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Research Article

Investigate Anti-Arthritic Activity Through Extraction And Phytochemical Screening Of Euphorbia Nivulia

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ABSTRACT

The study aimed to investigate the phytochemical constituents, anti-arthritic potential, and histopathological effects of the ethanolic extract of Leaves of Euphorbia nivulia in a Freund's adjuvant-induced arthritis model in rats. The ethanolic extract of Leaves of Euphorbia nivulia exhibits promising anti-arthritic activity in the experimental model. The observed effects may be attributed to the presence of bioactive compounds detected in phytochemical screening.The study provides valuable insights into the therapeutic potential of Euphorbia nivulia in managing arthritis, contributing to the development of natural and effective anti-arthritic agents. Complete Freund's Adjuvant induced arthritis and anti-arthritic property of the extract Ethanolic extract of Leaves of Euphorbia nivulia. The effect of Ethanolic extract of Leaves of Euphorbia nivulia were determined after administration at two dose level (100and 200 mg/kg b.w.) in arthritis induced rats and assessed by histopathological studies.From the results, it may be concluded that herbal ethanolic extract of leaves of Euphorbia nivulia possess significant anti-arthritic effect may be due to the effect of antioxidants like Flavonoids, Poly Phenols and Saponins present in the plant.

INTRODUCTION

Rheumatoid arthritis is a chronic, systemic, inflammatory autoimmune disorder causing symmetrical polyarthritis of large and small joints, typically presenting between the ages of 30-50 years, that is associated with progressive disability, Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia, autoantibody production, cartilage and bone destruction and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders(Majithia and Geraci,2007).

Symptoms:

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Symptoms of arthritis are gradually developed. The first symptoms are often felt in small joints like fingers and toes, although shoulders and knees can be affected early, and muscle stiffness can be a prominent early feature (Scott et al., 2010) Symptoms of RA includes

- Joint pain with warmth, swelling, tenderness and stiffness of the joint after resting
- Morning stiffness that last for at least 1 hr.
- Low-grade fever.
- Inflammation of small blood vessels can cause small nodules under the skin, but they are generally painless.

In rheumatoid arthritis, destructive molecules produced by an abnormal immune system response which is responsible for continuous inflammation of the synovium. Collagen is gradually destroyed, narrowing the joint space and finally damaging bone. In a progressive rheumatoid arthritis, destruction of the cartilage accelerates. Further pannus (thickened synovial tissue) formation occurs due to the accumulation of fluid and immune system cells in the synovium.

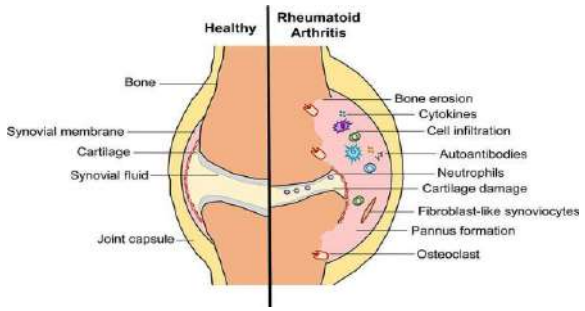


Fig.1.:Pathophysiology of Rheumatoid arthritis

Diagnosis

Diagnosing rheumatoid arthritis (RA) in the early stages can be difficult. There is no single test that can clearly identify rheumatoid arthritis. Instead, doctors diagnose rheumatoid arthritis based on factors that are strongly associated with the disease that is mentioned above.

Treatment:

The main aim of treatment is focused towards decreasing the disease activity or decreasing the inflamed condition with some remission if possible, along with a minimization of joint destruction and finally improving the physical condition and quality of life. Pharmacological Strategies: Generally, a strategic treatment plan is employed for the treatment of the disease which includes four different classes of drugs: non-steroidal anti- inflammatory agents (NSAIDs), corticosteroids, disease modifying anti-rheumatic drugs (DMARDs) and biological agents. The primary aim of this study is to investigate the phytochemical composition of Euphorbia nivulia extract, assess its anti-arthritic potential, and contribute to the understanding of its therapeutic properties. The specific objectives include:

Extraction of Phytochemicals:

- To extract bioactive compounds from Euphorbia nivulia using suitable solvents.
- To determine the phytochemical profile, including alkaloids, flavonoids, terpenoids, tannins, saponins, and other relevant constituents.

Phytochemical Screening:

- To conduct qualitative screening tests to identify and confirm the presence of specific phytochemical groups in the extract.
- To employ standard procedures for alkaloid, flavonoid, terpenoid, tannin, and saponin detection.

Anti-Arthritic Activity:

- To evaluate the anti-arthritic potential of Euphorbia nivulia extract using suitable in vivo models.

MATERIALS AND METHODS

Procurement of Plant Material

Leaves of fresh, well grown Euphorbia nivulia plant were collected during the month of feb 2024 from field near our collage and adjoining areas of Bhopal region, After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant.

Fig No 2 Euphorbia Nivulia Plant

Fig No 3 Fresh Leaves

Cleaning

After procurement of plant material, they were cleaned properly. The process involved the following steps. Firstly the decayed or deteriorated plant material was removed. This was followed by washing with tap water and distilled water. The washed plant material was wrapped in blotting paper in order to remove extra water.

Drying

Soon after cleaning, plant material was kept for drying under the shade. The main purpose of drying is to remove the water content from Leaves so that the Leaves can be stored. The dried Leaves were finely powdered using electric grinder, sieved and packaged in polyethylene bags until when needed.

Extraction by maceration method

Maceration was a popular and inexpensive homemade technique for the preparation of tonic since a long time (Mukherjee, 2007). Generally, the maceration procedure consists of multiple steps in extraction. The whole or coarsely powdered crude drug undergoes grinding to increase the surface area for proper mixing of powdered materials with the solvent. Above process is done in a closed vessel where an appropriate solvent (menstruum) is added. Next, the solvent is strained off followed by pressing the solid residue of the extraction process known as marc to recover an optimum amount of occluded solution. Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes:

1. Promotes diffusion,
2. Separates concentrated solution from the sample surface by adding new solvent to the menstruum for increasing the extraction yield.

The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant materials were subjected to extraction by ethanol solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using water bath. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

The percentage yield of each extract was calculated by using following formula:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered plant material}} \times 100$$

Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the extract of Euphorbia nivulia, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins,

flavonoids, steroids, proteins and amino acids (Kokate, 1994).

Determination of Alkaloids

S. No.	Identification test	Procedure	Observation
1	Mayer's Test	Test solution + Mayer reagent (Potassium mercuric iodide solution)	White or yellow precipitate
2	Dragendorff's Test	Test solution + Dragendorff's reagent (Potassium iodide + bismuth nitrate)	Showed orange red precipitate
3	Wagner's Test	Test solution + Wagner's reagent (iodine solution)	Brown or reddish brown precipitate
4	Hager's Test	Test solution + Hager's reagent (saturated solution of picric acid)	Gives characteristic crystalline ppt

Determination of Glycosides

S. No.	Identification test	Procedure	Observation
1	Raymond's Test	Test solution + 1 ml of 50% ethanol + 0.1% solution of dinitrobenzene in ethanol + 23 drops of 20% sodium hydroxide solution	Appearance of violet color, which changes into violet.
2	Killer Killani Test	2 ml of extract + glacial acetic acid + one drop of 5% $FeCl_3$ + conc. H_2SO_4.	Reddish brown color appeared at the junction of the two liquid layers and upper layer appeared bluish green.
3	Legal Test	Test solution dissolved in few drops of pyridine + a drop of 2% sodium nitroprusside + a drop of 20% sodium hydroxide solution.	Deep red color produced.

Identification tests for Carbohydrates

S. No.	Identification test	Procedure	Observation
1	Molisch's Test	2-3 ml. extract +few drops of α-naphthol solution (20% in ethyl alcohol) + 1 ml. conc. H_2SO_4 added along the side of the test tubes.	Violet ring was formed at the junction of two liquids.
2	Fehling's Test	Extract heated with dil. HCl +NaOH + Fehling's solution A & B	Brick red precipitate was formed
3	Benedict's Test	Extract +equal volume of Benedict's reagent. Heat for 5 min.	Solution appears Green, Yellow or Red

Identification tests for tannins

S. No.	Identification test	Procedure	Observation
1	Vanillin- HCl Test	Extract+ vanillin-HCl reagent (1 g vanillin + 10 ml. alcohol + 10 ml. conc. HCl)	Formation of pink or red color.
2	Gelatin Test	Extract solution + aqueous solution of gelatin	White buff color precipitate was formed.

Identification tests for flavonoids

S. No.	Identification test	Procedure	Observation
1.	Lead acetate test	Filter paper strip was dipped in the alcoholic solution of extract. Ammoniated with ammonia solution	Color changed from white to orange.
2.	Shinoda Test	Extract + 5 ml. 95% alcohol + few drops of conc. HCl + 0.5 g magnesium turning.	Pink color observed

Identification tests for resins

S. No.	Identification test	Procedure	Observation
1.	Color detection with ferric chloride	Extract + alcohol + few drops of $FeCl_3$ solution.	Green color appears
2	Turbidity Test	Extract solution (2 g of drug in methanol) +5 ml distilled water.	Turbidity appears

Identification tests for steroids

S. No.	Identification test	Procedure	Observation
1.	Liebermann- Burchard Test	2 ml. extract + Chloroform + 1-2ml. acetic acid + 2 drops H_2SO_4 from the side of the test tube	First red, then blue and finally green color appeared.
2.	Salkowski Reaction	2 ml. of extract +2 ml. chloroform + 2 ml. conc. H_2SO_4. Shake well.	Chloroform layer appeared red color and acid layer shows greenish fluorescence.

Identification tests for proteins and amino-acids

S. No.	Identification test	Procedure	Observation
1	Biuret's Test	3 ml. of extract + 4% NaOH + 2-3 drops of 1% copper sulphate solution.	Presence of red/violet coloration
2	Precipitation test	Mix with absolute alcohol White ppt. 3. Ninhydrin's Test Extract + Ninhydrin's reagent in boiling water bath for 10 min.	Violet color appeared.

Identification tests for phenol

S. No.	Identification test	Procedure	Observation
1	Ferric Chloride Test	3 ml. of extract + ferric chloride solution	Presence of bluish black colour.

Quantitative studies of phytoconstituents

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method. i.e 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter.02 ml (1mg/ml) of this extract was for the estimation of phenol. 02 ml of extract and each standard was mixed with 01 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 01 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added (Shamsa et al., 2008). The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

In-vivo anti-arthritic activity Materials and Methods

Animals

Albino Wistar rats of either sex (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55– 65%). Rats received standard rodent chow and water ad libitum. Animas were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rat was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Chemicals

Freund's complete adjuvant (Sigma-Aldrich Chemical Co.) was used for experiments.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD). Ethanolic extract of leaves of Euphorbia nivulia (5, 50, 300, and 2000 mg/kg) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-arthritic effect.

Anti-arthritis activity

Freund's adjuvant induced arthritis in rats: Animals were divided into five groups containing six animals each. Arthritic syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10mg of heat killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw.

Group I

Served as normal and received 2% gum acacia

Group II

Served as arthritis control-untreated received 2% gum acacia.

Group III

Received Aspirin (200 mg/kg p.o) served as reference standard

Group IV

Received extract of Ethanolic extract of Leaves of Euphorbia nivulia of doses of 100mg/kg p.o.

Group V

Received extract of Ethanolic extract of Leaves of Euphorbia nivuliaof doses of 200mg/kg p.o.

The drug treatment was started from 14th day of adjuvant induction and terminated on 28th day. The changes in paw volume was measured weekly by using Plethysmograph. At the end of experiment histopathology was done to check the inflammation.

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P<0.05$, $P<0.01$, and $P<0.001$ were considered to be statistically significant.

RESULT

Results of % yield of extracts

Among the polar solvents Ethanol used for extraction of Euphorbia nivulia. The percent yield, colour and consistency of each extracts have been summarized table 3.1,

Extracts	Colour	Consistency	Yield (%w/w)
Leaves of Euphorbia nivulia			
Pet ether	Dark brown	Semisolid	2.3%
Ethanol	Brown	Solid	5.8%

The evaluation of the % yield of crude extracts from the leaves of Euphorbia nivulia provides efficiency of the extraction process and the characteristics of the obtained extracts. In this study, two different solvents, namely petroleum ether and ethanol, were employed for extraction, each resulting in distinct yields and physical properties. The pet ether extract, characterized by its dark brown color and semisolid consistency, demonstrated a percentage yield of 2.3%. This suggests that the pet ether extraction method was less efficient in capturing the bioactive compounds present in the leaves of Euphorbia nivulia. The lower yield may be attributed to the selectivity of pet ether for specific types of compounds, potentially missing out on a broader range of phytochemicals. On the other hand, the ethanol extract exhibited a higher percentage yield of 5.8%, indicating a more effective extraction of bioactive constituents from the plant material. The

brown color and solid consistency of the ethanol extract suggest a concentration of compounds with different polarities compared to those extracted by pet ether. Ethanol is known for its ability to dissolve a diverse range of phytochemicals, including polar compounds like flavonoids and tannins. The variations in percentage yield and physical properties between the two extracts underscore the importance of solvent selection in optimizing the extraction process. It also highlights the complex nature of phytochemical composition in Euphorbia nivulia leaves, with different compounds being soluble in specific solvents based on their polarity.

Qualitative phytochemical tests for Euphorbia nivulia extract

Phytoconstituents	Results of phytochemical tests
i) Primary Metabolites	
Carbohydrates	Present
Amino acids	Present
Proteins	Present
Resins	Absent
ii) Secondary Metabolites	
Steroids	Absent
Glycosides	Absent
Flavonoids	Present
Tannins and Phenol	Present

The presence or absence of various phytoconstituents is indicative of the potential bioactive compounds that contribute to the medicinal properties of the plant. In the realm of primary metabolites, the presence of carbohydrates, amino acids, and proteins was detected in the Euphorbia nivulia extract. These compounds are fundamental to the plant's metabolic processes and are often associated with nutritive value. Carbohydrates, as energy-providing molecules, play a crucial role in various physiological functions. The presence of amino acids and proteins suggests the extract may contain essential building blocks for cellular structures and enzymatic activities. Moving to secondary metabolites, the absence of steroids and glycosides was observed in the phytochemical tests. Steroids are known for their diverse physiological roles, including anti-inflammatory properties, while glycosides often contribute to the plant's defense mechanisms. Their absence does not diminish the potential therapeutic value of the extract but provides specificity in understanding its chemical profile. Flavonoids, tannins, phenols, and alkaloids were detected in the Euphorbia nivulia extract. Flavonoids, recognized for their antioxidant properties, can contribute to the extract's ability to combat oxidative stress. Tannins and phenols, with their astringent properties, may influence the extract's pharmacological activities. Alkaloids, known for their diverse bioactivities, could be significant contributors to the overall medicinal potential of the extract The combination of these phytoconstituents suggests a complex chemical profile in the Euphorbia nivulia extract, indicative of its potential multifaceted therapeutic effects.

Quantitative study of bioactive compounds

Estimation of total phenolic content

Gallic acid is used as a standard compound and the total phenols were expressed as mg/100mg gallic acid equivalent using the standard curve equation: $y = 0.015x + 0.003$, $R2 = 0.999$, Where y is absorbance at 765 nm and x is total phenolic content in the ethanolic extract of Euphorbia nivulia. The results were expressed as the number of equivalents of Gallic acid (mg/100mg of extract).

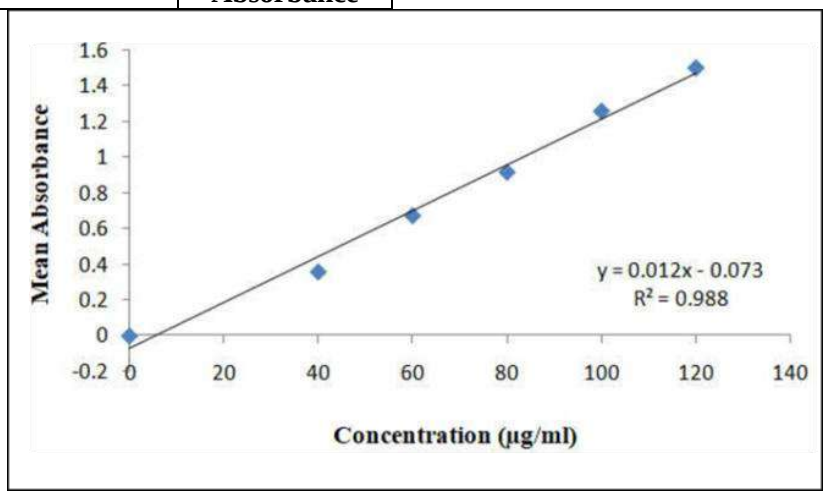
Sr. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	10	0.214
3	20	0.405
4	30	0.576
5	40	0.762
6	50	0.944

Graph of calibration curve of Gallic acid

Estimation of total alkaloid content

Alkaloid content was calculated from the regression equation of the standard plot y = 0.012x + 0.073, R2=0.998) and is expressed as Atropine equivalents (AE) (fig. 7.2). Total alkaloid content was (mg/100mg) quercetin equivalent in ethanolic extract of Euphorbia nivulia.

S. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	40	0.358
3	60	0.672
4	80	0.915
5	100	1.256
6	120	1.498



Graph of calibration curve of Atropine

Estimation of total phenolic and alkaloid content of Euphorbia nivulia

The estimation of total phenolic and alkaloid content in the Euphorbia nivulia extracts provides quantitative insights of bioactive compounds present in the plant. Phenolics and alkaloids are known for their diverse pharmacological activities, and their quantification adds valuable information to the overall understanding of the extract's potential health benefits. The ethanolic extract of Euphorbia nivulia exhibited a total phenol content of 0.225 mg/100 mg of dried extract. Phenolic compounds, including flavonoids, are renowned for their antioxidant properties. Antioxidants play a pivotal role in neutralizing free radicals and oxidative stress, thereby contributing to the prevention of various chronic diseases. The observed phenolic content suggests that the ethanolic extract of Euphorbia nivulia holds antioxidant potential, which could be harnessed for therapeutic applications. Simultaneously, the total alkaloid content in the ethanolic extract was found to be 0.165 mg/100 mg of dried extract. Alkaloids, with their diverse biological activities, contribute significantly to the pharmacological profile of medicinal plants. They are known for their analgesic, anti-inflammatory, and antimicrobial properties. The quantified alkaloid content in the Euphorbia nivulia extract indicates its potential to exert such bioactivities, supporting traditional uses of the plant in various folk medicine practices. The balance between phenolic and alkaloid content in the ethanolic extract suggests a potential synergy of these compounds, leading to a broader spectrum of bioactive effects.

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total alkaloid content(mg/100mg of dried extract)
1.	Ethanolic	0.225	0.165

Estimation of total phenolic and alkaloid content

Results of In Vivo Anti-arthritis activity

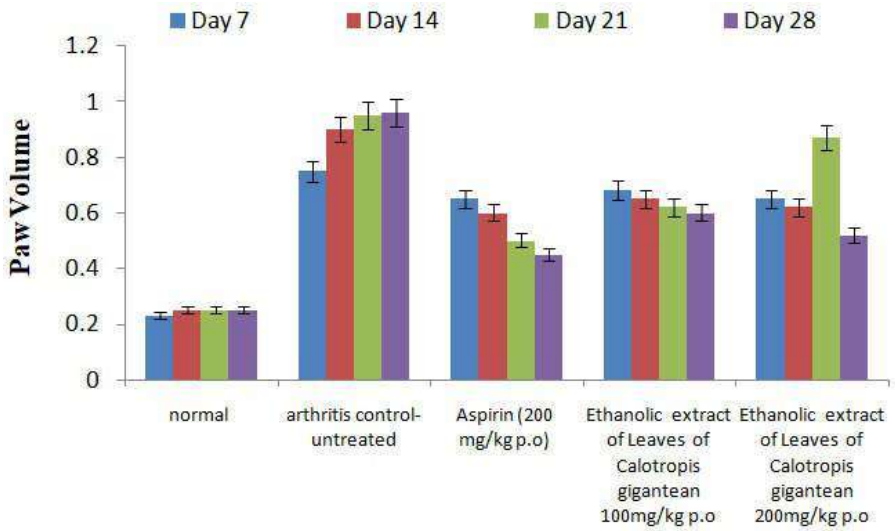
Paw Volume (mL)				
Group	**Day 7**	**Day 14**	**Day 21**	**Day 28**
I Normal	0.23±0.60	0.25±0.50	0.25±0.50	0.25±0.40
II Arthritis control untreated	0.75±0.20	0.90±0.35	0.95±0.30	0.96±0.40
III Aspirin (200 mg/kg p.o)	0.63±0.20	0.60±0.25**	0.50±0.50***	0.45±0.60***
IV Ethanolic extract of Leaves *niv*100mg/kg p.o	0.70±0.40	0.65±0.15*	0.62±0.50*	0.60±0.20*
V Ethanolic extract of Leaves *niv* 200mg/kg p.o	0.65±0.30**	0.63±0.40**	0.55±0.15***	0.52±0.40***

Values expressed as mean ± SEM (n=6) *P<0.05, **P<0.01, *** P<0.001 as compared to arthritis Control

Anti-arthritis activity of ethanolic extract of Leaves of Euphorbia nivulia against Freund's adjuvant induced arthritis in rats.

The evaluation of the anti-arthritis activity of the ethanolic extract of Leaves of Euphorbia nivulia against Freund's adjuvant-induced arthritis in rats involved the measurement of paw volume over a 28-day period. Arthritis, characterized by joint inflammation and swelling, was induced in the rats in Group II (arthritis control-untreated). The reference group (Group I) consisted of normal rats, while Groups III, IV, and V were treated with aspirin (positive control), ethanolic extract of Leaves of Euphorbia nivulia at 100mg/kg, and 200mg/kg, respectively. The results demonstrate a notable effect of the ethanolic extract of Leaves of Euphorbia nivulia on reducing paw volume, indicating its potential anti-arthritic activity. In comparison to the arthritis control group, both doses of the extract (100mg/kg and 200mg/kg) exhibited a significant decrease in paw swelling. This suggests that the extract may possess anti-inflammatory properties, supporting its traditional use in folk medicine for inflammatory conditions.

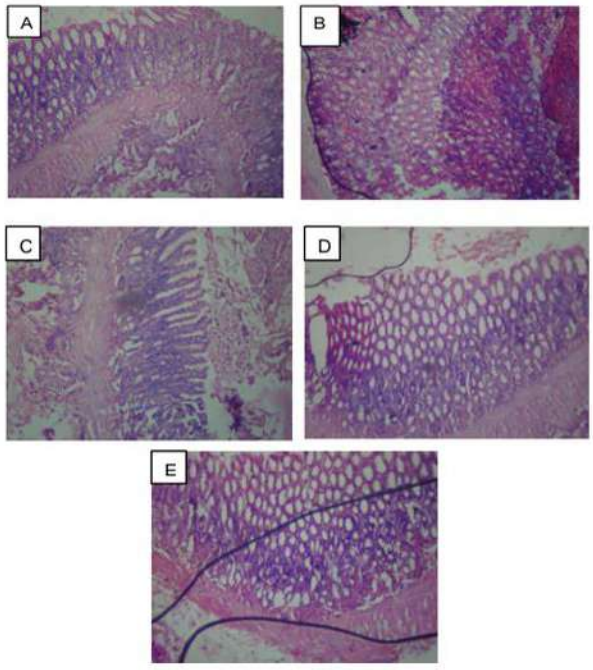
The positive control group treated with aspirin also showed a reduction in paw volume, reinforcing the anti-inflammatory potential of the ethanolic extract. The observed dose- dependent response, where the higher concentration (200mg/kg) demonstrated more pronounced effects, hints at the extract's concentration-dependent efficacy. The anti-arthritic activity could be attributed to the presence of bioactive compounds such as flavonoids, alkaloids, and phenolic compounds, as indicated by the qualitative phytochemical tests and quantitative estimation of total phenols and alkaloids. These compounds are known for their anti-inflammatory and analgesic properties, which could contribute to the observed therapeutic effects.



The ethanolic extract of Leaves of Euphorbia nivulia exhibits promising anti-arthritic activity in a Freund's adjuvant-induced arthritis model in rats. Further investigations into the specific mechanisms and active compounds responsible for this activity are essential for understanding the extract's potential as a natural remedy for arthritis.

Histopathology

Soft tissue swelling, massive influx of inflammatory cells and accumulation of abundant mononuclear cells, bone demineralization, cartilage erosions, and joints space narrowing were observed in the arthritic control group. In local collection of lymphocytes and bony trabeculae are seen at the extract of ethanolic extract of Leaves of Euphorbia nivulia of doses of 100mg/kg p.o. whereas mature lamellar bone was found in extract of Ethanolic extract of Leaves of Euphorbia nivulia of doses of 200mg/kg p.o.and with the standard drug.



Histopathology Observation: (A) normal; (B) control; (C) standard; (D) Ethanolic extract of Leaves of Euphorbia nivulia 100 mg/kg; (E) Ethanolic extract of Leaves of Euphorbia nivulia 200 mg/kg

SUMMARY AND CONCLUSION

Summary

The study aimed to investigate the phytochemical constituents, anti-arthritic potential, and histopathological effects of the ethanolic extract of Leaves of Euphorbia nivulia in a Freund's adjuvant-induced arthritis model in rats. The extraction process using ethanol resulted in a brown, solid extract with a yield of 5.8%. Qualitative phytochemical tests revealed the presence of primary metabolites such as carbohydrates, amino acids, and proteins, while secondary metabolites included flavonoids, tannins, phenols, and alkaloids. The total phenol content of the ethanolic extract was estimated to be 0.225 mg/100mg of dried extract, and the total alkaloid content was found to be 0.165 mg/100mg of dried extract. Calibration curves for gallic acid and atropine were prepared to quantify the phenolic and alkaloid content, respectively. The anti-arthritic activity was assessed by measuring paw volume over 28 days. The extract, administered orally at doses of 100mg/kg and 200mg/kg, demonstrated a significant reduction in paw swelling compared to the arthritic control group. The effects were dose- dependent, with the higher concentration showing more pronounced results. Aspirin, the standard drug, also exhibited anti-arthritic activity. Histopathological examination further supported the anti-arthritic effects. The arthritic control group displayed typical signs of arthritis, including soft tissue swelling, inflammatory cell influx, bone demineralization, and joint space narrowing. However, treatment with the ethanolic extract at both doses and the standard drug showed improvements, including a reduction in lymphocyte accumulation and evidence of mature lamellar bone. The ethanolic extract of Leaves of Euphorbia nivulia exhibits promising anti-arthritic activity in the experimental model. The observed effects may be attributed to the presence of bioactive compounds detected in phytochemical screening. Further research is warranted to isolate and identify specific compounds responsible for the anti-arthritic effects and to explore the underlying mechanisms. The study provides valuable insights into the therapeutic potential of Euphorbia nivulia in managing arthritis, contributing to the development of natural and effective anti-arthritic agents.

CONCLUSION

Complete Freund's Adjuvant induced arthritis and anti-arthritic property of the extract Ethanolic extract of Leaves of Euphorbia nivulia. The effect of extract Ethanolic extract of Leaves of Euphorbia nivulia were determined after administration at two dose level (100 and 200 mg/kg b.w.) in arthritis induced rats and assessed by histopathological studies. From the results, it may be concluded that herbal ethanolic extract of leaves of Euphorbia nivulia possess significant anti-arthritic effect may be due to the effect of antioxidants like Flavonoids, Poly Phenols and Saponins present in the plant. All these biological activities may be said to be a promising findings brought out by the present study. These contributions can be used as parameters for the authentication of plant as well as for developing newer drugs based on their activity. It can be optimistic that the present work suggests an herbal drug of multiple therapeutic advantages and likely to be a powerful anti- arthritic drug.

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