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RESEARCH ARTICLE

ANTIARTHRITIC POTENTIAL OF *CLEOME GYNDRA* LEAVES EXTRACT, IN BALB/C MICE MODELS

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ABSTRACT

Background: Arthritis, leadingto pain and swelling of joints,is commonly treated using drugs or non-drug methods. But, an effective or permanent cure has not yet been achieved. **Aim:** Thus, this study aims at evaluating the anti-arthritic activity of *Cleomegyndra* (CG) in BALB/C Mice. **Methodology:** The toxicity of the plant extract was analysed, followed by the animal weights and antiarthritic activity of *C. gyndra*in mice, based on toxicity results. **Results:** The toxicity analysis revealed non-toxicity at 100 mg/kg, whereas 500 to 2000 mg/kg showed severe damagein multiple organs. Thus, Groups G1 to G5 were compared. G1 was control, G2 was the diseased group, G3, G4 and G5was disease induced and treated with 500 mg/kg,100 mg/kgand 50 mg/kg, respectively. The animal body weights showed that Groups G1 to G4 showed approximately similar weights, both before and after treatments. In contrast, G5 exhibited reduced weight in the mouse models after treatment than before treatment. This is attributed to the effect of arthritis, and the dose of CG being very low (50 mg/kg).The histopathological analysis of bone tissues showed that G1 showed no damage (arthritic score - 0) for bone and cartilage erosion, and synovial inflammation, where G2 exhibited severe damage (scores 2-3). G3 exhibited maximum effects (score - 1), G4 exhibited mild to moderate (score 1-2), andG5 exhibiteda moderate arthritic score of 2. **Conclusion:** The plant extract showed a dose-dependent anti-arthritic activity. Thus, the study recommends *C. gyndra* as a potential antiarthritic therapeutic.

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INTRODUCTION

Arthritis consists of over 100 conditions, classified under four majorcategories as osteoarthritis, infectious arthritis, posttraumatic arthritis, and inflammatory arthritis (1). It leads to disability, medical issues and is inefficient to lead a normal lifestyle (2), and is a worldwide inflammatory disease, causingpain, swelling, heat, and redness in the joints, leading to discomfort and functional disabilities. Its prevalence varied based on region, race, income of the family, age, urbanization level, and sex(3).According to the World Health Organization,it is stated that nearly 73%and 70 % of the people affected by osteoarthritis and rheumatoid arthritis, respectively, areabove 55 years of age, where 55 to 60 % among them are women (4) (5). Though arthritis is also an age-related disease, it also affects children sometimes (6). Thus, treatment options include medicines, nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), or non-drug methods such as physical activities and exercises, and surgical treatments are Performed (7) (8). Currently, Diclofenacis the predominantly consumed NSAID drug. Though it relieves pain and reduces inflammation, its exposure in the long term leads to peptic ulcers, kidney issues, gastrointestinal bleeding, and cardiovascular events, even causing death (7). Thus, innovative solutions such as nanoparticles, biological agents like TNF monoclonal antibodies,are recently gaining importance(9). However, a complete cure for the disease has not been identified. Thus, many herbal plants are focused on identifying the best compound for arthritic treatment, but it remains unachieved. *Cleome gynandra*is a well-known traditional Indian medicine that belongs to the Cleomaceae family (10). Multiple studies have reported the therapeutic potential of over 200 species of *Cleome* (10). Especially, the species *Cleomegyandra* is well-known for its anti-inflammatory activities (11) (12). It is also known to contain various bioactives such as alkaloids, tannins, flavonoids, carotenoids, steroids and terpenoids (12). Various studies have been proved for its potential to be anticancer, antidiabetic, antifungal, antileishmanial, anti-inflammatory and antidiabetic activities(13)(10)(12).Other species of the family *Cleome,* such

as *C. amblyocarpa, C. arabica, C. viscosa, C. chelidonii, C. rutidosperma, C. coluteoides, C. brachycarpa, C. spinosa*, have been reported to possess multiple therapeutic applications, including antioxidant activity, anti-inflammatory, analgesic, and antimicrobial activities (10) (14) (15)(11) (16). Ethanolic extract of *C. gyndra* showed anti-arthritic activity by reducing the lysosomal enzymes that induce arthritis (17). Thus, this study delves deeper into determining the efficacy of *C. gyndra*leaves extract to exhibit anti-arthritic activity, by analyzing the toxicity of the compounds and histopathologic analysis.

METHODOLOGY

Animal conditions: BALB/C male mice of weight 25 ± 2 grams, 8 weeks old, inbred from the Trichy Research Institute of Biotechnology Animal Facility,wereused in this study. The ethical committee clearance reference number is 2295/PO/RcBiBt/S/2024/CCSEA.The BALB/C mice were housed in cages under temperature and humidity-controlled laboratory conditions of a 12-hour dark and light cycle. They were fed with standard pelleted feed and water ad libitum. The mice were maintained as per OECD 423 guidelines until the experiment. The mice were grouped for toxicity studies and the anti-arthritic activity of the extracts.

Grouping details: The mice were grouped into five groups for toxicity studies, each containing three mice. The five groups were as follows:Group I – Control; Group II – 2000 mg/kg; Group III – 1000 mg/kg; Group IV – 500 mg/kg; Group V – 100 mg/kg. For the drug analysis, the mice were again divided into 5 groups, each group containing 6 mice. The grouping details were as follows; Group 1 (G1) – Control; Group 2 (G2) – arthritisinduced (injected with collagen and lipopolysaccharide); Group 3 (G3) – arthritisinduced + CG plant extract at 500 mg/kg; Group 4 (G4) – arthritisinduced + CG plant extract at 100 mg/kg, and Group 5 (G5) – arthritisinduced + CG plant extract at 50 mg/kg.

Toxicity studies: The plant extract *Cleome gynandra*(CG), at different concentrations (10 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg) was tested for its toxicity. The plant extract wasadministered orally to the mice afteran overnightfast,during which they were fed only water. Then the mice were observed for their toxic symptoms such aslocomotion, behavioural changes, convulsion and mortalityfor another 14 days.

Treatment efficiency of the CG plant extract: The mice were treated with CG plant extracts at different concentrations of 500 mg/kg, 100 mg/kg and 50 mg/kg. The procedure was similar to the toxicity analysis. After treatment, the mice were analysed for their weights, and then sacrificed. The bone tissues of the mice were prepared and analysed for their histopathological characteristics. Score criteria were used to determine the severity of the damage in bone tissues. Scores 0, 1, 2, and 3 denoted healthy, mild, moderate, and severe, respectively, for bone erosion, synovial inflammation, and cartilage erosion.

RESULTS AND DISCUSSION

Analysing the weight of the organs is crucial to determine the course of the disease, as active disease conditions will result in weight loss, whereas weight gain indicates recovery (18). Variations in the weight of the animals were comparedin this study, both before and after treatment. The groups G1, G2, G3 and G4 showed similar variation in both groups. Weight reduction was found in the G5 alone after treatment. The reduction of weight could be attributed to the insufficient dosage of 50 mg/kg, to the disease. Figure 1 displays the graphical comparison of animal weights before and after treatment.Similar weight loss was observed in arthritic rats when treated with *Nigella sativa* oil(18). Toxicology evaluation of the plant extracts is recommended to identify their toxicity before human use.

Table 1: Score criteria for histopathological analysis of antiarthritic activity among groups G1 to G5.

S. No	Description	Score criteria for the groups				
		G1	G2	G3	G4	G5
1.	Bone erosion	0	2	1	1	2
2.	Synovial inflammation	0	3	1	2	2
3.	Cartilage erosion	0	3	1	2	2

Score describes the severity of the disease: 0 - Healthy; 1 - Mild; 2 - Moderate; 3 -Severe.

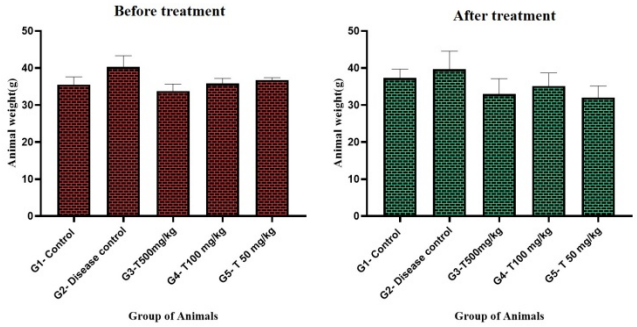

Figure 1. The comparison of animal weights before and after treatment

The evaluation provides insights to select a safe dose for human administration (19).Thus, all the organs of the mouse models were analysed for their histopathological parameters to identify changes in the tissues (20). The histopathological analysis of the toxicity study showed dose-dependent variations among various organs. At a dose of 100 mg/kg, the heart showed mild myocardial edema and cardiomyocyte hypertrophy, where the spleen exhibited slight disorganization, showing splenic macrophages. Kidney represented obliterated Bowman's spaces and multifocal hemorrhages, where liver showed mild hydropic degeneration of hepatocytes, and lungs displayed mild hyperemia.In contrast, progressive severity was identified at higher doses of 500 to 2000 mg/kg. CG extract at 500 mg/kg showed multifocal hyperemiain the heart and lungs, whereas, kidneys showed mild to moderate hyperemia.The spleen exhibited slight disorganized white pulp with hyperemia, and the liver showed multifocal mild to moderate vacuolation of hepatocytes. Fattydegeneration of hepatocytes, hepatocellular necrosis, and sinusoidal expansion along mononuclear infiltration were identified in the liver at doses of 1000 and 2000 mg/kg.Additionally, 2000 mg/kg showed oedema in the liver tissues. Spleen showed disorganized white pulp at 1000 mg/kg, where 2000 mg/kg exhibited the presence of splenic macrophages in addition. At 1000 mg/kg, the heart represented multifocal mild myocardial hypertrophy, but at 2000 mg/kg, severe damage showing multifocal mild myocardial degeneration, hyperemia, and wavy myocardial fibers was observed. Mild multifocal hyperemia (1000 and 2000 mg/kg) and oedema(2000 mg/kg) were observed in the lungs. Similarly, the kidney exhibited multifocal mild to moderate hyperemia (1000 and 2000 mg/kg), and multifocal mild to moderate tubular and glomerular necrosis with infiltration of mononuclear cells (2000 mg/kg). Figure 2 displays the organs showinghistopathological changes at different concentrations. This confirms that systemic toxicity was observed in histopathology on increased doses.The lethal dose LD50 of the *Cleome viscosa*was more than 5000 mg/kg, and no animal death was observed within 24 hours (19).

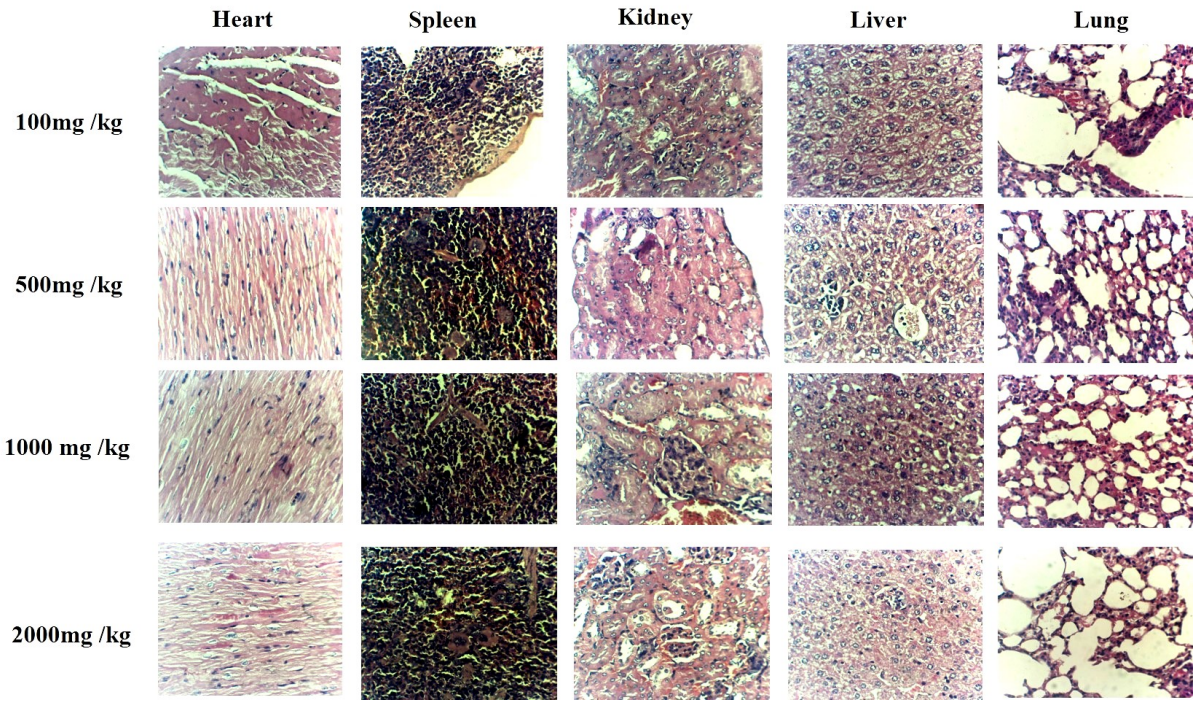

Figure 2. Histopathological analysis of organs after toxicity analysis

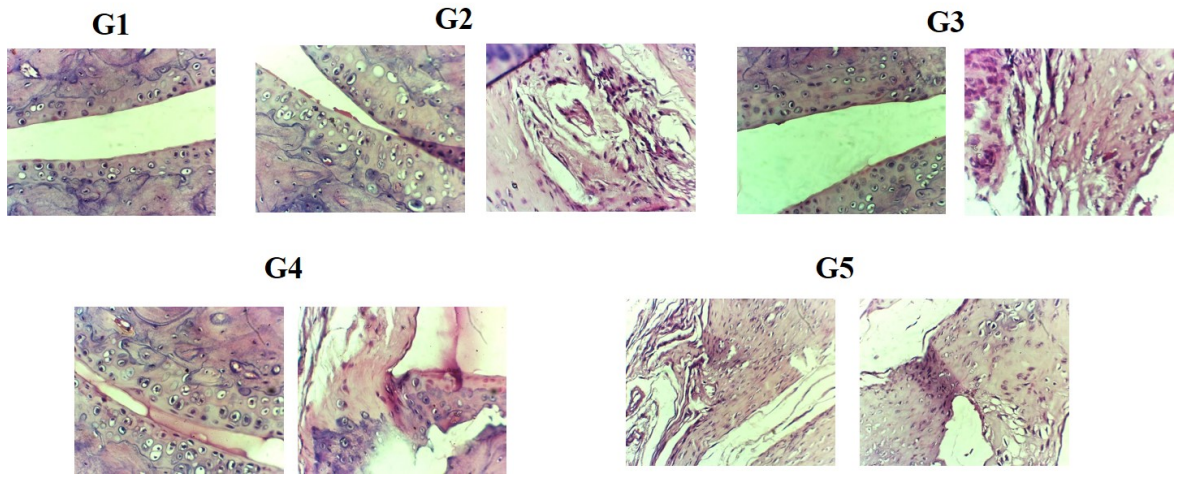

Figure 3. Histopathological analysis of the bone tissues of groups G1 to G5 after treatment

The bone tissues of the mice were analysed for the efficacy of CG extracts in treating arthritis. Groups G1 and G2 were used as the control and diseased sample, respectively. Groups G3 (500 mg/kg), G4 (100 mg/kg), and G5 (50 mg/kg) were treated with different doses of CG extract to determine their antiarthritic activity.Figure 3 represents the changes observed in the bone tissues after different treatments. Score criteriawere used to determine the severity of damage in terms of bone erosion, synovial inflammation, and cartilage erosion (Table 1). G1 was found to be healthy with 0 score in all three observations. G2 exhibited a score of 2 for bone erosion, whereas synovial inflammation and cartilage erosion showed a score of 3. Among the treatments, G3 showed the lowest score, 1 for all the observations, whereas G5 exhibited the highest score, 2 for all the observations. G4 alone showed mild damage of score 1 in bone erosion, and moderate damage of score 2 in synovial inflammation and cartilage erosion. The control group (G1) exhibited normal articular structure, showing smooth and clear cartilage and joint space, respectively.G2 exhibited moderate to severe damage to the cartilage and bones. The hyperplasia of the synovial lining and infiltration of inflammatory cells were noted. There was also a reduction of joint space, and diffusion of soft tissue swelling was noticed. G3 showed mild bone and cartilage damage, and hyperplasia of the synovial lining was observed. Whereas the joint space appeared normal, and soft tissue showed mild swelling. G4 exhibited mild to moderate damage to bone and cartilage. Slight reduction and mild swelling of the soft tissues were identified. G5 represented moderate cartilage and bone damage, and hyperplasia of synovial lining. There was also loss of joint space and moderate soft tissue swelling.*Cleomearabica* fruit extracts showed better anti-inflammatory effects in the paw tissues of Wistar rats (20).The compounds isolated from *C. amblyocarpa* also exhibited effective anti-inflammatory properties by reducing the paw edema in Wistar albino rats (10). A combination of *Cleome gynandra* and *Melicopeptelefolia*also exhibited anti-inflammatory activity; however, it was comparatively less effective thandiclofenac, a known drug(12). *C. brachycarpa* also exhibited 29% edema inhibition at a dose of 200 mg/kg in Wistar rats(15). Both *C. brachycarpa*(15) and a combination of *Cleome gynandra* and *Melicopeptelefolia*(12)showed better anti-inflammatory activity. However, both these studies showed a reduced effect than the standard drug, diclofenac. In contrast, the current study exhibited nearly equal efficiency to the control. The anti-arthritic activity of *C. gyndra*has been proved through in vivo studies, indicating the leukocyte migration (17). However, no in-depth information regarding bone and cartilage erosion and synovial inflammationhas beenstudied; hence, this study. The study highly

recommends that *C. gyndra*possesses increased anti-arthritic activity and can be applied in therapeutics.

CONCLUSION

*C. gyndra*was found to be non-toxic at a dose of 100 mg/kg, based on the toxicity analysis of different organs at various doses. Increased concentration of 500 to 2000 mg/kg showed severe toxicity. Based on this, the dosage of animal studies was standardized. Upon treatment, the animal weights reversed in G3 and G4, nearly to the weights that were before treatment. Whereas G5 showed a slight weight reduction, indicating the inefficient dosage to reverse arthritis. Histopathological analysis of the bone tissues of the treated groups indicated that G2 showed severe damage, while G3 exhibited only mild damage, G4 and G5 showed mild to moderate, and moderate damage, respectively. Based on the analysis, this study concludes that *C. gyndra* can be effectively used in the antiarthritic therapeutic activity.

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