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To Evaluate Anti-Arthritic Potential of *Syzygium samarangense* Plant Extracts in Wistar Rats

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Abstract **Rheumatoid** arthritis (RA) is a long-lasting infection, which can distress many of the organs and tissues but mainly affect the joints. The occurrence of RA may relate to changes in various hormones, genetics as well as environmental features. The existing work was performed for the investigation of Anti-arthritic action of Methanolic extract of *Syzygium samarangense* leaves (MESL) as well as roots (MESR) in Freund's complete adjuvant (CFA) induced arthritis in rats. For this study, MESL & MESR were prepared and subjected to phytochemical analysis as well as tested against CFA injected RA in rats on two dosage levels of 100 as well as 200 mg/kg of weight of body. Various parameters such as body weight, paw volume, arthritis index, hematological, histopathological as well as antioxidant parameters were evaluated for anti- arthritic activity of extracts. MESL and MESR showed anti-arthritic activity in dose-related means, by decreasing the volume of paw along with increasing the weight, while comparing with control group. MESL (100 and 200mg/kg) and MESR (100 and 200mg/kg) exhibited significant anti-arthritic action through elevating the level of Hemoglobin, RBC as well as a declining number of Platelets, WBC, Erythrocyte Sedimentation Rate, C-Reactive Protein along with Rheumatoid Factor. The higher and lower doses of both extracts showed decreases in oxidative stress in dose-related means compared to control group. The result of histopathological analysis showed that MESL (100 and 200mg/kg) and MESR (100 and 200mg/kg) reduce ankle joint destruction. Results of different parameters also showed that MESL has higher potency to decrease arthritis compared to MESR.

Keywords Antioxidant, Freund's Complete Adjuvant, Jambu, Rheumatoid Arthritis, Rheumatoid Factor, *Syzygium samarangense*

1. Introduction

RA is a long-lasting as well as autoimmune ailment considered to have synovial inflammation, symmetric polyarthritis, hyperplasia, bone and cartilage injury [1]. Worldwide, about 1% of adult people have RA [2]. The causes of RA are unidentified till date, but it might be because of the stimulation of macrophages, inflammatory B cells or T cells which attack on the joint synovium and damage cartilage as well as joints [3, 4]. The inflamed cells liberate numerous mediators of inflammations as well as cytokines, which may create serious damage in tissues and in RA [5]. Also, T-cells start the immunological reaction as well as facilitate continuing synovitis to cause joint damage.

Though the numerous drugs available for the RA treatment [6], still newer medication is essential along with fewer adverse effects [3]. Available treatment, like nonsteroidal anti-inflammatory drugs (NSAIDs) [7], steroids [8], IL-1 antagonists [9], and TNF-α [10] have displayed restricted achievement for RA treatment. These treatments only regulate the acute RA, nevertheless their

role in long-lasting RA is disappointed. Furthermore, the said treatments are having side effects too such as cardiovascular risks, GIT turbulences as well as allergies [11]. Therefore, it is important to search for a new chemical entity which has a least side effect, easy availability and cheaper cost. Drugs from plant origin are considered as a comparatively safe and cost-effective option for treatment of any disease.

Syzygium samarangense known as Jambu, belonging to family Myrtaceae, is extensively cultured in the Pacific regions as well as the Asia [12]. They are also found in India, Indonesia, and Thailand particularly in Maharstra, Assam, Bihar along with coastline of western ghats [13]. The plant *S. samarangense* generally contains flavonoids, resorcinol derivatives, tannins, acylphloroglucinols, sterols, phenolic compounds, and terpenoids [14]. The plant also contains ellagic acid, gallic acid, botulin, squalene, sitosterol, lupeol, lupenyl stearate, β-sitosterol stearate, mixture of cycloartenol stearate, minerals and vitamins [15]. Different plant parts are effectively useful for the remedy in cold, cracked tongue, itches, diabetes and dysentery, [16,17]. Fruit of plant comprises carotene, vescalagin along with anthocyanin, which are used as anti-microbial, antioxidant, and hypoglycemic effects [18]. Leaves of plant comprise myricetin, epigallocatechin, stropopinine, aurentiacin that behave as immunomodulatory and anti-inflammatory [19,20]. The plant is also useful as an astringent, halt diarrhea and to treat fever [21]. The plant is generally consumed fresh as well as processed as wines, nata, jellies, jams and vinegar [22,23]. Plant also exhibits different pharmacological actions like antimicrobial, analgesic, anti-HIV, thrombolytic, antidiabetic, spasmolytic, hepatoprotective, cytotoxic, anticancer, anxiolytic, protease inhibitory effect and anthelmintic [24]. The plant having active phytochemical constituent flavonoid, which is reported to be useful as analgesic [20,23], immunomodulators [14,23], anti-inflammatory [14,20,23] and as antioxidants [13,20]. Form this scientific data, it can be determined that flavonoid might be an accountable phytochemical constituent for anti-arthritic action. Even *S. samarangense* does not show any adverse effect on fur and skin, mucous membrane, eyes, circulatory rate, respiratory rate, and CNS changes as well as autonomic changes or mortality while observed in the acute toxicity examination on dosage of 2gm/kg of weight of body. Therefore, the plant is harmless for the use as medicinal plant without any lethal effect as well as the LD50 of *S. samarangense* thus, projected to be more than 2000 mg/kg [20, 25].

For the study of RA, CFA-induced RA is an extensively used model and owns numerous features when shared with human RA [26]. This model has significant importance for pharmacological as well as pathophysiological regulations in swelling progressions, along with the assessment of anti-arthritic properties of medicines [27]. The model tracks a biphasic time progression, containing an acute local inflammation that diminishes later to 3 – 4 days and a chronic RA displays a relapsing-remitting progression afterwards of two weeks and can continue for numerous months [28]. Still knowledge about biphasic pattern in RA is unclear but it might be because of the early stimulus produced through CFA followed by the late hypersensitivity reaction.

The present experiment showed the anti-arthritic activity of MESL and MESR in CFA injected RA in rats. For RA induction, single dose of 0.1 ml of CFA was inserted intradermally into a footpad of left hind paw of rats. After the induction of arthritis MESL and MESR were examined on the dosage levels of 100 as well as 200 mg/kg of weight of body for various parameters such as weight, paw volume, diameter of joint, arthritis index, hematological, histopathological along with antioxidant parameters.

2. Materials and Methods

2.1. Materials

CFA was obtained from Sigma Aldrich. Diclofenac sodium was procured locally from medical store, at Bardoli, Methanol was procured from SRP chemicals, Surat.

2.2. Methods

2.2.1. Plant Collection and Authentication

Syzygium samarangens plant was procured from Surat, Gujarat. *Syzygium samarangens* was authenticated by Dr. B. R. Patel, Associate Professor of Botany, The Patidar Gin Science College, Bardoli, Dist. Surat, Gujarat. (Authentication No: 05/2022 Botany) on date 29th of January 2022.

2.2.2. Experimental Animal Approval

The Animal Study protocol (Protocol No. CPCSEA/SNLPCP/IAEC/22/01/129) was approved by Institutional Animal Ethics Committee (IAEC). The IAEC meeting was held on 22nd January 2022 at Shree Naranjibhai Lalbhai Patel College of Pharmacy, Umrakh, Gujarat.

2.2.3. Preparation of Plant Extract

For extract preparation leaves and root were shaded dry by infrequent shifting and made powder through grinder. Power of leaves as well as root were passed over sieve #40 then kept in the air tight vessels. After that powdered materials were refluxed with methanol for 3 hrs individually. Both the filtrates were evaporated differently in a water bath for the complete removal of methanol. Obtained both extracts were stored in air tight bottles separately [29].

2.2.4. Phytochemical Screening of *Syzygium samarangens*

The Methanolic extracts of leaves and root of *Syzygium samarangens* were used to perform different

phytochemical tests for confirming the presence of various phytochemical components [30].

2.2.5. CFA-induced Arthritis Model

Either sex of albino wistar rats (n=6) of weighing 200-250 gm was arbitrarily divided in seven groups.

Group 1 – Normal rats treated with normal feed and water ad libitum

Group 2 – Rats treated with 0.1 ml CFA (positive control group)

Group 3 – Rats treated with standard drug 20 mg/kg Diclofenac sodium

Group 4 – Rats treated with lower dose 100 mg/kg of MESL

Group 5 – Rats treated with higher dose 200mg/kg of MESL

Group 6 – Rats treated with lower dose 100 mg/kg of MESR

Group 7 - Rats treated with higher dose 200mg/kg of MESR

RA was induced via single intra-dermal vaccination of 0.1 ml of CFA into a footpad of the left hind paw of rats. Here 1ml CFA comprises 1mg of Mycobacterium tuberculosis (MB). For the RA induction, animals were firstly anesthetized by diethyl ether before injection of CFA, because the viscid nature of exerts, which causes trouble during administration. Anti-arthritic action of MESL as well as MESR was assessed in vaccinated paw through examination of parameters like body weight and paw volume on 0,7th,14th,21th and 28th day. On 28th day, blood was collected through retro-orbital puncture to estimate different hematological parameters such as WBC, RBC, Platelets, C-reactive protein, rheumatoid factor, Erythrocyte sedimentation rate,and hemoglobin [14, 20, 31, 32]. Histopathological examination was also carried out on ankle join of rats.

2.2.6. Determination of Arthritis Index [64]

On 28th day, the sternness of lesions was examined visually as well as scored as following scheme (Table 1).

AI for separate rat was evaluated as a summation of scores. And the mean scores of extracts treated rats were observed against the control group rats. Here for the scoring, animals were observed visually for the beginning of RA in exterior joints, ears, tail, as well as eyes. The rats scored individually and collective scoring of redness along with swelling was considered. The score for RA was from 0 to 4, where 0, 1, 2, 3 and 4 specified no swelling or erythema, minor swelling or erythema, moderate edema, pronounced edema by partial joint usage as well as higher edema with rigidity of joint respectively [32].

2.2.7. Determination of Anti-oxidant Parameters

On 28th day animals were terminated through cervical dislocation. Livers of animals were separated and cleaned with chilled saline. Homogenates of liver were made in 0.1M tris-HCL buffer of PH 7.4 and taken for determination of superoxide dismutase, glutathione as well as malondialdehyde.

2.2.8. Histopathological Analysis of Ankle Joints

On 28th day, ankle joint of hind paw of each animal was isolated and fixed them instantly in 4% neutral formalin. For histopathological analysis, ankle joint bone sections were taken by the predictable tissue preparation procedures. Prepared sections were observed using light microscope (45×) afterward the staining was done by hematoxylin and eosin for existence of inflammatory cells, formation of pannus, joint destruction and hyperplasia of synovium [32,34].

Table 1. Scoring Pattern of Arthritis Index

Organ	Indication	Score
Ears	Absence of nodules & redness	0
	Presence of nodules & redness	1
Nose	No swelling of connective tissue	0
	Intensive swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
Forepaws	Absence of inflammation	0
	Inflammation of at least one joint	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

2.2.9. Statistical Analysis

Statistical examination of obtained was performed the use of Graph pad Prism software 8. Data are articulated as mean ± SEM (Standard Error of Mean) for all experimental groups (n=6). Observed results were investigated by the use of one-way analysis of variance (ANOVA) followed by Dunnet's multiple evaluation test. $p<0.05$ was taken as consequential level [35].

3. Result

3.1. Extraction Yield

Extraction from the leaves and root of *Syzygium samarangens* was performed by maceration method followed by evaporation of methanol and we got the extraction yield of 18.6% W/W and 14% W/W respectively for *Syzygium samarangens* leaves and root.

3.2. Phytochemical Screening of *Syzygium samarangens* Extracts

Various phytochemical elements were present in methanolic extract of *Syzygium samarangens* leaves and root (Table 2).

3.3. Body Weight

Animals' weight that was recorded just before the CFA induction can be considered as 0 day and thereafter on days 7th, 14th, 21st and 28th. The result showed that weight of animals was decreased after the induction of RA as in comparison of the normal group rats. After the treatment with MESL (100mg/kg; 246 ±0.58 and 200 mg/kg; 248 ±0.61), MESR (100 mg/kg; 238 ±0.422 and 200 mg/kg; 242 ±0.543) as well as diclofenac sodium the body weight of animals was increased while compared to only CFA treated rats (212 ±0.44) on 28th day (Table 3).

Table 2. Phytochemical screening of *Syzygium samarangens* extracts

Sr.no.	Phytochemical constituents	Methanolic leaves extract	Methanolic root extract
1	Alkaloids	-	-
2	Carbohydrates	-	-
3	Glycosides	-	-
4	Flavonoid	+	+
5	Phenolic compound	+	+
6	Saponins	+	-
7	Steroids	-	+
8	Steroidal saponin	-	+
9	Terpenoids	+	+

Table 3. Body weight of animals after the treatment with MESL and MESR

Sr. No.	Groups	Day 0 (gm)	Day 7 (gm)	Day 14 (gm)	Day 21 (gm)	Day 28 (gm)
1.	Normal	248 ±0.61	250 ±0.65	253 ±0.73	254 ±0.61	256 ±0.57
2.	Control	248 ±0.76#	235 ±0.76#	229 ±0.58#	220 ±0.44#	212 ±0.44#
3.	20 mg/kg Diclofenac Sodium	248 ±0.60*	233 ±0.98*	237 ±1.05*	244 ±0.47*	247 ±0.92*
4.	MESL (100 mg/kg)	251 ±0.67*	228 ±0.94*	237 ±0.70*	241 ±0.53*	246 ±0.58*
5.	MESL (200 mg/kg)	253 ±0.66*	234 ±0.94*	239 ±0.792	244 ±0.75*	248 ±0.61*
6.	MESR (100 mg/kg)	249 ±0.63*	230 ±0.55*	231 ±0.54*	236 ±0.41*	238 ±0.42*
7.	MESR (200 mg/kg)	250 ±0.68*	234 ±1.13*	236 ±0.74*	239 ±0.65*	242 ±0.53*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

3.4. Paw Volume

It was evaluated through plethysmometer (UGO BASILE) prior to CFA injection and thereafter on days 7th, 14th, 21th and 28th. Result showed the consequential ($P < 0.05$) decreases in paw volume after the treatment of different extracts along with standard drug compared to control animals. Diclofenac 20 mg/kg displayed a consequential decrease in paw volume from 14th day afterward (Table 4).

3.5. Arthritic Index

Arthritis in all groups except normal, exhibited a significantly higher arthritic index after the treatment with CFA. Treatment with diclofenac sodium, MESL and MESR (100 mg/kg and 200 mg/kg) consequential ($p<0.05$) reduced arthritic index in rats n comparison to arthritic control (4.01±0.047). After the treatment diclofenac (1.71±0.06), MESL100 mg/kg (2.97±0.61), MESL200 mg/kg (1.91±0.061) and MESR 100 mg/kg (3.01±0.149), MESR 200 mg/kg (2.66±0.160) reduced arthritic score respectively (Table 5).

Table 4. Paw volume of animals after the treatment with MESL and MESR

Sr. No.	Groups	Day 0 (ml)	Day 7 (ml)	Day 14 (ml)	Day 21 (ml)	Day 28 (ml)
1.	Normal	2.18±0.003	2.16±0.015	2.19±0.006	2.19±0.007	2.19±0.008
2.	Control	2.19±0.004#	2.80±0.006#	3.30±0.005#	3.41±0.006#	3.48±0.003#
3.	20 mg/kg Diclofenac Sodium	2.19±0.005*	2.56±0.006*	2.49±0.006*	2.38±0.158*	2.30±0.013*
4.	MESL (100 mg/kg)	2.20±0.006*	2.52±0.008*	2.47±0.006*	2.44±0.006*	2.37±0.006*
5.	MESL (200 mg/kg)	2.20±0.006*	2.50±0.006*	2.44±0.007*	2.39±0.007*	2.36±0.006*
6.	MESR (100 mg/kg)	2.20±0.006*	2.50±0.004*	2.47±0.004*	2.46±0.004*	2.39±0.004*
7.	MESR (200 mg/kg)	2.20±0.006*	2.53±0.008*	2.49±0.006*	2.42±0.006*	2.33±0.010*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

Table 5. Arthritic Index Score after the treatment with MESL and MESR

Sr. No.	Groups	Arthritic Index
1.	Control	4.01±0.047
2.	20 mg/kg Diclofenac Sodium	1.71±0.060*
3.	MESL (100 mg/kg)	2.97±0.061*
4.	MESL (200 mg/kg)	1.91±0.115*
5.	MESR (100 mg/kg)	3.01±0.149*
6.	MESR (200 mg/kg)	2.66±0.160*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control.

3.6. Hematological & Serum Parameters

3.6.1. Red Blood Cells (RBC)

After the treatment with CFA, there was a decrease in the number of RBC in all the animals. But the number of RBC was significantly increased after the treatment with standard as well as different doses of MESL and MESR (Table 6).

3.6.2. Erythrocyte Sedimentation Rate (ESR)

Results of ESR showed that sedimentation rate was raised in RA control animals in comparison to normal animals. Diclofenac, MESL as well as MESR showed suggestively ($p<0.05$) decreased ESR (Table 7).

3.6.3. White Blood Cells (WBC)

Result of WBC count showed that treatment with MESL, MESR as well as Diclofenac sodium considerably ($p<0.05$) decreased the WBC count. Where Arthritic control raised WBC (14.3±0.06). Treatment with Diclofenac sodium decreased WBC count (8.4±0.05), MESL 100 and 200mg/kg (13.28±0.17 and 12.75±0.28) as well as MESR 100 and 200 mg/kg showed (13.31±0.284 and 13.06±0.108) respectively. MESR showed less activity compared to MESL (Table 8).

Table 6. Red Blood Cells count after the treatment with MESL and MESR

Sr. No.	Groups	RBC (10^6 cells/mm^3)
1.	Normal	6.76±0.049
2.	Control	4.48±0.030#
3.	20 mg/kg Diclofenac Sodium	6.20±0.073*
4.	MESL (100 mg/kg)	4.70±0.036*
5.	MESL (200 mg/kg)	5.38±0.047*
6.	MESR (100 mg/kg)	5.11±0.033*
7.	MESR (200 mg/kg)	5.73±0.049*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

Table 7. ESR level after the treatment with MESL and MESR

Sr. No.	Groups	ESR levels(mm/hr)
1.	Normal	1.55±0.042
2.	Control	5.98±0.087#
3.	20 mg/kg Diclofenac Sodium	3.16±0.042*
4.	MESL (100 mg/kg)	4.33±0.055*
5.	MESL (200 mg/kg)	3.76±0.096*
6.	MESR (100 mg/kg)	4.73±0.033*
7.	MESR (200 mg/kg)	4.21±0.047*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

Table 8. White Blood Cells count after the treatment with MESL and MESR

Sr. No.	Groups	WBC level (cells/mm^3)
1.	Normal	7.15±0.076
2.	Control	14.05±0.076#
3.	20 mg/kg Diclofenac Sodium	8.00±0.057*
4.	MESL (100 mg/kg)	13.28±0.177*
5.	MESL (200 mg/kg)	12.75±0.281*
6.	MESR (100 mg/kg)	13.31±0.284*
7.	MESR (200 mg/kg)	13.06±0.108**

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

3.6.4. Haemoglobin (Hb)

Arthritic control group showed a decreased Hb level by 10.75±0.04. But after the treatment with MESL (100 and 200 mg/kg) and diclofenac Hb level was significantly ($p<0.05$) increased to 12.05±0.067 and 13.70±0.055 as well as 14.1±0.06 respectively (Table 9).

3.6.5. Platelets

Platelets in arthritic control (1481±0.666) increased which on treatment with MESL, MESR and Diclofenac sodium decreased significantly ($p<0.05$) (Table 10).

3.6.6. Rheumatoid Factor (RF)

RF in control animals increased up to 56±1.33 IU/ml. RF was remarkably ($p<0.05$) reduced in treatment groups. Decrease in RF level in MESL 100mg/kg (52.83±0.307) and 200mg/kg (51.59±0.33) gave promising result for arthritis treatment. Standard treatment drug Diclofenac sodium reduced RF level to (32±0.5) (Table 11).

Table 9. Heamoglobin level after the treatment with MESL and MESR

Sr. No.	Groups	Hb level (g/dL)
1.	Normal	14.56±0.088
2.	Control	10.75±0.042#
3.	20 mg/kg Diclofenac Sodium	14.10±0.068*
4.	MESL (100 mg/kg)	12.05±0.067*
5.	MESL (200 mg/kg)	13.70±0.055*
6.	MESR (100 mg/kg)	11.18±0.074*
7.	MESR (200 mg/kg)	12.11±0.044*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

Table 10. Platelets count after the treatment with MESL and MESR

Sr. No.	Groups	Platelets level (10^3 cells/mm^3)
1.	Normal	902±0.600
2.	Control	1481±0.666#
3.	20 mg/kg Diclofenac Sodium	1198±0.577*
4.	MESL (100 mg/kg)	1321±33.037*
5.	MESL (200 mg/kg)	1133±22.604*
6.	MESR (100 mg/kg)	1363±20.693*
7.	MESR (200 mg/kg)	1220±22.51*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

Table 11. Rheumatoid Factor (RF) level after the treatment with MESL and MESR

Sr. No.	Groups	RF level (IU/ml)
1.	Normal	14.83±0.477
2.	Control	56.50±1.335#
3.	20 mg/kg Diclofenac Sodium	32.66±0.557*
S	MESL (100 mg/kg)	52.83±0.307*
5.	MESL (200 mg/kg)	51.59±0.333*
6.	MESR (100 mg/kg)	53.66±0.210*
7.	MESR (200 mg/kg)	52.92±0.816*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

3.6.7. C-reactive Protein (CRP)

CRP level in control group is increased to 7.31±0.04 mg/lit. Treatment with diclofenac (3.11±0.04) as well as MESL (100mg/kg (6.30±0.24) and 200mg/kg (5.78±0.26)) significantly ($p<0.05$) decreased CRP level (Table 12).

3.7. Antioxidant Properties

Superoxide dismutase (SOD), Reduced glutathione (GSH) and Malonaldehyde (MDA) are measured after day 28. Oral administration of MESL and MESR considerably ($p<0.05$) again established the decreased level of SOD along with GSH, maybe through opposing the scavenging properties of free radicals. Treatment with MESL and MESR remarkably ($p<0.05$) diminished the level of MDA in RA animals in comparison to control animals, telling the healing probability of plant extract (Table 13).

3.8. Histopathological Analysis of Ankle Joints

Histopathological examination displayed the alterations in normal ankle joint as well as the joints of CFA injected rats. In this work, histopathological examination of hind paw joints of RA control rats displayed projecting aberrations such as bone destruction as well as widespread infiltration of the cells at the articular surface. Treatment with MESL and MESR showed the mark decreases in all above-mentioned situations, representing their antiarthritic activity.

Table 12. C-reactive Protein (CRP) level after the treatment with MESL and MESR

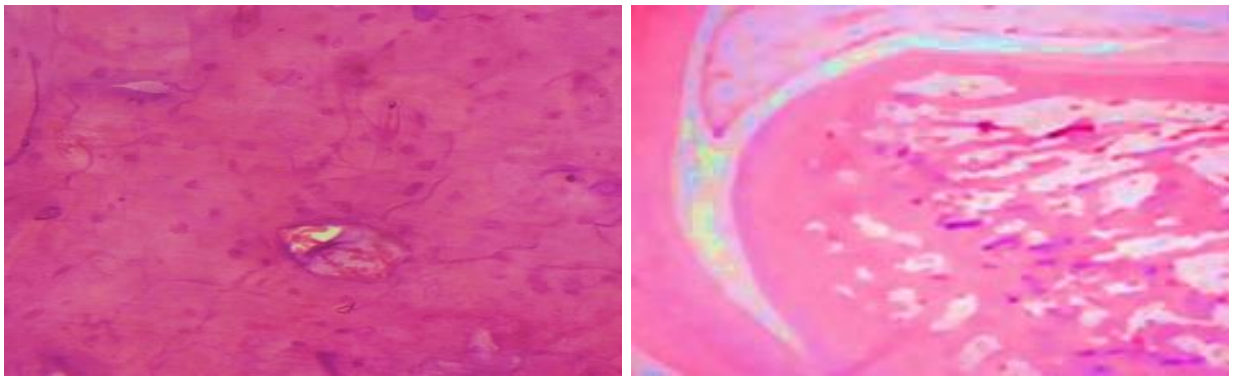
Sr. No.	Groups	CRP level (mg/lit)
1.	Normal	2.38±0.030
2.	Control	7.31±0.047#
3.	20 mg/kg Diclofenac Sodium	3.11±0.047*
4.	MESL (100 mg/kg)	6.30±0.243*
5.	MESL (200 mg/kg)	5.78±0.240*
6.	MESR (100 mg/kg)	6.53±0.299*
7.	MESR (200 mg/kg)	6.39±0.191*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.

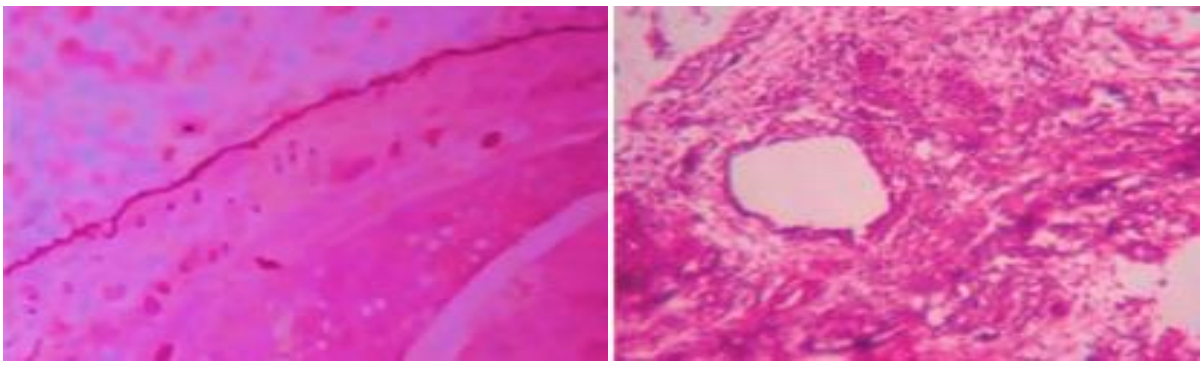
Table 13. Antioxidant properties of MESL and MESR

Groups	SOD (Units/mg protein)	GSH (µg GSH/mg protein)	MDA (nmol /mg protein)
Normal	4.2±0.049	74.98±0.047	2.05±0.042
Control	2.45±0.042#	42.96±0.049#	3.70±0.004#
20 mg/kg Diclofenac Sodium	3.50±0.036*	64.20±0.073*	2.71±0.030*
MESL (100 mg/kg)	2.90±0.036*	54.86±0.333*	3.08±0.040*
MESL (200 mg/kg)	3.20±0.036*	69.08±0.371*	2.48±0.155*
MESR (100 mg/kg)	2.86±0.204*	49.51±0.166*	3.43±0.055*
MESR (200 mg/kg)	3.06±0.111*	61.04±0.377*	3.21±0.040*

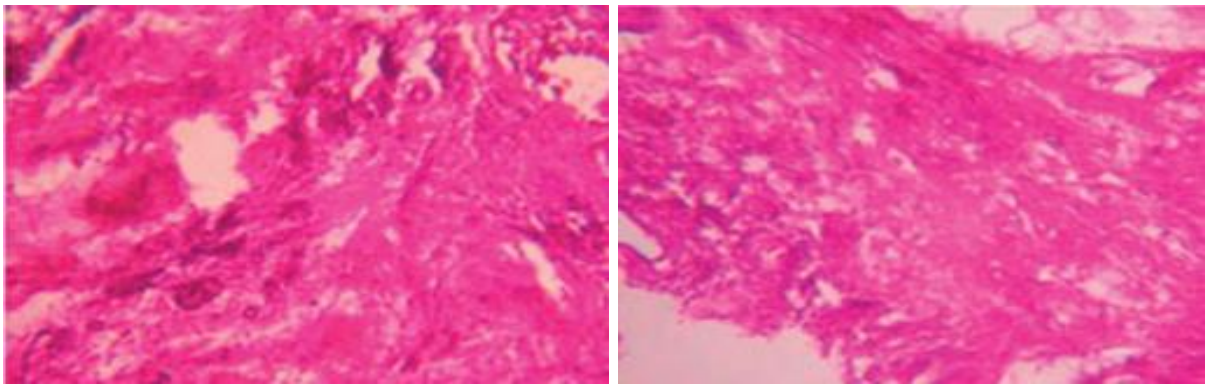
Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # P<0.05 as compared to Normal.



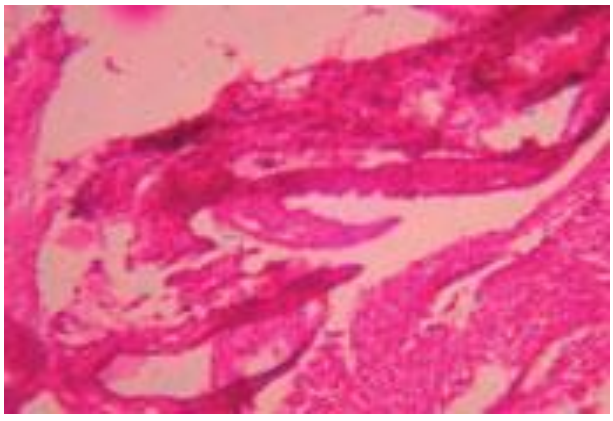
A) Normal B) Control



C) Standard D) MESL 100



E) MESL 200 F) MESR 100



G) MESR 200

Figure 1. Histopathological analysis of ankle joints

4. Discussion

RA is a long-lasting inflammatory ailment disturbing around 1% of the residents in advanced countries [1]. Infiltration of Inflammatory cell, limb swelling, bone erosion, proliferative synovitis as well as cartilage erection are common clinical features of arthritis in human [36] and CFA injected RA in rats. Because of this resemblance in pathologic conditions, the CFA injected RA in rats is a commonly used model for RA to assess the effectiveness of antiarthritic drugs. Currently, as a medication of RA non-steroidal anti-inflammatory drugs (NSIADs), disease modifying anti rheumatic drugs (DMARDs) and corticosteroids are used, which has meant for postponing structural abnormalities of RA as well as regulating the pain [37]. But still these medications are having serious adverse effects as well as more cost.

Nowadays, to treat the RA, alternative medicines are receiving more popularity. Numerous herbal plants give symptomatic relaxation whose effects are equivalent to that of available conservative medicines. And from these plants, new semi-synthetic and natural medicinal compounds are being introduced in market to rectify as much of synthetic medications adverse effects and to deliver a promising relief in particular diseases, which has already been in use from ancient times as "Ayurveda"- the home of Indian medicinal directory [38].

Several screening models are useful for the learning of RA pathogenesis [39]. Significant measures for the selection of model comprise: 1) effectiveness of the agents for induction of RA, 2) easy to execution, data reproducibility, length of examination period as well as 3) comparable pathogenesis and pathology resemblance to the RA in humans. CFA induced arthritis in animal is an advantageous means to identify the pathophysiology of RA, due to the observation of same signs and symptoms in human as well as animals. Therefore, the current work discovered that intraplanter injection of CFA containing MB induced inflammation and arthritic lesion between 8 to 21 days in animal. CFA contains deactivated as well as dry MB homogenized into the liquid paraffin, which efficiently inspires cell intermediated protection and finally causes production of immunoglobulin. CFA injected RA displays marked inflammation in the hind paw that can last for a week at a place with prostaglandin secrets [26].

Here, the treatment of MESL (100 and 200 mg/kg) as well as MESR (100 and 200 mg/kg) displayed anti-arthritic outcome for the inflammatory parameters by reducing them significantly in competition to control group.

This study showed that animals of a control group reduced their weight as compared to normal group animals. This may be due to raise in a cytokine hormone like leptin after the induction of CFA that may cause decrease in consumption of feed and weight loss occurs [40]. In RA reduction in body weight occurs and due to that physical activity is declined along with decreased in strengthen in muscle as well as everyday routine. The body weight gains may due to the resorption of 14 C-glucose as well as 14 C-leucine in animal's intestine, which reduces during inflammation. But with the help of anti-inflammatory medicines, this problem can be decreased and intestine regains its capacity of absorption. Here after treatment with MESL and MESR (100 and 200 mg/kg) weight of animals gets improved compared to control group.

Paw volume is useful for the assessment of grade of inflammation. Here after the administration of MESL and MESR, paw volume was decreased that might be because of extracts immunity, avoiding systemic spread as well as decreasing the destruction of joint rats. Studies are also reported that chronic infection occurs due to inflammatory mediators like prostaglandins F (PGDF), granulocyte macrophage colony stimulating factor (GM-CSF), interferons as well as cytokines and causes ache and deterioration of bone as well as cartilage with serious damage [41]. Here paw volume of rats was increased after CFA administration. But after the treatment with MESL and MESR (100 and 200 mg/kg) there was a decrease in paw volume of animals.

This experiment shown that ESR was raised in control animals' comparative to normal animals. It may be because of the increased level of fibrinogen and α, β-globulin, as well as elevated ESR. Acute phase protein (APP) in ESR shows the same property for elevated inflammation or stress like operation as well as tissue death [42], wound and injection. In this work, treatment with MESL and MESR (100 and 200 mg/kg) displayed reduction of ESR. The capability of MESL and MESR to decrease the ESR might be due to the presence of flavonoid because they concerned about inflection of pro-inflammatory expression of gene, as well as reduces in swelling of joints [43].

Increase in WBC in RA may be because of increase in respective colony stimulating factors cascading numerous cytokines [44]. But after the treatment with different doses of different extracts the WBC count decreased in comparison to only CFA induced group. In RA as erythropoietin gets decreased, Hb level also declined. The reduced action of bone marrow erythropoietin as well as premature demolition of RBC reasons for decreased count of RBC in RA [45]. Here after the treatment with MESL and MESR (100 and 200 mg/kg) RBC count as well as Hb level was increased compared to CFA treated control group. RA induces thrombocytosis yielding in increased platelets production associated with active intravascular coagulation and thickening of blood. This hematological alteration is balanced by MESL and MESR (100 and 200 mg/kg) as well as diclofenac sodium by decreasing platelet levels [46].

The measure of serum RF has a straight connection with inflammation development. Here, the noticeable rise in the amount of RF was observed in control group, which was considerably decreased on treatment with MESL and MESR. The anti-arthritic action of extract might be

because of formation of autoantibodies for CFA fragment and thus protecting the breakage of cartilage.

The increase in CRP level showed inflammation in body. CRP encourages the excretion of inflammatory mediators like cytokines (IL-1b, IL-6) as well as TNF-α [47]. Here, after the treatment with MESL and MESR (100 and 200 mg/kg), level of CRP gets decreased in relation to the arthritic control group rats. Reports have also revealed that the CRP level has a straight connection with RA growth along with the severity of ailment like RF [48].

GSH acts as a defense mechanism in contrast to injury produced by reactive oxygen species as well as organic peroxides, which reduce the oxidoreduction procedures. The production of oxygen free radicals during progression of arthritis causes reduction at levels of SOD and GSH, because of their ingestion at a time of lysis of cell as well as in oxidative stress [44]. The decreased GSH level in liver of RA rats may also be because of unnecessary ingesting of GSH through a body for the protection of oxidative impairment. But after the treatment with MESL and MESR (100 and 200 mg/kg) the GSH and SOD value gets raised.

Increased MDA level was observed in arthritic rats during the study, showing the augmented oxidative stress in this disease. Increased MDA level in only CFA injected rats is related to the injury facilitated by free radicals. MDA is a biomarker which might provide evidence on the general MDA level of cell. Scientific data displays that oxidative injury stimulated by ROS is a vital tool that causes damaging and proliferative synovitis and articular degradation [31, 49]. Again, decreased level of antioxidants is a crucial parameter of RA that can worsen the condition. Here, after the treatment with extracts the level of MDA gets decreased which showed the curative effect of plan extracts.

Thus, MESL and MESR may repress COX-1 and COX-2. But the plant may have more repressing probable to COX-2. The plant extracts might also be decreased lipooxygenase (LOX) and suppressed synthesis of leukotriene as well as hemolysis of RBC. Even treatment with S. samarangense may meaningfully reduce TNF-α, NF-κB p65, as well as IL-6 expression in comparison to control group in dose dependent manner. By this molecular mechanism, MESL & MESR might prevent the arthritis in rats [50, 51].

Apart from this, anti-arthritis activity of MESL and MESR (100 And 200 mg/kg) was confirmed through histopathological examination. MESL & MESR treatment decreased the edema formation, cellular infiltration as well as inflammation RA rats. The anti-inflammatory efficacy of syzygium samarangense leaves and roots extract showed a decreased level of edema along with prohibited development of RA in rats. Therefore, the results of experiment suggest that MESL & MESR may cure the arthritis as well as reduce the inflammation along with destruction of joints in RA animals.

5. Conclusions

Presented data indicates that administration of the MESL (100 and 200 mg/kg) and MESR (100 and 200 mg/kg) in rats showed anti-arthritic activity while evaluated for various parameters like weight of body, paw volume, AI, hematological, histopathological as well as antioxidant parameters. Results suggested that MESL and MESR deliver pharmacological justification for the folk remedied of plant in contrast to RA. From above obtained data, it can be summarized that the MESL having more potent anti arthritic action might be because of the optimum phytochemicals. However, MESR showed slightly less anti-arthritic activity compared to MESL. Though the extracts having anti-arthritic activity, still there is a need to conduct further study for isolation of the precise phytoconstituents that may be accountable for the strong anti-arthritic action.

Conflict of Interest

The authors hereby declare no interest in the given field.

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