Journal of Young Pharmacists, 2023; 15(2):264-277.
https://www.jyoungpharm.org

Original Article

Synergistic Effect of Different Extracts of *Asparagus officinalis* (Asparagaceae), *Garcinia travancorica* (Clusiaceae) and *Mucuna gigantea* (Fabaceae) Extracts on Analgesic, Anti-inflammatory and Anti-arthritic Activities in Rats

Satish Kumar[1,2], Pratima Srivastava[2], Sumeet Gupta[1,*]

[1]Department of Biology and Pharmacology, M.M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana, Haryana, INDIA.
[2]Aragen Life Sciences Private Limited, Hyderabad, Telangana, INDIA.

ABSTRACT

Background: The arial part of *Asparagus officinalis (A.O.)* (Family: Asparagus) stem and seeds, leaf parts of *Mucuna gigantea (M.G.)* (Family: Fabaceae) and fruit rinds and arial part of *Garcinia travancorica (G.T.)* (Family: Clusiaceae) have long been used to treat joint pain. However, its preclinical efficacy for rheumatoid arthritis has not been pharmacologically evaluated. In the current study, extracts of *A.O., M.G.*, and *G.T.* from petroleum ether, ethanolic extract, and aqueous extract were examined for their analgesic, anti-inflammatory, anti-arthritic, and phytochemical properties. **Materials and Methods:** Rats' tail flick method was used to assess analgesic activity, carrageenan-induced paw oedema model was used to assess anti-inflammatory activity, and protein Complete Freund's Adjuvant (CFA)-induced arthritis model was used to assess anti-arthritic potential. **Results:** We observed that many extracts had anti-inflammatory and anti-arthritic effects, and to a lesser extent, analgesic activities corresponding to the administered dose. The CFA model's findings showed improved defence against arthritic lesions and changes in body weight. Additionally, rheumatoid factor, altered WBCs count, and histological and radiographic changes were all markedly improved by *M.G., G.T.*, and *A.O.* **Conclusion:** All the three plants extract when given together, supports traditional combinatorial use of *M.G., G.T.* and *A.O.* as potent analgesic, a potential anti-inflammatory and anti-arthritic polypharmacy for the treatment of rheumatoid arthritis.

Keywords: Complete Freund's adjuvant model, Inflammation, Plant extract, Rheumatoid arthritis.

Correspondence:
Dr. Sumeet Gupta
Professor, Department of Pharmacology, MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University) Mullana-133207, Haryana, INDIA.
Email id: sumeetgupta25@gmail.com

Received: 20-11-2022;
Revised: 29-11-2022;
Accepted: 07-12-2022.

INTRODUCTION

Rheumatoid Arthritis (RA), an inflammatory condition that mostly affects flexible (synovial) joints, caused by inflammatory reactions in the joint capsule and subsequent enlargement of the synovial cells. Though, all ages are at risk for RA, but the occurrence has considerably increased in those over 40, especially in women, who have been reported to have two-or-three times higher risk than males of having the disease.[1] Although the exact etiology is unknown, but release of free radicals as a by-product of cellular metabolism may induce the generation of Interleukins (IL) and Tumour Necrosis Factor-α (TNF-α) from T-cells causes inflammation and destruction of tissues. The existing pharmacological data confirms the involvement of both inflammatory as well as pathophysiological targets in the progression of rheumatoid arthritis. Therefore, we Interleukin-1 (IL1), Interleukin-6 (IL6), Janus Kinase (JAK), Tumor Necrosis Factor Receptor (TNFR1) and Tumour Necrosis Factor-α (TNF-α) responsible for disease progression can be targeted.[2]

Because of limited uses of existing medicines, and associated side effects, the world is now focusing on herbal options for the treatment of RA. The information currently accessible on studies over the past 20 years demonstrates the anti-arthritic effects of plants and botanical products.[3] In the modern pharmacopoeia, over 25% of medications are now sourced from plants, and numerous others synthesised are equivalents from prototype chemicals identified from plant species.[4] Three plants (*A.O., G.T.*

DOI: 10.5530/jyp.2023.15.36

and *M.G.)* were selected and were evaluated for their individual and synergistic anti-arthritic activity when combined together.

A.O. belongs to asparagus family. Steroids, ferulic acid, amino acids, flavonoids, saponins, and fructans (asparagose and asparagosine) are all members of *A.O* family. Traditionally, the plant was used to treat kidney and Bladder stones, Asthma, Antiulcer, Antioxytoxic, Liver toxicity treatment, Anti-arthritic, Kidney damage, Oxidative stress, Antimicrobial, Antidiabetic and bloody cough.[5-7]

G.T. is a plant of Clusiaceae (formerly Guttiferae) family. Common name of *G.T.* is *Mangosteen. Garcinia* is comprising of 250 species. *Garcinia* is found on the Western ghats of southern India. *Garcinia* has been reported to possess Antibacterial, Antioxidant activity against *Streptococcus pyogenes*. Observed with *flavonoid*,[8] hexane was observed with *7*-epi-nemorosone and garcinol,[9] Xanthone gaudichaudione.[10]

Mucuna belongs to family Fabaceae. Common names of *M.G.* are Black Bean, Burney Vine, Seabean, Burny Bean, Velvet Bean.[11] *Mucuna* species contains other plants with various therapeutic effects for example *Mucuna nigricans, monosperma* and *pruriens. pruriens* contains wide range of Phyto-constituents like alkaloids, flavonoids, tannins and phenolic compounds which are responsible for different pharmacological activities and L-DOPA as well.[12] Many activities are already reported for *Mucuna*. The pathogenic mechanism and cellular immune response of arthritis caused with Complete Freund's adjuvant (CFA) have already been proven to be similar to human arthritis.[13] That's why CFA model was used to evaluate the Anti-arthritic activity. Objective of this study was to check the individual and synergistic effect of above selected plants as Anti-inflammatory, Analgesic and anti-arthritic agent.

MATERIALS AND METHODS

Plant material

Arial part of *A.O.* was procured form botanical Garden of Southern region, aerial part of *M.G.* (Stem, leaves, ripe rod with seeds) was collected from Kottapuram, Kerala and arial part of *G.T.* was procured form Delhi. Dr. Sunita Garg, former chief scientist and head of the raw materials herbarium and museum at N.I.S.C.A.I.R., New Delhi, India, authenticated the samples plant after identifying it using sources of information from the literature with reference number NISCAIR/RHMD/consult/-2021/3827-28, NISCAIR/RHMD/Consult/2020/3716-17 and NISCAIR/RHMD/consult/-2020/3652-53-1-28, respectively.

Chemical and drugs

Drugs of pharmaceutical quality were employed in this investigation. We bought carrageenan and Complete Freund's adjuvant from Sigma Chemicals (St Louis, USA). Prednisolone and methotrexate were gifts from Ind. Swift Laboratories (Baddi, India), while pure samples of diclofenac sodium and prednisolone and methotrexate were received from MacLeod's Lab (Mumbai, India). The study's other reagents and compounds were all of Analytical grade.

Preparation of extracts

Using a Soxhlet device, the dried powder was progressively extracted using petroleum ether, ethanol, and water. The vacuum dried process was used to get rid of the final bit of solvent. Prior to usage, the extracts were kept at or below 2-4°C. Dried extracts were used for qualitative phytochemical tests and dosing.

Animals

The animals were given free access to water and a standard pellet meal (Safe Diet, purchased from Samitek Instruments). Rats (*Sprague-Dawley* strain) weighing between 130 and 150 g were utilised after receiving the protocol B-098's permission from the Institute Animal Ethics Committee (1125/PO/Rc/S/07/CPCSEA).

Qualitative phytochemical screening

Different qualitative tests were performed for alkaloids Wagner's and Dragendroff's reagent test, for flavonoids Shinoda and ++Lead acetate test, for tannin Ferric chloride and Iodine tests, for sterols and triterpenoids Salkowski reaction test and for protein Millons and Buret test.[14]

Experimental Design

Different activities were performed as per the Table 1.

Tail flick method (Analgesic activity)

Analgesiometer was used to measure the analgesic effect in rats. A nichrome wire within the instrument is heated to the necessary temperature and kept there by heat regulators. The rat was housed in a rat cage with only its tail sticking out. The centre of the tail was positioned on the base such that it was barely above the hot wire but not touching it. The animal's sudden, recognisable flick or tail raising in response was used to measure the animal's latency (reaction time). The cut-off period was set at 10 sec to protect the animal's tissue.[15]

Carrageenan induced paw oedema in rats (Acute inflammation)

After one week of oral medication treatment, all groups except the control group received 0.1 ml of 1% w/v carrageenan injections into the rat paw. The volume of the paw oedema was taken with a digital plethysmometer. Anti-inflammatory activity was determined by calculating the proportion of oedema that was inhibited in comparison to the control. Using the below formula, the % inhibition of oedema was determined.[15]

Complete Freunds Adjuvant (CFA) induced arthritis (Chronic inflammation)

By injecting 0.1mL (0.1% w/v) of CFA into left hind paw, Arthritis was developed. On the fifth day, a second CFA booster was administered, whereas the normal group got the same amount of normal saline. The drug treatment programme began on day one and lasted for 21 days. Different parameters were observed on different time points. Methotrexate (0.5 mg/kg two twice a week) prednisolone (5 mg/kg once daily) were used as standard drug.[15] For the examination of rheumatoid factor, blood samples from the retro orbital plexus were taken on the twenty-first day, and serum was separated for analysis. On the 21st day, after blood withdrawal, the animals were euthanized with CO_2 and their legs were removed after the femur bone was cut. X-rays were taken immediately after the bone was collected. To assess bone deformities and soft tissue inflammation later on tissue histopathology was performed.

Statistical Analysis

Data were presented as mean standard deviation. Using a one-way ANOVA and Dunnett's multiple range test, the statistics of the ankle joint diameter, soft tissue thickness, % inhibition, and paw oedema was conducted. A chance of error with the values $^{a}p<0.0001$, $^{b}p<0.001$, and $^{c}p<0.05$ was deemed statistically significant.

RESULTS

Qualitative Phytochemical analysis

The presence of flavonoid, terpenoids, alkaloids, tannins, sterols, proteins and carbohydrates content was confirmed in *A.O.* flavonoid, terpenoids, alkaloids, tannins, sterols, proteins, amino

Table 1: Animals were divided into 16, 15 and 14 for Anti- Arthritic, Anti- Inflammatory and Analgesic Activity respectively (*n*=6).

Group No.	Anti-arthritic Activity	Anti-inflammatory Activity	Analgesic Activity
Group 1	Normal Control	Normal Control	Normal Control
Group 2	CFA induced Arthritic Control	Carrageenan induced Inflammation Control	Diclofenac (10 mg/kg)
Group 3	Methotrexate (0.5 mg/kg)	Diclofenac Treated (10 mg/kg)	*M.G.*, Petroleum ether extract, 150 mg/kg
Group 4	Prednisolone (5 mg/kg)	*M.G.*, Petroleum ether extract, 150 mg/kg	*M.G.*, Ethanolic extract, 150 mg/kg
Group 5	*M.G.*, Petroleum ether extract, 150 mg/kg	*M.G.*, Ethanolic extract, 150 mg/kg	*M.G.*, Aqueous extract, 150 mg/kg
Group 6	*M.G.*, Ethanolic extract, 150 mg/kg	*M.G.*, Aqueous extract, 150 mg/kg	*G.T.*, Petroleum ether extract, 150 mg/kg
Group 7	*M.G.*, Aqueous extract, 150 mg/kg	*G.T.*, Petroleum ether extract, 150 mg/kg	*G.T.*, Ethanolic extract, 150 mg/kg
Group 8	*G.T.*, Petroleum ether extract, 150 mg/kg	*G.T.*, Ethanolic extract, 150 mg/kg	*G.T.*, Aqueous extract, 150 mg/kg
Group 9	*G.T.*, Ethanolic extract, 150 mg/kg	*G.T.*, Aqueous extract, 150 mg/kg	*A.O.*, Petroleum ether extract, 150 mg/kg
Group 10	*G.T.*, Aqueous extract, 150 mg/kg	*A.O.*, Petroleum ether extract, 150 mg/kg	*A.O.*, Ethanolic extract, 150 mg/kg
Group 11	*A.O.*, Petroleum ether extract, 150 mg/kg	*A.O.*, Ethanolic extract, 150 mg/kg	*A.O.*, Aqueous extract, 150 mg/kg
Group 12	*A.O.*, Ethanolic extract, 150 mg/kg	*A.O.*, Aqueous extract, 150 mg/kg	Cassette, Petroleum ether extract, 50 mg/kg of Each
Group 13	*A.O.*, Aqueous extract, 150 mg/kg	Cassette, Petroleum ether extract, 50 mg/kg of Each	Cassette, Ethanolic extract, 50 mg/kg of Each
Group 14	Cassette, Petroleum ether extract, 50 mg/kg of Each	Cassette, Ethanolic extract, 50 mg/kg of Each	Cassette, Aqueous extract, 50 mg/kg of Each
Group 15	Cassette, Ethanolic extract, 50 mg/kg of Each	Cassette, Aqueous extract, 50 mg/kg of Each	
Group 16	Cassette, Aqueous extract, 50 mg/kg of Each		

Table 2: Effect on analgesic activity on rats.

Groups ($n = 6$)	0 min (s)	After 30 min (s)	After 60 min (s)	After 90 min (s)	After 120 min (s)	After 180 min (s)
Group 1 (Normal Control, Tween 80 (1% *v/v*) + 0.5% *w/v* CMC in Aqueous extract (99% *v/v*))	1.84 ± 0.35	1.76 ± 0.42	1.87± 0. 38	1.85± 0.38	1.93 ± 0.24	1.62 ± 0.42
Group 2 (Diclofenac sodium, 10 mg/kg)	1.69 ± 0.18	1.84 ± 0.19 (4.74)	2.71 ± 0.52[c] (45.05)	4.54± 0.37[a] (145.76)	5.84 ± 0.44 [a] (203.29)	3.63 ± 0.42[a] (124.97)
Group 3 (M.G., Petroleum ether, 150 mg/kg)	1.80 ± 0.17	1.96 ± 0.47 (11.56)	2.28 ± 0.40 (22.03)	2.87 ± 0.48[c] (55.14)	3.74 ± 1.08 [a] (94.20)	2.43 ± 0.43[b] (50.36)
Group 4 (M.G., Ethanol, 150 mg/kg)	1.72 ± 0.19	1.86 ± 0.23 (5.59)	2.20 ± 0.53 (17.75)	2.88 ± 0.70[c] (56.14)	3.19 ± 1.05 [c] (65.40)	2.08 ± 0.20 (28.59)
Group 5 (M.G., Water, 150 mg/kg)	1.71 ± 0.19	1.88 ± 0.31 (6.73)	2.52 ± 0.56 (34.61)	3.28 ± 0.43[a] (77.35)	3.22 ± 0.43 [b] (67.04)	2.41 ± 0.25[c] (48.92)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	1.69 ± 0.39	1.77 ± 0.22 (0.57)	1.78 ± 0.28 (-5.00)	2.30 ± 0.44 (24.73)	2.31 ± 0.37 (19.81)	2.80 ± 0.38[a] (73.17)
Group 7 (G.T., Ethanol, 150 mg/kg)	1.70 ± 0.27	1.76 ± 0.20 (-0.09)	1.94 ± 0.24 (3.84)	2.46 ± 0.61 (33.03)	2.69 ± 0.52 [c] (39.45)	2.63 ± 0.66[b] (62.95)
Group 8 (G.T., Water, 150 mg/kg)	1.70 ± 0.26	1.80 ± 0.23 (2.18)	2.59 ± 0.60 (38.63)	2.25 ± 0.57 (22.02)	2.31 ± 0.38 (19.72)	2.22 ± 0.29 (37.56)
Group 9 (A.O., Petroleum ether, 150 mg/kg)	1.66 ± 0.23	1.99 ± 0.18 (13.08)	2.13 ± 0.19 (14.18)	2.17 ± 0.49 (17.33)	2.51 ± 0.32 [c] (30.19)	2.60 ± 0.34[b] (60.68)
Group 10 (A.O., Ethanol, 150 mg/kg)	1.74 ± 0.34	1.91 ± 0.14 (8.34)	2.07 ± 0.41 (10.97)	2.40 ± 0.26[c] (29.78)	2.29 ± 0.21 (18.60)	2.66 ± 0.37[b] (64.60)
Group 11 (A.O., Water, 150 mg/kg)	1.67 ± 0.32	1.95 ± 0.24 (11.00)	2.00 ± 0.17 (6.87)	2.19 ± 0.18 (18.32)	2.27 ± 0.20 (17.99)	2.60 ± 0.23[b] (61.20)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	1.71 ± 0.33	2.20 ± 0.46 (25.21)	2.15 ± 0.47 (14.81)	2.21 ± 0.27 (19.68)	2.44 ± 0.44 (26.56)	2.69 ± 0.39[a] (66.46)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	1.65 ± 0.30	2.22 ± 0.47 (26.16)	2.22 ± 0.30 (19.00)	2.45 ± 0.30 (32.58)	2.39 ± 0.43 (23.79)	2.71 ± 0.32[a] (67.49)
Group 14 (Cassette, Water, 50 mg/kg of Each)	1.67 ± 0.28	2.57 ± 0.65 [c] (46.35)	2.17 ± 0.60 (15.97)	3.05 ± 0.59[b] (65.25)	2.57 ± 0.31 (33.48)	3.04 ± 0.32[a] (88.44)
F Value		2.577	2.358	13.07	20.91	9.048
P Value		0.0057	0.0112	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

acid and carbohydrates content was confirmed in *G.T.* extracts and saponins, flavonoid, terpenoids, alkaloids, tannins, sterols, proteins, amino acid and carbohydrates content was confirmed in *M.G.* extracts. The presence was confirmed using various phytochemical assays.

Analgesic Activity

In this study Sprague Dawley rats to evaluate the analgesic activity of *M.G., G.T.* and *A.O.* to check the synergistic effect here we compared 180 min for 150 mg/kg of each plant extracts with 50 mg/kg as cassette and we observed 50 mg/kg as cassette is equal/more significant than 150 mg/kg of individual plant. Response time was increased 124 with diclofenac whereas with different extracts also we observed 48.92 to 77.35% increase at 150 mg/kg in comparison to vehicle group. We observed synergistic effect with 50 mg/kg as cassette with three plant extracts 65.25 to 88.44% response time increase (Table 2).

Anti-inflammatory activity

Acute anti-inflammatory activity of *M.G., G.T.* and *A.O.* extracts. And it was observed that Aqueous extract, alcoholic and Petroleum ether extract has statistically high significant

Table 3: Effect on Acute Inflammation (Using plethysmometer).

Groups ($n = 6$)	Pre-Induction hour	1st hr	2nd hr	3rd hr	4th hr	6th hr
Group 1 (Normal Control, Tween 80 (1% *v/v*) + 0.5% *w/v* CMC in water (99% *v/v*))	0.690 ± 0.023 (-2.73)	0.695 ± 0.019 (1.65)	0.672 ± 0.015[a] (23.24)	0.667 ± 0.022[a] (26.74)	0.663 ± 0.023[a] (29.18)	0.663 ± 0.019[a] (23.90)
Group 2 (Carrageenan induced Inflammation Control)	0.672 ± 0.028	0.707 ± 0.022	0.875 ± 0.019	0.910 ± 0.011	0.937 ± 0.022	0.872 ± 0.028
Group 3 (Diclofenac, 10 mg/kg)	0.682 ± 0.023 (-1.49)	0.705 ± 0.018 (0.24)	0.723 ± 0.016[a] (17.33)	0.752 ± 0.038[a] (17.40)	0.735 ± 0.019[a] (21.53)	0.702 ± 0.017[a] (19.50)
Group 5 (M.G., Petroleum ether, 150 mg/kg)	0.683 ± 0.018 (-1.89)	0.720 ± 0.024 (-1.89)	0.853 ± 0.022 (2.48)	0.860 ± 0.013[b] (5.49)	0.858 ± 0.023[a] (8.36)	0.822 ±0.019[b] (5.74)
Group 8 (M.G., Ethanol, 150 mg/kg)	0.682 ± 0.018 (-2.12)	0.722 ± 0.012 (-2.12)	0.858 ± 0.012 (1.90)	0.863 ±0.010[b] (5.13)	0.878 ± 0.019[a] (6.23)	0.818 ±0.028[b] (6.12)
Group 11 (M.G., Water, 150 mg/kg)	0.680 ± 0.017 (-1.65)	0.718 ± 0.020 (-1.65)	0.802 ± 0.025[a] (8.38)	0.807 ±0.016[a] (11.36)	0.843 ± 0.020[a] (9.96)	0.808 ±0.023[a] (7.27)
Group 5 (G.T., Petroleum ether, 150 mg/kg)	0.687 ± 0.016 (-2.23)	0.723 ± 0.020 (-2.36)	0.842 ± 0.017 (3.81)	0.860 ± 0.018[b] (5.49)	0.868 ± 0.015[a] (7.30)	0.835 ± 0.019 (4.21)
Group 8 (G.T., Ethanol, 150 mg/kg)	0.678 ± 0.017 (-0.99)	0.718 ± 0.019 (-1.65)	0.855 ± 0.027 (2.29)	0.872 ± 0.021[c] (4.21)	0.853 ± 0.020[a] (8.90)	0.828 ± 0.013[c] (4.97)
Group 11 (G.T., Water, 150 mg/kg)	0.672 ± 0.020 (0.00)	0.715 ± 0.020 (-1.18)	0.862 ± 0.019 (1.52)	0.873 ± 0.015[c] (4.03)	0.877 ± 0.012[a] (6.41)	0.825 ± 0.019[c] (5.35)
Group 5 (A.O., Petroleum ether, 150 mg/kg)	0.682 ± 0.029 (-1.49)	0.713 ± 0.020 (-0.94)	0.760 ± 0.011[a] (13.14)	0.785 ± 0.019[a] (13.74)	0.740 ± 0.023[a] (21.00)	0.717 ± 0.016[a] (17.78)
Group 8 (A.O., Ethanol, 150 mg/kg)	0.678 ± 0.019 (-0.99)	0.715 ± 0.016 (-1.18)	0.740 ± 0.021[a] (15.43)	0.788 ± 0.023[a] (13.37)	0.748 ± 0.015[a] (20.11)	0.725 ± 0.019[a] (16.83)
Group 11 (A.O., Water, 150 mg/kg)	0.672 ± 0.031 (0.50)	0.718 ± 0.020 (-1.65)	0.740 ± 0.014[a] (15.43)	0.753 ± 0.015[a] (17.22)	0.743 ± 0.012[a] (20.74)	0.702 ± 0.018[a] (19.50)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	0.685 ± 0.021 (-1.99)	0.720 ± 0.020 (-1.89)	0.837 ± 0.024[c] (4.38)	0.865 ± 0.014[c] (4.95)	0.857 ± 0.012[a] (8.54)	0.822 ± 0.019[c] (5.74)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	0.680 ± 0.019 (-1.24)	0.718 ± 0.025 (-1.65)	0.813 ± 0.014[a] (7.05)	0.815 ± 0.010[a] (10.44)	0.795 ± 0.014[a] (15.12)	0.775 ± 0.014[a] (11.09)
Group 14 (Cassette, Water, 50 mg/kg of Each)	0.675 ± 0.019 (-0.50)	0.720 ± 0.025 (-1.89)	0.800 ± 0.017[a] (8.57)	0.812 ± 0.017[a] (10.81)	0.807 ± 0.022[a] (13.88)	0.802 ± 0.023[a] (8.03)
F Value	0.3827	0.8268	63.97	68.02	95.50	58.59
P Value	0.9760	0.6383	<0.0001	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

anti-inflammatory effect from 2 hr to 6 hr at higher dose and less significant effect at lower doses also. Diclofenac showed 17.33 to 21.53% inhibition and we observed a significant range of 4.03 to 20.74% inhibition with different extracts for different medicinal plants at 150 mg/kg whereas synergistic effect with 50 mg/kg as cassette with three plant extracts with range 4.38 to 15.12% inhibition (Table 3).

Anti-arthritic Activity

Effect on animal body weight

In arthritic animal's body decreased weight was observed in comparison to normal control group whereas in treated animal's Methotrexate, Prednisolone and different extracts were able to keep the animals body weight in normal range. Significant with

Table 4: Effect on body weights.

Groups ($n = 6$)	Day 1	Day 7	Day 15	Day 21
Group 1 (Normal Control, Tween 80 (1% *v/v*) + 0.5% *w/v* CMC in Water (99% *v/v*))	128.96 ± 10.72 (-0.030	179.67 ± 10.99 (-5.46)	244.19 ± 13.66 (4.25)	299.30 ± 12.00 (9.74)
Group 2 (CFA induced, Arthritic Control)	128.99 ± 10.20	190.05 ± 13.41	234.24 ± 13.40	272.74 ± 8.44
Group 3 (Methotrexate, 0.5 mg/kg)	129.10 ± 9.75 (0.08)	181.75 ± 12.26 (-4.37)	236.55 ± 15.15 (0.98)	294.53 ± 12.33 (7.99)
Group 4 (Prednisolone, 5 mg/kg)	129.43 ± 9.06 (0.34)	182.93 ± 9.67 (-3.75)	236.80 ± 10.34 (1.09)	286.80 ± 12.49 (5.16)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	129.34 ± 8.80 (0.27)	183.39 ± 9.79 (-3.50)	234.85 ± 14.47 (0.26)	288.17 ± 18.89 (5.66)
Group 9 (M.G., Ethanol, 150 mg/kg)	129.53 ± 8.23 (0.42)	182.34 ± 7.38 (-4.05)	231.83 ± 8.96 (-1.03)	287.32 ± 16.55 (5.35)
Group 12 (M.G., Water, 150 mg/kg)	129.60 ± 7.99 (0.47)	176.50 ± 13.04 (-7.13)	226.16 ± 14.80 (-3.45)	280.51 ± 14.88 (2.85)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	129.89 ± 7.49 (0.70)	184.44 ± 9.95 (-2.95)	237.02 ± 11.86 (1.19)	284.28 ± 18.10 (4.23)
Group 9 (G.T., Ethanol, 150 mg/kg)	129.89 ± 7.05 (0.70)	184.32 ± 9.30 (-3.01)	233.80 ± 12.26 (0.19)	281.80 ± 22.89 (3.32)
Group 12 (G.T., Water, 150 mg/kg)	129.94 ± 6.77 (0.74)	191.71 ± 7.36 (0.88)	239.24 ± 8.08 (2.13)	291.86 ± 12.35 (7.01)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	129.71 ± 7.00 (0.56)	188.57 ± 8.57 (-0.77)	238.25 ± 15.54 (1.71)	282.87 ± 20.50 (3.71)
Group 9 (A.O., Ethanol, 150 mg/kg)	129.77 ± 6.83 (0.60)	192.10 ± 5.46 (1.08)	243.53 ± 6.39 (3.97)	299.04 ± 9.20[c] (9.65)
Group 12 (A.O., Water, 150 mg/kg)	129.84 ± 6.95 (0.66)	199.14 ± 6.05 (4.79)	254.41 ± 10.06 (8.61)	294.82 ± 18.86 (8.10)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	121.2 ± 6.94 (0.64)	181.63 ± 5.74 (-5.43)	250.07 ± 8.74 (5.41)	302.54 ± 9.52[c] (10.04)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	121.39 ± 6.90 (0.65)	192.7 ± 7.09 (2.82)	234.83 ± 12.76 (4.74)	289.47 ± 14.96[c] (9.50)
Group 14 (Cassette, Water, 50 mg/kg of Each)	121.59 ± 6.50 (0.40)	180.19 ± 7.47 (1.52)	215.37 ± 16.26 (4.06)	297.39 ± 9.94[c] (10.24)
F Value	0.009904	2.867	1.848	1.866
P Value	>0.9999	0.0012	0.0418	0.0394

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

normal control was 9.74% more than arthritic control animals. Methotrexate animals were observed with increased body weight 7.99% more than arthritic control animals. Whereas synergistic effect with 50 mg/kg as cassette with three plant extracts with range 9.50 to 10.24% significant increase in body weight was observed (Table 4).

Effect on paw oedema in Arthritic animals

Methotrexate-28.33%, Prednisolone-32.18% inhibition of oedema observed. We observed a significant effect with a range of 16.19 to 27.01% inhibition of paw oedema on with different extracts at 150 mg/kg whereas synergistic effect with 50 mg/kg as cassette with range 19.38 to 22.03% significant inhibition of paw oedema was observed (Table 5).

Effect on Paw diameter in Arthritic animals

Methotrexate showed 30.78%, Prednisolone showed 32.97% inhibition of oedema. We observed a significant effect with a range of 23.54 to 30.13% inhibition of paw oedema on with different extracts at 150 mg/kg whereas synergistic effect with 50 mg/kg as

Table 5: Effect on paw oedema (Using plethysmometer).

Groups ($n = 6$)	Day 1	Day 7	Day 14	Day 21
Group 1 (Normal Control, Tween 80 (1% *v/v*) + 0.5% *w/v* CMC in Water (99% *v/v*))	0.74 ± 0.02	0.94 ± 0.02[a] (49.09)	1.26 ± 0.06[a] (48.11)	1.50 ± 0.09[a] (40.41)
Group 2 (CFA induced, Arthritic Control)	0.75 ± 0.03	1.84 ± 0.03	2.42 ± 0.33	2.51 ± 0.26
Group 3 (Methotrexate, 0.5 mg/kg)	0.74 ± 0.05	1.84 ± 0.05 (0.09)	1.92 ± 0.17[a] (20.65)	1.80 ± 0.16[a] (28.33)
Group 4 (Prednisolone, 5 mg/kg)	0.73 ± 0.01	1.82 ± 0.10 (0.73)	1.77 ± 0.12[a] (26.77)	1.70 ± 0.15[a] (32.18)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	0.75 ± 0.01	1.76 ± 0.05 (4.26)	1.91 ± 0.12[a] (21.34)	1.93 ± 0.16[a] (23.22)
Group 9 (M.G., Ethanol, 150 mg/kg)	0.74 ± 0.03	1.81 ± 0.06 (1.45)	1.86 ± 0.10[a] (23.19)	1.84 ± 0.12[a] (26.68)
Group 12 (M.G., Water, 150 mg/kg)	0.75 ± 0.01	1.82 ± 0.03 (0.82)	1.88 ± 0.07[a] (22.51)	1.83 ± 0.04[a] (27.01)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	0.75 ± 0.022	1.81 ± 0.05 (1.45)	1.97 ± 0.19[a] (18.58)	1.86 ± 0.11[a] (25.95)
Group 9 (G.T., Ethanol, 150 mg/kg)	0.74 ± 0.02	1.82 ± 0.03 (0.73)	2.04 ± 0.11[b] (15.90)	1.97 ± 0.05[a] (21.70)
Group 12 (G.T., Water, 150 mg/kg)	0.72 ± 0.03	1.84 ± 0.06 (-0.18)	2.20 ± 0.07 (9.36)	2.03 ± 0.15[a] (19.04)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	0.73 ± 0.02	1.84 ± 0.03 (0.00)	2.02 ± 0.08[b] (16.66)	1.91 ± 0.08[a] (23.95)
Group 9 (A.O., Ethanol, 150 mg/kg)	0.75 ± 0.02	1.84 ± 0.02 (0.09)	2.10 ± 0.11[c] (13.42)	2.12 ± 0.19[b] (16.19)
Group 12 (A.O., Water, 150 mg/kg)	0.73 ± 0.03	1.84 ± 0.04 (0.00)	2.03 ± 0.15[b] (16.10)	1.94 ± 0.17[a] (22.89)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	0.77 ± 0.05	1.82 ± 0.03 (1.09)	2.20 ± 0.14 (9.02)	2.03 ± 0.19[a] (19.38)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	0.74 ± 0.02	1.83 ± 0.02 (0.27)	2.02 ± 0.18[b] (16.59)	1.98 ± 0.16[a] (21.30)
Group 14 (Cassette, Water, 50 mg/kg of Each)	0.74 ± 0.02	1.82 ± 0.03 (1.00)	1.99 ± 0.13[a] (18.03)	1.95 ± 0.17[a] (22.23)
F Value	1.090	145.7	16.72	11.89
P Value	0.3789	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

cassette with range 24.22 to 27.23% significant inhibition of paw oedema was observed (Table 6).

Effect on arthritis symptoms

Visual scorings were given based on walking, inflammation, joint deformities and pain assessment using Grimace scale (0= Normal to 5= Severe). Methotrexate showed 51.85%, Prednisolone showed 81.48% recovery. We observed a significant effect with a range of 22.22 to 48.15% inhibition of visual parameters on with different extracts at 150 mg/kg whereas synergistic effect with 50 mg/kg as cassette with range 33.33 to 44.44% significant inhibition of visual parameters was observed on day 20 (Table 7).

Effect on Spleen weight

Methotrexate showed 18.28%, Prednisolone showed 43.38% recovery. We observed a significant effect with a range of 22.22 to 25.85% reversal of spleen weight on with different extracts at 150 mg/kg whereas synergistic effect with 50 mg/kg as cassette with range 16.35 to 26.98% significant inhibition of reversal of spleen weight was observed (Table 8).

Effect on RA Factor

Methotrexate showed 38.43%, Prednisolone showed 49.32% recovery. We observed a significant effect with a range of 30.11 to 67.47% on RA factor inhibition with different extracts at 150 mg/kg whereas synergistic effect with 50 mg/kg as cassette with range 32.02 to 45.25% significant effect on RA factor inhibition was observed (Table 9).

Effect on WBC's

Methotrexate-43.32%, Prednisolone-65.21% decrease in WBC's count. We observed a significant effect with a range of 39.40 to 61.29% on WBC count reversal with different extracts at 150 mg/kg whereas synergistic effect with 50 mg/kg as cassette with range 39.17 to 53.46% significant effect on WBC count reversal was observed (Table 10).

Effect on Joint Diameter in X-Ray

Methotrexate showed 18.52%, Prednisolone showed 18.86% decrease joint diameter. We observed a significant effect with a range of 10.41 to 18.51% on X-ray joint inflammation inhibition

Table 6: Effect on Joint diameter (Using Vernier calliper).

Groups ($n = 6$)	1st Day (mm)	7th Day (mm)	10th Day (mm)	15th Day (mm)	20th Day (mm)
Group 1 (Normal Control, Tween 80 (1% *v/v*) + 0.5% *w/v* CMC in Water (99% *v/v*))	4.33 ± 0.23	4.45 ± 0.26[a] (48.32)	5.15 ± 0.40[a] (48.27)	5.37 ± 0.21[a] (46.14)	5.54 ± 0. 13[a] (43.82)
Group 2 (CFA induced, Arthritic Control)	4.29 ± 0.12	7.84 ± 0.87	9.95 ± 0.59	9.98 ± 0.27	9.86 ± 0.40
Group 3 (Methotrexate, 0.5 mg/kg)	4.31 ± 0.28	7.48 ± 0.44 (4.65)	8.14 ± 0.82[a] (18.14)	7.22 ± 0.450[a] (27.68)	6.83 ± 0.21[a] (30.78)
Group 4 (Prednisolone, 5 mg/kg)	4.40 ± 0.26	7.55 ± 0.40 (3.76)	7.90 ± 0.24[a] (20.58)	6.84 ± 0.41[a] (31.44)	6.61 ± 0.50[a] (32.97)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	4.22 ± 0.19	7.54 ± 0.38 (3.87)	8.20 ± 0.31[a] (17.53)	7.58 ± 0.33[a] (24.04)	6.96 ± 0.37[a] (29.39)
Group 9 (M.G., Ethanol, 150 mg/kg)	4.39 ± 0.34	7.46 ± 0.24 (4.85)	8.31 ± 0.240[a] (16.47)	7.22 ± 0.51[a] (27.68)	6.98 ± 0.40[a] (29.20)
Group 12 (M.G., Water, 150 mg/kg)	4.22 ± 0.19	7.18 ± 0.13 [c] (8.42)	8.84 ± 0.64[c] (11.11)	8.86 ± 0.91[c] (11.18)	7.37 ± 0.55[a] (25.27)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	4.28 ± 0.24	7.39 ± 0.42 (5.74)	7.98 ± 0.22[a] (19.78)	7.43 ± 0.33[a] (25.58)	6.89 ± 0.47[a] (30.13)
Group 9 (G.T., Ethanol, 150 mg/kg)	4.30 ± 0.31	7.24 ± 0.34 (7.72)	8.22 ± 0.80[a] (17.31)	7.21 ± 0.47[a] (27.70)	7.54 ± 0.78[a] (23.54)
Group 12 (G.T., Water, 150 mg/kg)	4.39 ± 0.13	7.31 ± 0.23 (6.76)	8.15 ± 0.53[a] (18.03)	7.65 ± 0.51[a] (23.30)	7.50 ± 0.37[a] (24.00)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	4.21 ± 0.07	7.41 ± 0.16 (5.46)	8.65 ± 0.27[c] (13.06)	7.65 ± 0.58[a] (23.35)	7.27 ± 0.27[a] (26.25)
Group 9 (A.O., Ethanol, 150 mg/kg)	4.21 ± 0.10	7.34 ± 0.37 (6.44)	8.86 ± 0.74[c] (10.93)	7.35 ± 0.42[a] (26.34)	7.41 ± 0.48[a] (24.84)
Group 12 (A.O., Water, 150 mg/kg)	4.19 ± 0.17	7.68 ± 0.52 (2.13)	8.17 ± 0.50[a] (17.86)	7.55 ± 0.42[a] (24.29)	7.44 ± 0.59[a] (24.52)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	4.19 ± 0.09	7.40 ± 0.31 (5.67)	8.97 ± 0.94[c] (9.80)	7.59 ± 1.40[a] (23.96)	7.47 ± 1.57[a] (24.22)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	4.18 ± 0.13	7.70 ± 0.22 (1.77)	8.71 ± 0.58[c] (12.38)	7.66 ± 0.33[a] (23.25)	7.18 ± 0.50[a] (27.23)
Group 14 (Cassette, Water, 50 mg/kg of Each)	4.20 ± 0.12	7.62 ± 0.38 (2.83)	8.23 ± 0.40[a] (17.21)	7.34 ± 0.58[a] (26.41)	7.31 ± 0.70[a] (25.84)
F Value	0.8796	23.59	18.26	15.90	11.84
P Value	0.5887	<0.0001	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

with different extracts at 150 mg/kg whereas synergistic effect with 50 mg/kg as cassette with range 11.60 to 15.19% significant effect on X-ray joint inflammation inhibition was observed (Table 11, Figure 1).

Histopathological Study

The histopathological examination of the knee joints following CFA injection is shown in Figure 2. In the Non-arthritic control group, the architecture of the joint seems normal; however, in the arthritic control group, there is a very wide joint space, erosion of the synovial membrane, and destruction of bone and articular cartilage. However, standard drugs, *M.G., G.T.*, and *A.O.* treatments with aqueous, ethanolic extract, and petroleum ether extracts stopped the degradation of the articular architecture in the treated animals.

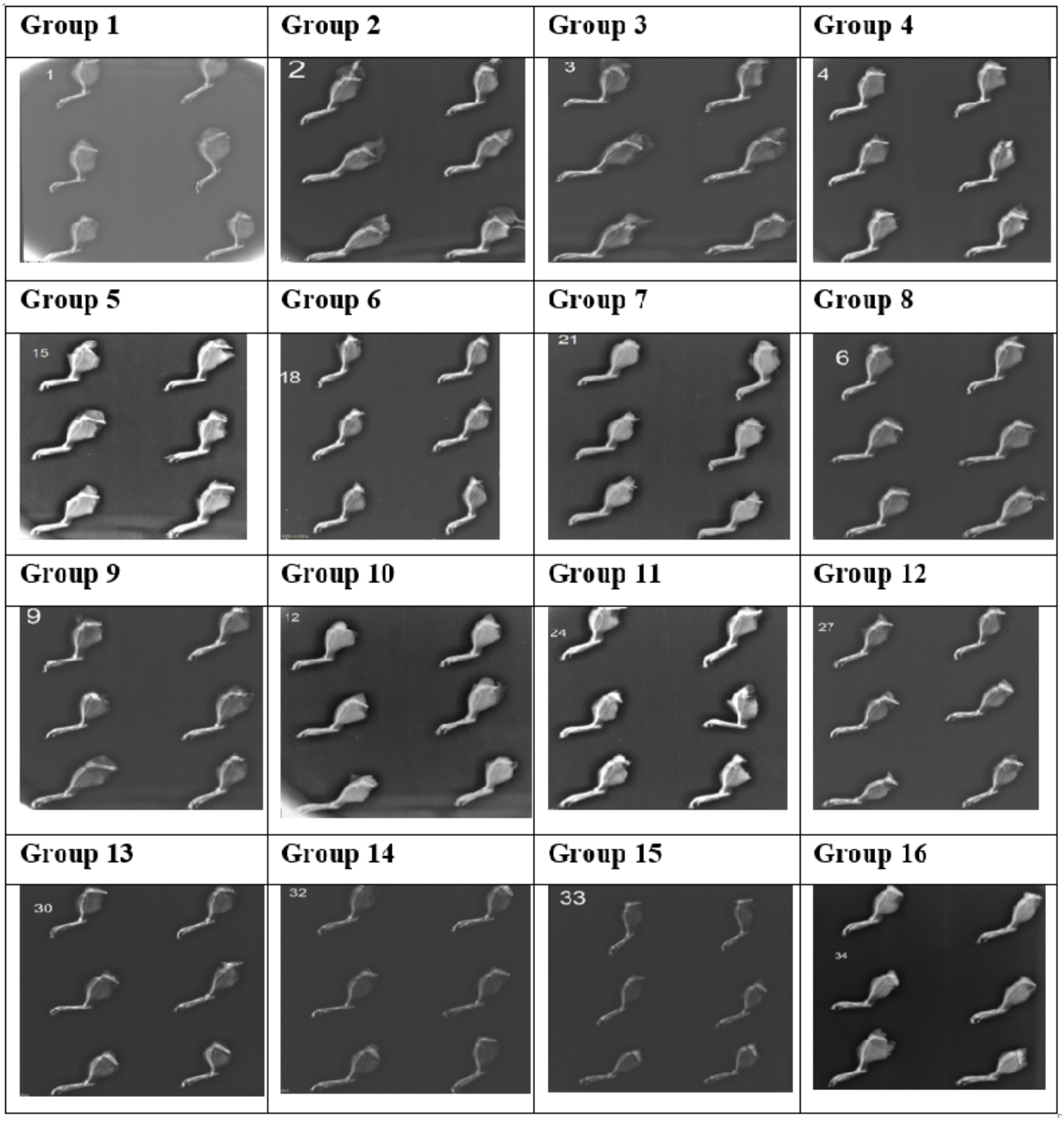

Figure 1: X-ray of rat joints.

DISCUSSION

Tail flick experiments were used to assess the extract's central effects in producing anti-nociception. The tests are also distinguished by their inclination to respond to pain stimuli provided via different brain pathways. The tail flick method requires more brain function and is thought to be a supraspinally organised reaction.[16,17] Flavones derived from various plants, such as quercetin and rutin, have been reported for Antinociception activity, it is possible that flavonoids are to accountable for the extract's analgesic activity.[18]

Arachidonic acid curved to Prostaglandin (PG) in the existence of cyclooxygenase COX. Different PG's (PGE2, PGD2, PGI2 (prostacyclin), and TxA2 (thromboxane A2), PGF2) ultimately leads to inflammation.[19] Inflammation in CFA-induced arthritis

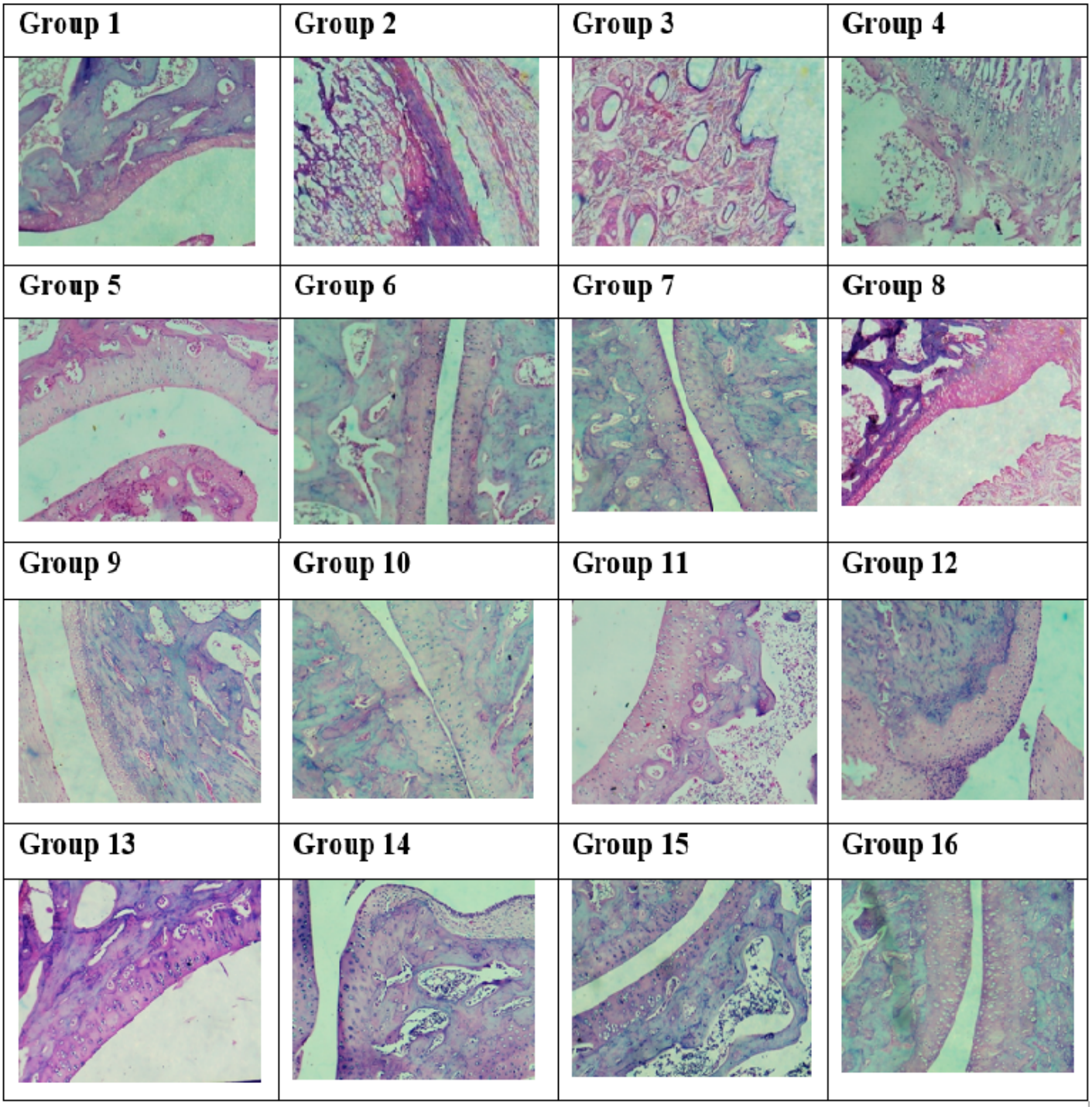

Figure 2: Histopathology.

occurs in two different phases, first is the acute inflammation which is a local reaction because of early lesions. Second phase starts after 14 days which is chronic inflammation with secondary lesions. Cytokines IL-1, histamine, TNF, prostaglandins, kinins and serotonin released in first phase drift in the affected region and cause edoema this leads to arthritis.[20] In rheumatoid arthritis, a number of autoantibodies, including Anti-Cyclic Citrullinated Peptide Antibody (ACPA), have been recognised as key biomarkers for tracking the development of bone and cartilage degradation. Certain genetic variables controlled the production of ACPA,[21] which is directly implicated in the synthesis of the cytokines needed to mediate the pathogenesis of rheumatoid arthritis. The principal inflammatory cytokine that affects rheumatoid arthritis disease development through the TNFR1 receptor is TNF.[22] The cytokines IL-1 and prostaglandins, kinins, TNF-α, histamine, and serotonin are few of the mediators released because of leukocytes when they travel to the damaged area and cause edoema, IL-6 is the main factor in the worsening

Table 7: Effect on movement (Using Arthritis Visual Score).

Groups ($n = 6$)	1st Day	5th Day	10th Day	15th Day	20th Day
Group 1 (Normal Control, Tween 80 (1% *v/v*) + 0.5% *w/v* CMC in Water (99% *v/v*))	0.00	0.00 ± 0.00[a] (100.00)	0.00 ± 0.00[a] (100.00)	0.00 ± 0.00[a] (100.00)	0.00 ± 0.00[a] (100.00)
Group 2 (CFA induced, Arthritic Control)	0.00	2.67 ± 0.52	4.50 ± 0.55	4.50 ± 0.55	4.50 ± 0.55
Group 3 (Methotrexate, 0.5 mg/kg)	0.00	2.83 ± 0.41 (-6.25)	4.33 ± 0.52 (3.70)	2.67 ± 0.52[a] (40.74)	2.17 ± 0.76[a] (51.85)
Group 4 (Prednisolone, 5 mg/kg)	0.00	2.67 ± 0.52 (0.00)	3.50 ± 0.55[c] (22.22)	2.17 ± 0.41[a] (51.85)	0.83 ± 0.75[a] (81.48)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	0.00	2.67 ± 0.52 (0.00)	4.33 ± 0.52 (3.70)	2.50 ± 0.55[a] (44.44)	2.50 ± 0.55[a] (44.44)
Group 9 (M.G., Ethanol, 150 mg/kg)	0.00	2.50 ± 0.55 (6.25)	4.67 ± 0.52 (-3.70)	2.33 ± 0.52[a] (48.15)	2.33 ± 0.52[a] (48.15)
Group 12 (M.G., Water, 150 mg/kg)	0.00	2.67 ± 0.52 (0.00)	4.67 ± 0.52 (-3.70)	2.83 ± 0.75[b] (37.04)	3.00 ± 0.63[c] (33.33)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	0.00	2.67 ± 0.52 (0.00)	4.50 ± 0.55 (0.00)	2.83 ± 0.41[b] (37.04)	2.33 ± 0.52 (48.15)
Group 9 (G.T., Ethanol, 150 mg/kg)	0.00	2.67 ± 0.52 (0.00)	4.67 ± 0.52 (-3.70)	3.50 ± 0.55 (22.22)	3.33 ± 0.82[c] (25.93)
Group 12 (G.T., Water, 150 mg/kg)	0.00	2.67 ± 0.52 (0.00)	5.00 ± 0.00 (-11.11)	3.50 ± 0.55 (22.22)	3.17 ± 0.75[c] (29.63)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.67 ± 0.52 (-3.70)	3.33 ± 0.52[c] (25.93)	2.67 ± 0.52[b] (40.74)
Group 9 (A.O., Ethanol, 150 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.83 ± 0.41 (-7.40)	3.17 ± 0.41[c] (29.63)	3.50 ± 0.55 (22.22)
Group 12 (A.O., Water, 150 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.50 ± 0.55 (0.00)	3.33 ± 0.52[c] (25.93)	2.83 ± 0.75[c] (37.04)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	0.0000	3.00 ± 0.00 (-12.50)	4.83 ± 0.41 (-7.41)	3.17 ± 1.47[c] (29.63)	3.00 ± 1.10[c] (33.33)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	0.0000	2.50 ± 0.55 (6.25)	4.67 ± 0.52 (-3.70)	2.83 ± 0.75[b] (37.04)	2.50 ± 0.55[a] (44.44)
Group 14 (Cassette, Water, 50 mg/kg of Each)	0.0000	2.67 ± 0.52 (0.00)	4.67 ± 0.52 (-3.70)	2.50 ± 0.55[a] (44.44)	2.50 ± 0.84[a] (44.44)
F Value		14.20	36.43	13.39	14.02
P Value		<0.0001	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

Table 8: Effect on Spleen Weight.

Groups (n = 6)	Spleen Weight (g)
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Water (99%v/v))	0.73 ± 0.08[c] (19.67)
Group 2 (CFA induced, Arthritic Control)	0.91 ± 0.08
Group 3 (Methotrexate, 0.5 mg/kg)	0.75 ± 0.09[c] (18.28)
Group 4 (Prednisolone, 5 mg/kg)	0.52 ± 0.08[a] (43.38)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	0.65 ± 0.10[b] (28.98)
Group 9 (M.G., Ethanol, 150 mg/kg)	0.69 ± 0.11[c] (24.66)
Group 12 (M.G., Water, 150 mg/kg)	0.70 ± 0.07[c] (23.04)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	0.73 ± 0.10 (20.34)
Group 9 (G.T., Ethanol, 150 mg/kg)	0.68 ± 0.05[c] (25.85)
Group 12 (G.T., Water, 150 mg/kg)	0.71 ± 0.11[c] (22.75)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	0.70 ± 0.14[c] (23.59)
Group 9 (A.O., Ethanol, 150 mg/kg)	0.71 ± 0.09[c] (22.09)
Group 12 (A.O., Water, 150 mg/kg)	0.73 ± 0.10[c] (20.20)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	0.76 ± 0.07[c] (16.35)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	0.73 ± 0.07[c] (20.69)
Group 14 (Cassette, Water, 50 mg/kg of Each)	0.67 ± 0.04[b] (26.98)
F Value	4.425
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

Table 9: Effect on RA Factor.

Groups (n = 6)	IU/mL
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Water (99%v/v))	7.60 ± 1.15[a] (65.51)
Group 2 (CFA induced, Arthritic Control)	22.03 ± 0.59
Group 3 (Methotrexate, 0.5 mg/kg)	13.57 ± 4.19[c] (38.43)
Group 4 (Prednisolone, 5 mg/kg)	11.17 ± 5.35[b] (49.32)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	12.37 ± 6.09[c] (43.87)
Group 9 (M.G., Ethanol, 150 mg/kg)	13.00 ± 2.44[c] (41.00)
Group 12 (M.G., Water, 150 mg/kg)	7.17 ± 1.79[a] (67.47)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	12.43 ± 1.58[c] (43.57)
Group 9 (G.T., Ethanol, 150 mg/kg)	14.20 ± 4.60[c] (35.55)
Group 12 (G.T., Water, 150 mg/kg)	17.53 ± 5.20 (20.42)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	9.70 ± 3.05[a] (55.98)
Group 9 (A.O., Ethanol, 150 mg/kg)	15.40 ± 3.65[c] (30.11)
Group 12 (A.O., Water, 150 mg/kg)	12.93 ± 7.01[b] (41.30)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	12.07 ± 5.76[c] (45.23)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	14.97 ± 3.91[c] (32.02)
Group 14 (Cassette, Water, 50 mg/kg of Each)	12.80 ± 4.42[c] (41.91)
F Value	4.328
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]P<0.0001, [b]P<0.001, [c]P<0.05 compared with negative control group. ns: non-significant.

process of the bones and cartilage.[23,24] Any of the intermediate can be the target for drug to produce the Anti-arthritic activity. Paw diameter and volume were assessed using a vernier calliper and both parameters demonstrated a substantial decrease in inflammation following standard and extract therapy. A primary serologic indicator of arthritis is Rheumatoid Factor (RF), an auto-antibody that targets the Fc region of IgG.[25] Serum Rheumatoid Factor (RF) is an immunoglobulin molecule that is regarded a "Non-self" molecule that can cause immune system reactions. Extracts in the form of cassette were observed with statistically significant reduction in inflammation and with sufficient synergistic effect. The current study's findings also show a strong correlation between the weight loss, the arthritic index and degree of inflammation.[26] Reversal of body weight was not significant with 150 mg/kg individual extract, but statistically significant reversal of body weight was observed with cassette dosing. Which again hints the synergistic effect of cassette. The most prevalent molecular target in the treatment of RA is the suppression of inflammatory cytokines.[27] Significant radiological changes, such as bone erosion and joint space constriction were reversed in treatment groups.

As the spleen weight gain is a classic indication of arthritis Immediately following the experiment, the animal's spleen was removed, and the organ's weight was recorded. It was determined how much the animal's organs weighed.[28]

The release of the IL-Ib inflammatory response induces a slight to moderate rise in WBC counts in arthritic situations. IL-Ib stimulates the generation of granulocytes as well as macrophage colony-stimulating factor.[29] WBC's count was decreased in treated groups which aging tells about the significant decrease in arthritic conditions. With fewer side effects and comorbidities,

Table 10: Effect on WBC's

Groups ($n = 6$)	Cells/cumm
Group 1 (Normal Control, Tween 80 (1% *v/v*) + 0.5% *w/v* CMC in Water (99% *v/v*))	6066.67 ± 1795.36[a] (58.06)
Group 2 (CFA induced, Arthritic Control)	14466.67 ± 750.56
Group 3 (Methotrexate, 0.5 mg/kg)	8200.00 ± 2271.56[a] (43.32)
Group 4 (Prednisolone, 5 mg/kg)	5033.33 ± 702.38[a] (65.21)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	8766.67 ± 680.69[c] (39.40)
Group 9 (M.G., Ethanol, 150 mg/kg)	7066.67 ± 2307.23[a] (51.15)
Group 12 (M.G., Water, 150 mg/kg)	8600.00 ± 2338.80[c] (40.55)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	8566.67 ± 1365.04[b] (40.78)
Group 9 (G.T., Ethanol, 150 mg/kg)	7666.67 ± 723.42[a] (47.00)
Group 12 (G.T., Water, 150 mg/kg)	8466.67 ± 1887.68[b] (41.47)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	4566.67 ± 1553.49[a] (68.43)
Group 9 (A.O., Ethanol, 150 mg/kg)	6133.33 ± 680.69[a] (57.60)
Group 12 (A.O., Water, 150 mg/kg)	5600.00 ± 1135.78[a] (61.29)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	8800.00 ± 3100.00[b] (39.17)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	6733.33 ± 2830.78[a] (53.46)
Group 14 (Cassette, Water, 50 mg/kg of Each)	9400.00 ± 1276.71[c] (35.02)
F Value	10.38
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a]p<0.0001, [b]p<0.001, [c]p<0.05 compared with negative control group. ns: non-significant.

Table 11: Effect on Joints in X-Ray.

Groups ($n = 6$)	mm
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Water (99%v/v))	6.29 ± 0.35 (38.43)
Group 2 (CFA induced, Arthritic Control)	10.21 ± 0.64
Group 3 (Methotrexate, 0.5 mg/kg)	8.32 ± 0.68[a] (18.52)
Group 4 (Prednisolone, 5 mg/kg)	8.29 ± 0.51[a] (18.86)
Group 6 (M.G., Petroleum ether, 150 mg/kg)	9.68 ± 0.49 (5.25)
Group 9 (M.G., Ethanol, 150 mg/kg)	9.15 ± 0.64[c] (10.41)
Group 12 (M.G., Water, 150 mg/kg)	9.42 ± 0.36 (7.75)
Group 6 (G.T., Petroleum ether, 150 mg/kg)	8.78 ± 0.48[b] (14.05)
Group 9 (G.T., Ethanol, 150 mg/kg)	8.34 ± 0.60[a] (18.37)
Group 12 (G.T., Water, 150 mg/kg)	9.54 ± 0.59 (6.61)
Group 6 (A.O., Petroleum ether, 150 mg/kg)	8.54 ± 0.57[a] (16.38)
Group 9 (A.O., Ethanol, 150 mg/kg)	8.83 ± 0.37[a] (13.53)
Group 12 (A.O., Water, 150 mg/kg)	8.32 ± 0.31[a] (18.51)
Group 12 (Cassette, Petroleum ether, 50 mg/kg of Each)	8.66 ± 0.44[a] (15.19)
Group 13 (Cassette, Ethanol, 50 mg/kg of Each)	9.03 ± 0.38[c] (11.60)
Group 14 (Cassette, Water, 50 mg/kg of Each)	8.99 ± 0.46[b] (11.95)
F Value	17.60
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

the natural substance has a major influence. Natural remedies could be used to overcome the limitations of currently available synthetic medicines.

CONCLUSION

At the end of this study, the conclusion that we can deduce is that *M.G., G.T.* and *A.O.* plant containing the synergistic effect at 50 mg/kg as cassette when compared with 150 mg/kg of individual plant in analgesic, anti-inflammatory and Anti-arthritic activities. *M.G., G.T.* and *A.O. In vivo* tests have been done to assess these qualities. These findings support the traditional use of these herbs together to treat chronic inflammatory disorders, and also suggest that they might be prospective candidates for the discovery of novel anti-inflammatory, analgesic and Anti-arthritic medicines.

ACKNOWLEDGEMENT

We would like to acknowledge all authors for idea, execution and implementation. We would also appreciate management for giving the opportunity to writing this manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Mahdi HJ, Khan NAK, Asmawi MZB, Mahmud R, A/L Murugaiyah VAL. *In vivo* anti-arthritic and Antinociceptive effects of ethanol extract of *Moringa oleifera* leaves on complete Freund's adjuvant (CFA)-induced arthritis in rats. Integr Med Res. 2018;7(1):85-94. doi: 10.1016/j.imr.2017.11.002, PMID 29629295.
2. Dayer JM, Oliviero F, Punzi L. A brief history of IL-1 and IL-1 Ra in rheumatology. Front Pharmacol. 2017;8:293. doi: 10.3389/fphar.2017.00293, PMID 28588495.
3. Hajja G, Bahlouli A. Medicinal plants in the prevention and treatment of rheumatoid arthritis. MOJBB. 2018;5(1):60-4. doi: 10.15406/mojbb.2018.05.00084.
4. Kala CP, Dhyani PP, Sajwan BS. Developing the medicinal plants sector in northern India: challenges and opportunities. J Ethnobiology Ethnomedicine. 2006;2(1):32. doi: 10.1186/1746-4269-2-32.
5. Al-Snafi AE. The pharmacological importance of A.O. A Review. Pharm Biol. 2015;5(2):93-8.
6. Kim BY, Cui ZG, Lee SR, Kim SJ, Kang HK, Lee YK, et al. Effects of A.O. Extracts on Liver Cell Toxicity and Ethanol Metabolism. J Food Sci. 2009;7(47):H204-8.
7. Khorasani A, Sani W, Philip K, Taha RM, Rafat A. Antioxidant and antibacterial activities of ethanolic extracts of A.O. cv. Mary Washington: Comparison of *in vivo* and *in vitro* grown plant bioactivities. Afr J Biotechnol. 2010;9(49):8460-6.
8. Anu AAP, Nandu TG, Shiburaj S, Rameshkumar KB. Antioxidant and antibacterial activities of *Garcinia* species in the Western Ghats. JNTBGRI. 2017.
9. Warriar P, Barve K, Prabhakar B. Anti-arthritic effect of garcinol enriched fraction against adjuvant induced arthritis. Recent Pat Inflamm Allergy Drug Discov. 2019;13(1):49-56. doi: 10.2174/1872213X12666181120091528, PMID 30457056.
10. Gao XM, Yu T, Cui MZ, Pu JX, Du X, Han QB, *et al*. Identification and evaluation of apoptotic compounds from *Garcinia oligantha*. Bioorg Med Chem Lett. 2012;22(6):2350-3. doi: 10.1016/j.bmcl.2012.01.068, PMID 22365754.
11. Raina AP, Misra RC. Chemical evaluation of *Mucuna* species for L-dopa content-an Anti-Parkinson's drug yielding medicinal plant from India. Indian J Trad Knowl. 2018;17(1):148-54.
12. Kunchit K, Ahnond B, Jarupa V, Monnipha S. The effects of seed extract of *Mucuna gigentean*. On the expression of neural markers in mesenchymal stem cells. J Med Plants Res. 2012;6(7):1297-303.
13. Nair V, Singh S, Gupta YK. Evaluation of disease modifying activity of *Coriandrum sativum* in experimental models. Indian J Med Res. 2012;135(2):240-5. PMID 22446868.
14. Matotoka MM, Masoko P. Phytochemical screening and pharmacological evaluation of herbal concoctions sold at Ga Maja Limpopo Province. S Afr J Bot. 2018;117:1-10. doi: 10.1016/j.sajb.2018.04.013.
15. Kumar S, Hitendra, Bhatia M, Garg LN, Gupta S. Acute and chronic inflammation studies of *Strobilanthes callosus* leaves extract on rat model. Inflammopharmacology. 2013;21(3):233-9. doi: 10.1007/s10787-012-0150-8, PMID 22983841.
16. Foroud M, Vesal N. Evaluation of the Antinociceptive effects of morphine, tramadol, meloxicam and their combinations using the tail flick test in rats. Vet Res Forum. 2015;6(4):313-8. PMID 26973767.
17. Wani TA, Kumar D, Prasad R, Verma PK, Sardar KK, Tandan SK, *et al*. Analgesic activity of the ethanolic extract of *Shorea robusta* resin in experimental animals. Indian J Pharmacol. 2012;44(4):493-9. doi: 10.4103/0253-7613.99322, PMID 23087512.
18. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci. 2016;5:e47. doi: 10.1017/jns.2016.41, PMID 28620474.
19. Giménez-Bastida JA, Shibata T, Uchida K, Schneider C. Roles of 5-lipoxygenase and cyclooxygenase-2 in the biosynthesis of hemiketals E2 and D2 by activated human leukocytes. FASEB J. 2017;31(5):1867-78. doi: 10.1096/fj.201601136R, PMID 28096231.
20. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204-18. doi: 10.18632/oncotarget.23208. PMID 29467962.
21. Rönnelid J, Turesson C, Kastbom A. Autoantibodies in rheumatoid arthritis-laboratory and clinical perspectives. Front Immunol. 2021;12:685312. doi: 10.3389/fimmu.2021.685312, PMID 34054878.
22. Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20(2):87-103. doi: 10.1615/critreveukargeneexpr.v20.i2.10, PMID 21133840.
23. Gabay C. Interleukin-6 and chronic inflammation. Arthritis Res Ther. 2006;8(Suppl 2);2:S3. doi: 10.1186/ar1917. PMID 16899107.
24. Carlson NG, Wieggel WA, Chen J, Bacchi A, Rogers SW, Gahring LC. Inflammatory cytokines IL-1 alpha, IL-1 beta, IL-6, and TNF-alpha impart neuroprotection to an excitotoxin through distinct pathways. J Immunol. 1999;163(7):3963-8. doi: 10.4049/jimmunol.163.7.3963, PMID 10490998.
25. Vinuesa CG, Sanz I, Cook MC. Dysregulation of germinal centres in autoimmune disease. Nat Rev Immunol. 2009;9(12):845-57. doi: 10.1038/nri2637, PMID 19935804.
26. Vijayalaxmi A, Bakshi V, Begum N, *et al*. Anti-arthritic and anti-inflammatory activity of beta caryophyllene against Freund's complete adjuvant induced arthritis in Wistar rats. Bone Rep. Recommendations. 2016;1:2.
27. Gou HX, Ye J, Wang YR, Xu XL, Shen QX, Xue JW, *et al*. The anti-inflammatory activity of Toonaciliatin K against adjuvant arthritis. BioMed Res Int. 2017;2017:9436280. doi: 10.1155/2017/9436280, PMID 29181410.
28. Cui X, Wang R, Bian P, Wu Q, Seshadri VDD, Liu L. Evaluation of anti-arthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. Artif Cells Nanomed Biotechnol. 2019;47(1):3391-8. doi: 10.1080/21691401.2019.1649269, PMID 31394949.
29. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. Int J Mol Sci. 2019;20(23):6008. doi: 10.3390/ijms20236008, PMID 31795299.

Cite this article: Kumar S, Srivastava P, Gupta S. Synergistic Effect of Different Extracts of *Asparagus officinalis* (Asparagaceae), *Garcinia travancorica* (Clusiaceae) and *Mucuna gigantea* (Fabaceae) Extracts on Analgesic, Anti-inflammatory and Antiarthritic Activities in Rats. J Young Pharm. 2023;15(2):264-77.