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Pedalium Murex Ethanolic Seed Extract Loaded Phytoniosomes Mitigates Monosodium Urate – Induced Gouty Arthritis in Rats

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KEYWORDS

Gouty Arthritis, Pedalium murex, Niosomes, Monosodium Urate Crystals, Hematological Parameters, Biochemical Parameters

ABSTRACT:

The current investigation was carried out with the aim to investigate the anti gout arthritic activities of the ethanolic seed extract Pedalium murex (ESEPM)of and the ethanolic seed extractof Pedaliummurex loaded niosomes nESEPM in monosodium urate crystals induced gouty arthritic rats. The animals were pretreated with ESEPM and nESEPM (200 mg/kg b.w) for 10 days and gouty arthritis was induced on the 10thday.MSU crystals were infused into the synovial region on both sides of the ankle joint at a concentration of 80 mg/ml. The degree of protection by the ESEPM and nESEPM against the development of arthritis was evaluated by ankle swelling. The hematological and biochemical parameters were measured. The histopathological evaluation of the synovial membrane was also performed. In the current study the ESEPM and nESEPM drug treated rats exhibited low degree of ankle swelling compared to the MSU crystal induced arthritic rats. There was a significant reduction in the WBC cells and ESR levels as well as enzymes such as ALT, AST and xanthine oxidase was observed in MSU crystal induced and the ESEPM and nESEPM drug treated rat groups comparable to the MSU crystal induced rat groups. Histopathological investigations are also in support of the protective nature of the ESEPM and nESEPM extract which shows the formation of less lesions in the synovial membrane in the drug treated group when comparable to MSU crystal induced groups.

Introduction

Gout is a complex disease that impacts joint flexibility. It is typically defined by repeated episodes of acute inflammatory arthritis, which presents as red, tender, hot, and swollen joints resulting in bursitis[1]. Gout is defined by elevated levels of uric acid in the body, leading to the development and accumulation of urate crystals, commonly referred to as tophi crystals, in joints, tendons, and nearby tissues. This condition is associated with hyperuricemia and in its chronic phase, it can potentially result in renal failure[2]. These crystals can cause an abrupt inflammatory response and result in long-term tissue damage, such as joint cartilage ulcers, osteophytosis, geodic,erosive lesions, and chronic inflammation of the synovial membrane. Severe joint inflammation is caused by the growth of uric acid crystals in the joints, leading to the release of inflammatory factors and cellular components that are crucial in the development of gout. Deposition of MSU crystals in the tissues leads to the activation of NF-κB[3]. Activation of NF-κB in the inflammasome signaling pathway results in the secretion of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) interleukin IL-4 and IL-2. Cytokines activate monocytes and neutrophils, causing

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inflammation in the synovial membrane of the joint, which results in severe joint pain, redness, swelling, and dysfunction. Prolonged and severe inflammation can result in tissue damage, leading to cartilage degeneration and joint damage[4].

An essential enzyme that contributes to hyperuricemia is xanthine oxidase, which catalyzes the conversion of hypoxanthine to uric acid. The main approaches to treating gouty arthritis include reducing inflammation and controlling elevated uric acid levels. Since xanthine oxidase inhibitors successfully reduce plasma and urine urate levels, they are essential in the treatment of hyperuricemia. Currently, anti-inflammatory medications including NSAIDs, and corticosteroids are used in clinical practice to treat gout, while allopurinol, a Xanthine oxidase inhibitor, is used to lower uric acid content. However, each of these therapies comes with a list of adverse effects, including liver and gastrointestinal damage. Food ingredients that inhibit the function of xanthine oxidase can reduce the production of uric acid and reduce inflammation. Plants are able to produce a wide variety of chemicals, and these molecules perform crucial biological tasks. These plant-based chemical compounds have positive effects on chronic illnesses[5].In today's competitive world, where conventional medicines have few documented adverse effects and governed the ancient era with highly helpful pharmacological actions, the use of herbal remedies is imperative.

Pedalium murex, often known as Gokhru (Yanai Nerunjil), is one of the most beneficial medicinal plants. This little herb, which is a member of the *Pedaliaceae* family, is found in coastal regions of south India, tropical Africa, and Mexico. The fruits are abundant in soluble proteins, glycosides such as sapogenin (diosgenin-0.06%), and polyphenolics such as flavonoids and phenolics[6]. *Pedalium murex* fruits play a significant role in the treatment of diabetes, gonorrhea, and urinary calculi, according to Patel et al.[7]. *Pedalium murex* has been found to contain pedalitin (5,6,3,4-tetrahydroxy-7-methoxyflavone), a flavonoid molecule, according to Subramanian et al.[8] Zafar and Gupta[9]reported the flavones dinatin (5,7,4-trihydroxy-6-methoxy flavones) that were extracted from the fruit of *Pedalium murex*. Niosomes are a type of self-assembling vesicular nanocarriers that are created when cholesterol, non-ionic surfactant, or other compounds are hydrated. These nanocarriers can be used for targeted or topical medication administration[10]. Herbal extracts and phytochemicals have been successfully encapsulated using the phytosome method, showing excellent outcomes in both in-vitro and in-vivo pharmacokinetic investigations[11]. The goal of the current study is to assess the preventative effects of ESEPM and nESEPM pre-treatment on the onset of gouty arthritis in rats produced by MSU crystals.

Materials and methods

Chemicals

The uric acid was acquired from Merck Millipore Company. Hemoglobin, glucose, cholesterol, liver enzymes, and uric acid levels were determined using Ranbaxy kits. Chemicals including ethanol, Tween 60, chloroform, cholesterol, dimethyl sulfoxide (DMSO), PBS, and other analytical chemicals were obtained from SRL Pvt Ltd.

Obtaining and preparing an ethanolic seed extract of *Pedalium murex* (ESEPM)

The seeds of *Pedalium murex* were gathered from Pondicherry.The sample was rinsed 2-3 times with distilled water and then air-dried in the shade for 7-14 days. The identification was confirmed by the Professor in department of Pharmacognosy, Dr.Mythreyi from schoolof pharmaceutical sciences,Chettinad Academy of Research and Education, Chennai, India. The same seed was placed in the specified centre as a voucher specimen for future use. Theethanolic seed extract of *Pedalium murex* (ESEPM) was obtained by soaking the seeds in 90% ethanol for three days at room temperature with intermittent shaking using the cold maceration technique. The extracts were filtered, and the excess solvent was removed using vacuum evaporation. The dry yield of ESEPM was calculated to be 0.94% (w/w).

Preparation of ethanolicseed extract of *Pedalium murex* loaded phytoniosome (nESEPM)

Pedalium murex phytoniosomes (nESEPM) were synthesized using the film hydration process[12].First,Tween 60 (10 mM) and cholesterol

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were dissolved in a 1:1 M chloroform solution, and a round-bottom flask was filled with 1.0 mg/ml of *Pedalium murex* extract. To get a thin layer of nESEPM, the excess chloroform was removed at 55°C using a rotary evaporator. This layer was then hydrated with phosphate buffer saline in a water bath at 55°C for two hours. To create smaller vesicles, the finished solution was further subjected to bath sonication for 20 minutes. By using distilled water for dialysis, phytoniosomes were separated from the released materials overnight.

Animals

Male albino Wistar rats, specifically bred to be pathogen-free, with a specified weight: 100-120 g and 6-7 weeks) were purchased from Mass Biotech Private Limited Chennai, India. The animals were kept in a controlled laboratory environment at a temperature of 25 ± 2°C, with a relative humidity of 50-55%, and subjected to 12-hour light/dark cycles. All the rats were provided with regular food and clear water. Animal experimentation and procedure (no. 2018-0042) follow ethical principles outlined by CPCSEA under the Ministry of Environment and Forests regulations from June 2007.

Studies on acute toxicity:

Researcher Ecobichon[13]conducted investigations on the acute toxicity of an ethanolic seed extract of *Pedalium murex* following OECD guidelines 420. No deaths were seen up to the final dose of 2000mg/kg body weight, with normal hematological and biochemical markers. For the current study, 200mg/kg b.w. of the extracts were chosen to assess their effectiveness in treating gouty arthritis, which is equivalent to one-tenth of the LD_{50} dose.

MSU crystal synthesis

MSU crystals were promptly formed by crystallizing the supersaturated uric acid solution with the previously outlined adjustments[14]. Briefly 24gms 200ml of pyrogen-free distilled water was used to dissolve sodium hydroxide. Dissolve 5 grammes of uric acid in 1000 milliliters of distilled water. Add 9 milliliters of 0.5 N sodium hydroxide and boil the solution to 120 degrees Celsius for 6 hours with moderate stirring. The solution was slowly cooled to room temperature and stored overnight at 4ºC for crystallization process. Followed by the crystal formation they were then sterilized by heating at 100ºC for 2 hrs. After incubation the Crystals were gathered using filtering, cleaned with 100% ethanol, dried under vacuum, and sterilized by autoclaving at 121°C for 20 minutes. The resulting MSU crystals (needle-shaped, length 5-20 µm) observed by compensated polarized light microscopy suspended in endotoxin-free PBS at a concentration of 25mg/mL and used for further assay.

Induction of gouty arthritis by MSU in male Albino Wistar rats

Anti- gouty arthritic activity was performed according to Getting et al., [15]method. MSU crystals will be sterilized by heating at 180˚C for 2 hours before the trials. Rats were anaesthetized using intraperitoneal injection of urethane at a dose of 1.0 g/kg. MSU crystals at a concentration of 80mg/ml was injected into the synovial area of both sides of the ankle joint in a 50 µl volume dissolved in sterile phosphate buffered saline (PBS). The animals were divided into five groups of five animals in each group and received the specified dose as part of the administration process.

Group I: Acted as the control group. Animals were administered an intra-articular injection of Sterilized PBS in the ankle using a vehicle.

Group II: Served as a negative control Gouty arthritis induced by MSU crystals and treated by vehicle on the 10^{th}day

Group III: Animals were pretreated with EESPM (200mg/kg B.W) for 10days and induced with MSU on the 10th day.

Group IV: Animals were pretreated with nEESPM (200mg/kg B.W) for 10days and induced with MSU on the 10th day.

Group V: Rats induced with Monosodium Urate Crystal were treated with indomethacin at a dosage of 3mg/kg as a positive control.

On the tenth day 60minutes after drug administration acute gouty arthritis was induced by injecting MSU crystals (20mg/ml) into the synovial space of both side of the ankle joint. Theanti-gout arthritic activity induced by MSU was measured at different time intervals for the period of 24hrs.After 24 hours of inducing gout, the rats will be euthanized with an

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overdose of anesthesia. Blood samples will be obtained using retro-orbital puncture to evaluate different hematological parameters. After 30 minutes at room temperature, the samples were centrifuged for 15 minutes at 3000 rpm/min to extract the serum for biochemical analysis. Ankle tissues were collected for histopathological analysis.

Determination ofAnti-gout arthritic activity

Arthritis progression will be evaluated by monitoring ankle swelling using a vernier caliper at 0hr (baseline, before the MSU injection).2hr, 4hr, 6hr, 8hr, 12hr, and 24 hr after the MSU crystal injection. Arthritis was assessed by measuring the mean increase in the diameter of the ankle swelling. The average increase in ankle swelling was recorded and the percentage of inhibition was estimated.

Percentage of inhibition = 100 (1-Vt / Vc)Where, Vc= volume of edema in control and Vt= volume of edema in test / standard compound.

Estimation of Hematological and Biochemical parameters:

The hematological parameters such as total RBC, total WBC, platelets and ESR were measured using established protocols. The biochemical parameters such as glucose, urea, uric acid, creatinine, total protein, total cholesterol were estimated by using commercial Kits obtained from Ranbaxy India Pvt Ltd. Estimation of liver enzymes such as ALT, AST, ALP and Xanthine oxidase were also performed using the above-mentioned commercial kits.

Histopathological observations

Ankle tissues were recovered by dissection of the ankle area,that involved skin and muscle removal and maintained in 10% of formalin solution. Followed by rinsing of dehydrated samples with xylol after treating with various amounts of alcohol. Then subjected to paraffin submerging process and sliced into 4-6 µM. Then stained by eosin and hematoxylin and examined under microscope to assess histopathological changes.

Statistical Analysis:

The values are shown as Mean±SE. The statistical analysis was conducted using the ANOVA approach, followed by Dunnet's 't' test. The p value is less than 0.05, indicating statistical significance.

Results

Anti-gout arthritic activity

Table- 1 and Figure -1 depict the effect of ESEPM and ESEPM loaded phytoniosomes(nESEPM) on the ankle diameter in the MSU induced gouty arthritis. Among the two extracts the nESEPM exhibited a maximum reduction of the swelling observed in the ankle region following the introduction of MSU crystals. At the end of experimental period the nESEPM extract showed 90.79% inhibition of joint swelling when comparable to its ethanolic extracts ESEPM which showed 57.62% inhibition at the same concentration after 24hr of treatment. In the present study Indomethacin showed an inhibition of 97.82% of joint swelling.

Table 1: Effect of ESEPM with and without niosomes on volume of ankle joints with acute gouty arthritis

Treatment	Mean increase in ankle diameter in (cm) and Inhibition (%) on					
	2hr	4hr	6hr	8hr	12hr	24hr
Group I (vehicle treated) (control)	0.32±2.46a*** (76.40%)	0.32±2.25a*** (58.62%)	0.32±2.25a*** (29.05%)	0.32±2.25a*** (23.28%)	0.32±2.25a*** (20.35%)	0.32±2.25a*** (16.46%)
Group IIMSU(80mg/kgbw) crystal induced (negative Control)	0.89±0.03	1.16±0.02	2.34±0.06	2.92±0.09	3.34±0.15	4.13±0.20
Group III ESEPM (200mg/kgbw) +	0.79±0.15b**	0.94±0.16b**	1.54±0.13b**	1.63±0.28b**	1.55±0.19b**	1.75±0.63b**

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MSU crystal treated	(11.23%)	(18.96%)	(34.18%)	(44.17%)	(53.59%)	(57.62%)
Group IV nESEPM (200mg/kgbw) + MSU crystal treated	0.64±0.13c*** (28.08%)	0.68±0.23c*** (41.47%)	1.32±0.17c*** (43.58%)	0.95±0.24c*** (67.46%)	0.45±0.15c*** (86.52%)	0.38±0.30c*** (90.79%)
Group V Indomethacin (3mg/kgbw) 200mg/kg/bw) + MSU crystal treated (positive control)	0.43±0.41d*** (51.68%)	0.34±0.04d*** (70.68%)	0.26±0.05d (88.88%)	0.14±0.15d *** (95.20%)	0.11±0.03d*** (96.70)	0.09±0.06d*** (97.82%)

The values represent the mean ± standard error of the mean of 6 animals in each group. An ANOVA test was conducted for comparison, followed by Dunnet's 't' test to determine statistical significance. Comparisons between Group I and Group II, Group II and Group III, Group II and Group IV, and Group II and Group V.P values: *p<0.05, **p<0.01, ***p<0.001, NS–Not Significant.

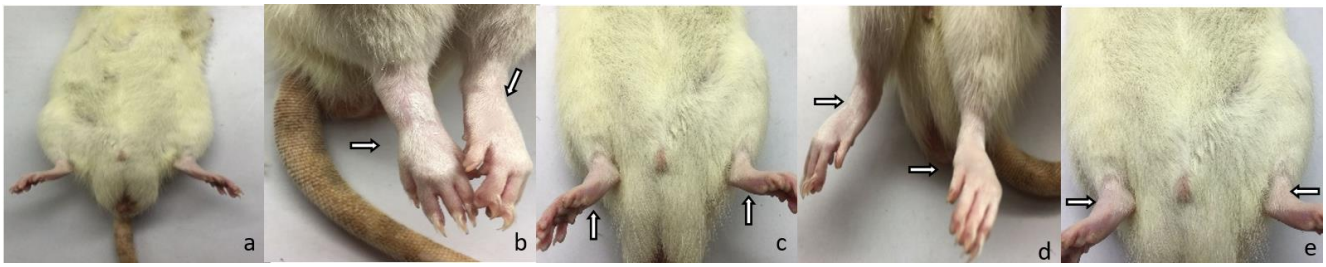

Figure 1: MSU induced gouty arthritis in rats- Showing macroscopic signs on swelling Observed in each group 24 hours after injecting MSU into ankle joints.

Group (a)representing negative control with no swelling,Group (b) representing MSU induced model with increased ankle swelling, Group (c) ESEPM treated rats with moderate ankle swelling, Group (d) nESEPM, treated rats with less ankle swelling and Group (e) representing positive control indomethacin treated rats with less ankle swelling.

Effect of ESEPM and nESEPM on Hematological parameters

The effect of ESEPM and nESEPM extracts on the Hematological parameters such as hemoglobin, WBC, RBC and ESR on rats with induced gout is demonstrated in Table-2. Group II rats exhibited a notable rise in white blood cell count and erythrocyte sedimentation rate compared to the control rats. Rats with gouty arthritis induced MSU, and treated with ESEPM and nESEPM drugs, exhibited reduced white blood cell count and erythrocyte sedimentation rate (ESR), suggesting the drugs' anti-arthritic properties. No significant difference was observed in the other hematological measures, such as hemoglobin, RBC, and platelets, in the current investigation.

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Table 2: Effect of ESEPM and nESEPM extracts on Hematological parameters in experimental rats.

Treatment	Hemoglobin g/dl	Total RBC Count (10^6/mm^3)	Total WBC Count (103/mm3)	Platelets count (10^5/mm^3)	ESR (mm/hr)
Group I (vehicle treated) (control)	12.54 ± 1.04a ^{ns}	4.24±0.18a ^{ns}	6350±377a**	2.65± 0.07a ^{ns}	12.02±1.08a**
Group IIMSU (80mg/kgbw) crystal induced (negative Control)	12.25 ± 2.00	4.30± 2.00	9422±399	2.45± 0.09	33.04±1.20
Group III ESEPM (200mg/kgbw) + MSU crystal treated	12.20 ± 2.00b ^{ns}	4.40±1.65 b^{ns}	8222±369b**	2.55± 0.03 b ^{ns}	16.02±1.10 b**
Group IV nESEPM (200mg/kgbw) + MSU crystal treated	12.37 ± 0.22c ^{ns}	4.15±2.00 c ^{ns}	7121±218c**	2.50 ± 0.04c ^{ns}	14.22±1.00c **
Group V indomethacin (3mg/kgbw) (200mg/kgbw) + MSU crystal treated (positive control)	12.08 ± 2.23d ^{ns}	4.12 ±1.77 d ^{ns}	6424±420d**	2.68 ± 0.08d ^{ns}	13.04±1.12d**

Values are mean ± SEM of 6 animals in each group. A statistically significant test for comparison was done by ANOVA followed by Dunnet's 't" test. Comparison between (a)-Group I vs Group II, (b)-Group II vs Group III, (c)-Group II vs Group IV and (d)-Group II vs Group V.P values*p<0.05,**p<0.01, ***p<0.001, NS–Not Significant.

Effect of ESEPM and nESEPM on Biochemical parameters in experimental rats

The effect of ESEPM and nESEPM extracts on the (Table-3) biochemical data, including glucose, urea, uric acid, creatinine, and total protein, in rats with induced gout. There is a notable rise in urea, uric acid, and creatinine levels in group II rats compared to the control rats. Rats with gouty arthritis produced by (group III and group IV) MSU, and treated with ESEPM and nESEPM drugs, exhibited reduced levels of urea, uric acid, and creatinine, suggesting the drugs' anti-arthritic properties. No significant variation was seen in the other biochemical markers, such as glucose, total cholesterol, and total protein levels in the current investigation.

Table 3:Effect of ESEPM and nESEPM on Biochemical parameters in experimental rats

Treatment	Glucose (mg/dl)	Urea (mg/dl)	Total cholesterol (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Total Protein (g/dl)
Group I (vehicle treated) (control)	84.00 ± 3.01a^{ns}	32.16±2.75a**	186.34±12.60a^{ns}	4.79± 0.12 a**	0.73±0.12a*	6.45±2.7a^{ns}
Group IIMSU (80mg/kgbw)	89.00 ±1.12	45.00±2.40	172.54±14.20	8.55±0.34	0.93±1.34	6.78±1.34

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crystal induced (negative Control)						
Group III ESEPM (200mg/kgbw) + MSU crystal treated	90.00±3.81b^{ns}	39.40±1.34 b***	163.17±10.82b^{ns}	6.39±0.17 b**	0.64±0.03b*	6.48±1.56b^{ns}
Group IV nESEPM (200mg/kgbw) + MSU crystal treated	85.50± 3.28 c^{ns}	38.89±1.89c**	157.23±15.32c^{ns}	5.45±1.05c**	0.67±0.12c*	6.89±0.23c^{ns}
Group V indomethacin (3mg/kgbw) (200mg/kgbw) + MSU crystal treated (positive control)	99.33±3.44d^{ns}	40.00±2.67d**	187.23±18.54c^{ns}	6.01± 1.34d**	0.78±0.43d*	6.23±2.13d^{ns}

The values are the mean ± standard error of the mean for 6 animals in each group. An ANOVA test was conducted for comparison, followed by Dunnet's 't' test to determine statistical significance. Comparison of Group I with Group II, Group II with Group III, Group II with Group IV, and Group II with Group V.P values: *p<0.05, **p<0.01, ***p<0.001 NS–Not Significant.

Effect of ESEPM and nESEPM on liver enzymes in experimental rats

Table 4 shows the variations in serum levels of liver marker enzymes such as AST, ALT, ALP, and xanthine oxidase. Elevated levels of blood transaminases AST and ALT were seen in the MSU treatedanimals of group II. The enzymes were restored to almost normal levels in animals from group IV that were pretreated with nESEPM (p<0.001). The high levels of xanthine oxidase enzymes seen in group II negative control animals were considerably (p<0.01, p<0.001) reduced in group III and IVanimals which are pretreated with ESEPM and nESEPM and intoxicated with MSU. Group I control rats the marker enzyme levels showed no significant differences, showing that there were no detrimental side effects from the administration of Tween-80.

Table 4: Effect of ESEPM and nESEPM on ALT, AST, ALP and xanthine oxidase in experimental rats

Treatment	**ALT U/L**	**AST U/L**	**ALP IU/L**	**Xanthine Oxidase ng/ml**
Group I (vehicle treated) (control)	34.24 ± 3.01a**	36.78±1.75a**	126.14±10.60a**	2.69± 0.18 a**
Group IIMSU (80mg/kgbw) crystal induced (negative Control)	79.00 ±2.22	67.89±3.10	182.22±16.70	6.45±0.53
Group III ESEPM (200mg/kgbw)	52.80±2.41b**	49.40±2.34 b***	172.24±11.82b**	4.19±0.23 b**

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+ MSU crystal treated				
Group IV nESEPM (200mg/kgbw) + MSU crystal treated	45.70± 2.18 c**	42.89±1.89c**	157.23±15.32c**	3.23±0.95c**
Group V indomethacin (3mg/kgbw) (200mg/kgbw) + MSU crystal treated (positive control)	76.16±3.44d^{ns}	60.30±1.88d*	182.23±16.34c^{ns}	6.01± 1.34d**

The values represent the mean ± standard error of the mean of 6 animals in each group. An ANOVA test was conducted forcomparison, followed by Dunnet's 't' test to determine statistical significance. Compare Group I with Group II, Group II with Group III, Group II with Group IV, and Group II with Group V.P values: *p<0.05, **p<0.01, ***p<0.001, NS–Not Significant.

Effect of ESEPM and nESEPM on histopathological alterations

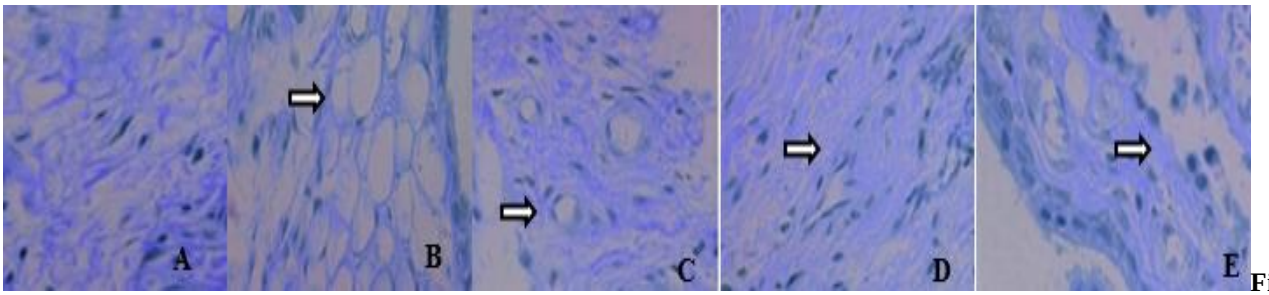

Figure 2: Effect of ESEPM and nESEPM on MSU induced gouty arthritis in rats- after 24hr Injecting MSU crystals into ankle joints.Arrows indicate thickening of synovial membrane (typical lesion formation)

(A) representing negative control indicated no noticeable lesions Group (B) representing MSU induced model indicating more lesion formation. Group (C) representing injection of ESEPM, Group (D) representing nESEPM, Group (E) representing positive control indomethacin.

Methylene blue was used for coloring the synovial membrane isolated from ankle joints. The results exhibited that group II MSU crystal insulted rats developed lesions in synovial membrane due to mononuclear cell infiltration and inflammatory reactions into joint capsule. The synovial membrane was deprived of lesions in ESEPM and nESEPM treated group III and group IV rats. This may attributedue to the lack of mononuclear cell infiltration into joint capsules which is responsible for the inflammatory reactions and thereby reduce the lesion formation.The outcome aligned with the measurements of the rats' joint diameter at various time points.

Discussion

Gouty arthritis is an inflammatory reaction caused by disrupted uric acid metabolism. The atypical uric acid increasepresents severe discomfort, paw edema, and swelling near the ankle joints. At present gouty arthritis is managed with non-steroidal anti-inflammatory medications, such as colchicine, indomethacin, and adrenocortical hormones[16]. However, the application of these drugs are in a state of causing severe side effects which insists the use of traditional medicines as one of the most popular alternative drugs that possess low toxicity profile with high beneficial efficacy. The current study focuses on the impact of an ethanolic seed extract of *Pedalium murex* (ESEPM) and its phytoniosomes loaded extract (nESEPM) in gout induced rat models that has been

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extensively utilized in screening medicines that reduce uric acid levels.

The latest study shows that MSU crystals are one of the most effectual pro-inflammatory stimuli, firmly related to pathology of gouty arthritis that is capable of provoking, elaborating, and sustaining sturdy inflammatory response in joint cavity [17]. Literature states MSU crystals promote the production of IL-4 and IL-2 the crucial inflammatory mediators that regulate the development, growth, and death of cells in gouty arthritis. Further MSU also secretes TNF-α which induces acute inflammation. The resolute inflammatory responses lead to future tissue damage. It is crucial to prevent the production of inflammatory mediators from MSU crystal-activated macrophages in order to control and manage gouty arthritis.

After 24 hrs of MSU administration there is a notable increase in the inflammation in the ankle joints of rats in the present study. These inflammatory features were similar to arthritis in humans[18]. On continuous observation severe ankle swelling was seen at 12 and 24 hrs, in MSU crystal administered group II rat, which was reduced in ESEPM and nESEPM treated group III and group IV rats. The drug treatment considerably reduced the ankle swelling due to MSU administration may accounted for the presence of phytochemicals such as 11 -EicosenoicAcid, Methyl ester,1,2Benzendicarboxylic Acid, mono(2-ethylhexyl) ester present in seed of *Pedalium murex*as stated by Patel et.al.,[19]that resulted in depletion of inflammatory activity in ankle joints in rats.

The study results showed elevated levels of white blood cells (WBC) and erythrocyte sedimentation rate (ESR) in the arthritis-induced group in comparison to the healthy control group. Drug pre- treatment with ESEPM and nESEPM for 10 days reduces the ESR and normalizes the total white blood cell count. According to Bihaniet al.,[20]the rise in the WBC count is due to the immune system that reacts to inflammatory stimuli mediated by IL-1β owing to MSU crystal injection in gout. The body responds to inflammation by sending white blood cells into the affected tissue, which can lead to damage and the release of enzymes or proteases from lysosomal membranes, exacerbating tissue damage and inflammation[21].The increase in ESR could be attributed to the elevated levels of pro-inflammatory cytokines such IL-1β, IL-6, and TNF-α,which induce the acute phase reactants from the hepatic origin[22]. Treatment with ESEPM and nESEPM could reduce the ESR and WBC cells thereby reduce the inflammatory reactions observed in the present study. The researcher Muhammad Imran and colleagues[23] conducted a review on the phytochemical and pharmacological properties of *Pedalium murex* Linn, as well as its traditional therapeutic applications. The main phytochemical groups found in various chemical extracts of *Pedalium murex*include reducing sugars, flavonoids, glycosides, phenolic compounds, phytosterols, carbohydrates, triterpenoids, aromatic oil, alkaloids, tannins, stable oil, resins. Saponins, and xanthoproteins. These compounds may contribute to the observed anti-inflammatory activity in the current study.

The increase in the biochemical parameters such as urea,uric acid and creatinie as well as enzymatic parameters AST, ALT, Xanthine oxidase and ALP in MSU crystal induced arthritic rats indicates the ankle joint tissue destruction. ESEPM and nESEPM drug pretreatment for 10 days alleviate these values to near normal indicating the curative nature of the plant. Decreasing the Uric acid and its crystallization reduces inflammatory responses observed in the course of acute gouty arthritis. Serum AST and ALT are known to be crucial in the synthesis of chemical mediators during inflammation, as demonstrated in various investigators[24]. Xanthine oxidase inhibitors are crucial in treating hyperuricemia as they effectively lower plasma and urine urate levels and prevent the formation of tophaceous deposits[25]. As per Devanesan et al., [26] studies the *Pedalium murex* seeds are found to be rich in soluble proteins, sapogenins and flavonoids. According to Kuraoka-Oliveira et al.,[27]the flavonoids have the ability to inhibit the proinflammatory cytokines and thereby treat joint ailments. The results suggest that the flavonoids present in the *Pedalium murex* have a beneficial impact on the anti-arthritic effect. Moreover, Rajasekaran et al.,[28]have isolated the flavonoids such as triacotanyldotriacontanoate,2′,4′,5′-trihydroxy5,7-dimethoxyflavones,tetratriacontanyloctacosanoate and heptatriacontan-4-one from the fruits. These

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flavonoids may act as the inhibitors of the inflammatory reactions triggered due to MSU crystals and thereby exert its anti-gout activity. Anti arthritic potential of any plant extract relies on histopathology analysis. The synovial membrane is considered as the main target for inflammatory reactions where the formation of inflammasome is instigated by activation of macrophages influenced by phagocytosis of MSU crystals as stated by lit et, al.[29].In the present study the administration of nESEPM administered rats showed more depletion in inflammatory activity than group loaded with normal ESEPM which may be due to their improved permeation of the phytoconstituents present in the extract through the lipid bilayer of the cells.

Conclusion

The current study's findings point to the anti-gout arthritic potential of ESEPM and nESEPM extracts, with nESEPM extracts showing particularly strong protective efficacy. This might be because, during the creation of the phytoniosomes, phospholipids and phytochemicals from the plant extract form a complex. The phytochemicals' enhanced absorption boosts both their bioavailability and the intended pharmacological impact. Pedalium murex seeds contain a variety of phytoconstituents, including flavonoids, triterpenoids, glycosides, phytosterols, phenolic compounds, alkaloids, tannins, xanthoproteins, aromatic oil and saponins,. These phytoconstituents work in concert to lessen the inflammatory reactions caused by the administration of MSU crystals, thereby exerting the anti-gout arthritic activity seen in this study.

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