibition of joint infiltration of Evans blue dye in the rat paw joints as compared to the control group (Table 4).

Effect on Histamine Release in Blood

In the histamine release in blood assay, the control group animals showed elevated histamine content in blood. In contrast, Diclofenac (10 mg/kg) and chamomile extract (100, 200 and 300 mg/kg) showed significant ($P<0.05$ to $P<0.001$) reduction of release of histamine into the blood (Table 4).

Effect on ESR

In control group animals exhibited severe inflammation, by generation of acute phase reactants such as fibrinogens and immunoglobulins, which are increased in inflammation and leads RBC's to fall more rapidly and hence increased ESR’s were observed. In contrast, Diclofenac (10 mg/kg) and methanol extract of Chamomile showed significant ($P<0.001$) decrease in ESR’s as compared to control group (Table 4).

Radiographic and Histopathological Changes

Significant changes have been observed after taking the radiographs. The smooth muscle swelling was decreased in the Diclofenac (10 mg/kg) and Matricaria recutita extract (100, 200 and 300 mg/kg) treated groups. Joint spaces as well as deformities were decreased in treated groups as compared to control. In case of histopathological assessment, the degree of presence of inflammatory cells, lymphocytes, plasma cells and polymorphonuclear cells have reduced in Matricaria recutita L. extract treated groups and the degree of cartilage erosion also reduced as compared to the control group (Figure 1).
Figure 1: The extent of rat paw pathological conditions was graded on a semi quantitative scale. Light microscopy 10x. Paw joint tissue was fixed in 10% formaldehyde and 5-μm paraffin sections were stained with hematoxylin and eosin. (a1 and a2) Grade 3: Destruction of cartilage and subchondral bone, disorganization of the joint space and replacement with mononuclear cells and fiber thickening, increased lymphocytes and plasma cells (control group); (b1, b2, e1 and e2) Grade 1: mild proliferation and infiltration of mononuclear cells, mild subchondral bone erosion and superficial cartilage damage, Diclofenac (10 mg/kg) and M.R. (300 mg/kg); (c1, c2, d1 and d2) Grade 2: moderate pannus formation with superficial cartilage erosion M.R. (100 and 200 mg/kg).
DISCUSSION

In the present study, the methanol extract of *Matricaria recutita* showed significant anti-inflammatory activity against acute carrageenan, archidionic induced rat paw edema and writhing acetic acid induced peritoneal inflammation and FCA induced chronic inflammation rats. The extract significantly reduced paw edema in carrageenan induced inflammatory model. The reduction of paw edema occurs at 3rd and 5th hr of the induction, this indicates early and late phase of pro-inflammatory agents inhibition (Crankhun and Meacock, 1971). The methanol extract of *Matricaria recutita* showed significant reduced writhing response and total protein content against acetic acid induced peritoneal inflammation in mice. An inflammatory activity also includes the increase in the vascular permeability, which causes increased exudation of plasma proteins with migration of leucocytes but, in our experiment we observed a significant inhibition in the peritoneal exudation of plasma proteins after treatment with chamomile extract.

The FCA induced arthritis in rats has been very common and widely used method for a chronic model of inflammation. In this model, we observe chronic and immediate pain behaviours such as reduced locomotion, more scratching and itching. Paw swelling is the major factor to assess the degree of inflammation and to investigate the efficacy of the test drugs. The increased release of inflammatory mediators such as histamine in the blood also shows the inflammatory condition. In contrast to inflammation, in our study the methanol extract of *Matricaria recutita* was found showing significant anti-inflammatory activity. Increase in locomotion and reduction in grooming and paw volume on 28th day of injection of FCA was observed as compared to control. The release of histamine content in blood also reduced significantly.

FCA induced arthritis is thought to occur by cell mediated auto immunity by effect of mycobacterium on cartilage proteoglycans in rats (Van Eden et al., 1985). MR also reduced the arthritis scores along with paw swelling which shows immunosuppressive effects. WBC increases in arthritic conditions (Maria et al., 1983) and WBC were reduced by MR extract. Depletion in Hb content in arthritis results from reduced response of the bone marrow erythropoietin and also due to premature destruction of red blood cells. Erythrocyte sedimentation rate (ESR) is influenced by an increase in the plasma concentration in response to inflammation (Van Eden et al., 1985) and so, the platelet count gets elevated too. *Matricaria recutita* treatment showed significant reduction in ESR and platelets but did not show elevated effect on Hb and RBC. Radiographic observations showed that by treating with *Matricaria recutita*, there is inhibition of joint related alterations in rat paws. Chamomile treated animals were showed that reduced the inflammatory cells such as lymphocytes, plasma cells and polymorphonuclear cells and cartilage erosion as compared to control group animals in histopathological studies.

CONCLUSION

The present study indicated that methanol extract of *Matricaria recutita* potentially possess the anti-inflammatory and anti-arthritic activity. *Matricaria recutita* showed significant anti-inflammatory activity against acute carrageenan, archidionic induced rat paw edema and writhing acetic acid induced peritoneal inflammation and FCA induced chronic inflammation rats. The extract significantly reduced paw edema in carrageenan induced inflammatory model. Further research required to study the exact mechanism of action of *Matricaria recutita* at molecular level.

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REFERENCES

Bogdan, C. (2001). Nitric oxide and the immune response. *Nature Immunology* 2(10): 907-916.

Buch, M., Emery, P. (2002). The etiology and pathogenesis of rheumatoid arthritis. *Hospital Pharmacy Journal* 9: 5-10.

Cai, X., Wong, Y.F., Zhou, H., Xie, Y., Jiang, Z.H., Bian, Z.X., Xu, H.X., Liu, L. (2006). The comparative study of Sprague-Dawley and Lewis rats in adjuvant induced arthritis. *Nauyn-Schmiedeberg’s Archives of Pharmacology* 373(2): 140-47.

Chandrashekar, V.M., Ranapariya, V.L., Ganapaty, S., Parashar, A., Muchandi, A.A. (2010). Neuroprotective activity of *Matricaria recutita* L against global model ischemia in rats. *Journal of Ethnopharmacology* 127(3): 645-51.

Corley, D.A., Kerhikowske, K., Verma, R., Baffler, D. (2003). Protective association of aspirin/NSAIDs and esophageal cancer. *A systemic review and meta analysis. Gastroenterology* 124(1):47-56.

Costa, M.D., Sutter, P.D., Gybel, J. (1981). Adjuvant induced arthritis in rats: A possible animal model of chronic pain. *Pain* 10(2):173-85.
Katti et al.,
Crunkhon, P., Meacock, S.C.R. (1971). Mediators of inflammation induced in the rat paw by carrageenan. *British Journal of Pharmacology* 42(3):392-402.

Dimartino, M.J., Griswold, D.E., Berkowitz, B.A., Poste, G., Lewis, A.J. (1987). Pharmacologic characterization of the anti-inflammatory properties of a new dual inhibitor of lipooxygenase and cyclo-oxygenase. *Agents Actions* 20: 113-23.

Franchis, Y.L., Hilida, L.W., Ethel, S.K.N. (2004). Time course and substance P effects on the vascular and morphological changes in adjuvant induced monoarthritis rats. *International Immunopharmacology* 4(2):299-310.

Holthusen, H., Arndt, J.O. (1994). Nitril oxide evokes pain in humans on intra-cutaneous injection. *Neuroscience Letter* 165(1-2):71-74.

Kalia, R., Rao, C.M., Kutty, N.G. (2007). Synthesis and evaluation of the anti-inflammatory activity of N[2-(3,5-di-tert-butyl-4-hydroxyphenyl)-4-oxo-thiazolidin-3-yl]-nicotinamide. *Arznei-Forschung* 57: 616-622.

Katz, L., Piliero, S.T. (1969). A study of adjuvant induced polyarthritis in the rat with special reference to associated immunological phenomena. *Annals of the New York Academy of Sciences* 147: 515-36.

Koster, R., Anderson, M., De Beer, E.J. (1959). Acetic acid for analgesic screening. *Federation proceedings* 18: 412.

Maria, M., Engeniusz, M., Miroslaw, K., Maria, K., Iwona, P. (1983). Adjuvant induced disease in rats, clinical findings and morphological and biochemical changes in the blood histological changes in internal organs. *Rheumatology* 2: 231-245.

Martens, D. (1995). Chamomile: the herb and the remedy. *The Journal of the Chiropractic Academy of Homeopathy* 6:15-18.

Motavalizadehkakhky, A. (2012). Antimicrobial activity and chemical composition of essential oils of chamomile from Neyshabur. *Iranian Journal of Medicinal Plant Research* 6: 820-24.

Newall, C.A., Anderson, L.A., Phillipson, J.D. (1996). Herbal medicines: A Guide For Healthcare Professionals. Pharmaceutical Press, London.

Sci. Technol. Arts Res. J., Jan-March 2014, 3(1): 01-07
Prashith Kekuda, T.R., Onkarappa, R., Raghavendra, H.L. (2013). Pharmacological Activities of Streptomyces Species PO-178 Isolated from Rhizosphere Soil of Agumbe, Karnataka, India. *Science, Technology and Arts Research Journal* 2(2): 83-91.

Prabhakar, K.R., Veerapur, V.P., Bansal, P., Vipan, K.P., Reddy, K.M., Barik, A., Reddy, B.K., Reddanna, P., Priyadarshini, K.I., Unnikrishnan, M.K. (2006). Identification and evaluation of antioxidant, analgesic/anti-inflammatory activity of the most active ninhydrin-phenol adducts synthesized. *Bioorganic and Medicinal Chemistry* 14(21):7113-7120.

Salaman, I. (1992). Chamomile: a medicinal plant. *The Herb, Spice and Medicinal Plant Digest* 10: 1-4.

Shore, P.A., Burkhalter, A., Cohn, V.H. (1959). A method for the fluorimetric assay of histamine in tissues. *Journal of Pharmacology and Experimental Therapeutics* 127: 182-186.

Sims, J.E., Smith, D.E. (2010). The IL-1 family: regulators of immunity. *Nature Reviews Immunology* 10: 89-102.

Tomita, T., Yoshimi, K., Philip, S.T. (2006). THR-0921, a novel peroxisome proliferation-activated receptor gamma agonist, reduces the severity of collagen-induced arthritis. *Arthritis Research and Therapy* 8(1):R7.

Trease, G.E., Evaans, W.C. (1989). Pharmacognosy, 13th ed. Bailliere Tindall, London. 683-684.

Turner, R.A. (1965). Analgesics. In: Turner RA, (ed.), Screening Methods in Pharmacology. London: Academic Press, p. 100.

Van Eden, W., Holoshitz, J., Nevo, Z., Frenkel, A., Kiazman, A., Cohen, I.R. (1985). Arthritis induced by a T-lymphocyte clone that responds to mycobacterium tuberculosis and to cartilage proteoglycans. *Proceedings of the National Academy of Sciences USA* 82(15):5117-5120.

Vodovozov, Y., Kim, P.K., Bagci, E.Z., Ermentrott, G.B., Chow, C.C., Bahar, I., Billiar, T.R. (2004). Inflammatory Modulation of Hepatocyte Apoptosis by Nitric Oxide: In vivo, In vitro, and In Silico Studies. *Current Molecular Medicine* 4(7):753-62