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Evaluation of Anti-Arthritic Potential of The Plant Extract of *Solanum melongena* in Freund's Complete Adjuvant- Induced Arthritis in Wistar Rats

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ABSTRACT

The objective of the study was to evaluate antiarthritic activity of ethanolic extract of *Solanum melongena* in Freund's complete adjuvant (FCA) induced arthritis in female Wistar rats. Ethanolic extract was prepared by maceration and subjected to preliminary phytochemical screening and tested against FCA induced arthritis in rats. Arthritis assessment was done by measuring- paw volume, joint diameter, body weight, arthritis score, pain threshold, thermal hyperalgesia, haematological and biochemical parameters. The ethanolic extract was administered at concentrations of 200 and 400 mg/kg body weight. Extract (200 and 400 mg/kg) significantly ($p<0.01$ and $p<0.001$, respectively) decreased paw volume, joint diameter and increased pain threshold, body weight and paw withdrawal latency compared to arthritic control group. Ethanolic extract (200 and 400 mg/kg) exhibits significant ($p<0.01$ and $p<0.001$, respectively) antiarthritic activity by increasing the haematological parameters like red blood cell (RBC), haemoglobin (Hb) and decreasing the white blood cell (WBC), platelets and serum C-reactive protein (CRP). The levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase were decreased and the level of total protein was increased by treatment with ethanolic extract (200 and 400 mg/kg). The present study suggests that *Solanum melongena* has antiarthritic potential, and it might be attributed to the phytoconstituents such as tannins, flavonoids and phenolic compounds present in the extract.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease which includes pathological changes such as hyperplasia of synovial membrane, infiltration of inflammatory cells, neovascularization, cartilage erosion and joint destruction (Chunxia et al. 2011). The prevalence of RA is about 1% of the world population and the epidemiology of arthritis in male to female ratio is 3:1 (Narendhirakannan et al. 2007). Cytokines play a major role in inflammation and damage the joint leading to tissue destruction during the development of RA which includes the tumor necrosis factor a (TNF-a), interleukin (IL) IL-1b and IL-6 (Yeom et al. 2006). Non-steroidal anti-inflammatory drugs (NSAID) and disease modifying anti rheumatoid drugs (DMARD) have many applications in treating diseases but are associated with side effects like gastrointestinal tract complications, ulcers and cardiovascular problems (Emmanuel & Montgomery 1971; Singh et al. 1996; Gaffo et al. 2006). Major issues of the currently available medicines for RA include poor efficacy, potential side effects and high cost of biological agents. Thus, an efficient and safe alternative from herbs has drawn special attention from scientists worldwide.

STUDY PLAN

Procurement of animals

Adult albino female rats of Wistar strain (150-200 g) were used in the pharmacological and toxicological studies. The animals were maintained and acclimatized to animal house conditions using paddy husk bedding at 25 ± 2^0C temperature and 50 ± 5% humidity with day night cycle (12 ± 1 h) in solid bottomed polypropylene cages. The rats were fed with balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai, India and water ad libitum was provided throughout the experimental period. The animals were sheltered for a week and prior to the experiment they were acclimatized to laboratory temperature. Food was withdrawn 2 hours before and during experimental duration. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline.

TOXICITY STUDIES

Acute oral toxicity study

Acute toxicity studies were conducted in female albino rats (150-200 g) body weight by *Staircase Method* of Ghosh [58]. The Experimental animals were subjected to acute oral toxicity studies as per revised OECD Organization of Economic Co-operation and Development guidelines (OECD No. 423) and acute class method.

IN VIVO ANTI-ARTHRITIC STUDY

Chemicals

Freund's complete adjuvant (Sigma Aldrich) and all other chemicals and reagents used for the study were of analytical grade procured from approved organization.

Animals

Female Wistar rats of body weight 150–200 g were used for the study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water ad libitum. All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines and all the experimental procedures were approved by IAEC.

Induction of arthritis

To induce arthritis, animals were first anesthetized with a small amount of ether vapor, and then a single injection of 0.2 ml Complete Freund's adjuvant dissolved in mineral oil (sterile) was injected delicately into the sub-plantar region of hind paw.

Treatment regimen

The anti–arthritic activity was performed according to Jubie et al. [59] method. After classifying and grouping animals according to their weight, each animal was marked and placed in a cage with a letter identifying the cage.

The animals were divided into five groups of six animals each. Each group was given a dose schedule as follows:

1. Group I -Vehicle control, 1% w/v DMSO, p.o; (nonarthritic);
2. Group II - Negative control (0.1ml Complete Freund's adjuvant);
3. Group III -Arthritic animals treated with standard, 0.75 mg/kg Methotrexate, p.o;
4. Group IV - Arthritic animals treated with 200 mg/kg of *Solanum melongena*, p.o;
5. Group V -Arthritic animals treated with 400 mg/kg of *Solanum melongena*, p.o;

Vehicle control animals were given 1% w/v of DMSO solution daily.

Negative control group was given a single injection of 0.2 ml Complete Freund's adjuvant in mineral oil into the sub-plantar region of hind paw on day 1 under light ether anesthesia.

PARAMETERS:

1. Paw edema volume

Procedure:-

Mercury Plethysmometer should be filled with mercury to a designated level. A mark was made on both the legs at lateral malleolus to facilitate uniform dipping and recording of paw volumes. The animal's paw was dipped into one column and the displacement of mercury was noted when the paw was dipped in mercury column up to a predetermined mark on the paw. The difference in paw volume indicates the degree of inflammation.

Evaluation:-

The hind paw volumes of all the animals were measured just before Freund's complete adjuvant injection on day 0 and thereafter at different time intervals (day 4, 13, 25, 40) using a plethysmometer instrument [12, 13 & 14]. The paw volume changes were calculated by subtracting initial paw volumes from the final paw volumes.

$$\text{Paw volume} = \frac{Vc - Vo - (Vi - Vo)}{Vc - Vo} \times 100$$

Where,

V_c = Paw volume after induction V_o = Paw volume before induction V_i= Paw volume after treatment

2. Arthritic score

Visual observation:-

The degree of arthritis was continuously monitored on day 0, 4, 13, 25 and 40 after injection of Freund's adjuvant. The arthritic score was monitored by set visual criteria and changes in the morphological feature of arthritis like redness, swelling and erythema was noted and scoring was done. [60,61]

3. Locomotor activity

Procedure:-

The locomotor activity of rats was recorded individually for each animal. Body movement was measured by observing the time taken by individual animal to move a certain distance. It was recorded on day 0, 4, 13, 25, 40 of the experiment.

Evaluation:-

The locomotor activity of an individual animal was measured and recorded.

4. Body weight

Procedure:-

Measuring balance was calibrated and the mark was kept at zero. Each animal was placed in the balance and the weight was recorded.

Evaluation:-

Body weight was recorded at different time intervals (day 0, 4, 13, 25, 40). Changes in body weight was calculated.

***IN VITRO* ANTI-ARTHRITIC STUDY**

1. Biochemical estimations

On day 41, after anesthesia (using ether vapor), cardiac puncture was done and a centrifuge tube was introduced to withdraw blood. Blood with and without anticoagulant was centrifuged for 15 min (3000 rpm) and the plasma and serum was collected. Total proteins such as albumin and globulin, Rheumatoid factor (R_f) and C-Reactive protein (CRP) levels were quantified.

Procedure:

A 50 µl aliquot of blood plasma was transferred to a test tube and the volume was made up to 2.0 ml with distilled water. A 50 µl aliquot of this solution was transferred to a second test tube and the volume was adjusted to 2.0 ml with 0.2 mol L^{-1} of acetic acid. Standard curve was prepared by taking 0.0, 20.0, 40.0, 60.0, 80.0, 100.0 and 120.0 µl of standard solution of BSA (1.5 g L^{-1}), a calibration curve with the concentrations from 0.0 to 84.0 µg mL^{-1} was obtained, the volumes were adjusted to 2.0 ml with acetic acid (0.2 mol L^{-1}). After, in all tubes, 100 µl of TBPEE (0.005% m/v) was added, shaken and incubated at 37ºC for 10 minutes. The tubes were then cooled to room temperature and, after 30 minutes the absorbances at 610 nm were read against the blank (0.0 µg mL^{-1}).

2. Protein Denaturation Inhibition Study

Procedure:

The method of protein denaturation was conducted as per the method described by Mizushima and Kobayashi [62]. The reaction mixture consisted of0.45 ml of bovine serum albumin (5% w/w aqueous solution) and 0.05ml of ethanolic extracts of *Solanum melongena* at variable concentrations ranging from 100 to 1000 µg in 10% v/v of polyethylene glycol. The pH adjustment was done to 6.3 by adding 0.1N HCl and the samples were incubated at 37^0C for 20

min and then heated at 57˚C for 3min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. The resulting turbidity was measured at 660 nm spectrophotometrically. For control tests, 0.05 ml distilled water was used instead of plant extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows. The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (positive control) in the present investigation.

$$\text{Percent Inhibition} = 100 - \frac{(O.D\ of\ test - O.D\ of\ product\ control)}{O.D\ of\ control} \times 100$$

3. Proteinase Inhibition Study

Procedure:

The proteinase enzyme inhibitory assay was studied by the method described by Oyedapo et al. [63]. The reaction mixture contained 0.06 mg trypsin, 1.0 ml of 25 mM Tris–Hydrochloric acid buffer (pH 7.4) and 1.0 ml of ethanolic extracts of *Solanum melongena* at variable concentrations ranging from 100 to 1000 µg in polyethylene glycol. The mixtures were incubated at 37^0 C for 5 minutes and then 1.0 ml of 0.8% (w/v) casein was added.

The mixtures were incubated for additional 20 minutes and 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged and absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated. The results were compared with acetyl salicylic acid (250 µg/ml) treated samples.

$$\text{Percent inhibition} = 100 - \frac{O.D\ of\ test - O.D\ of\ product\ control}{O.D\ of\ control} \times 100$$

OTHER METHODS

1.Histological analysis of ankle joints

The animals were sacrificed on day 41, the ankle joints were removed and preserved in 10% buffered formalin for 24 hours. It was followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 50 µm thickness. The sections were stained with hematoxylin and eosin H & E [64] and evaluated under light microscope for the presence of hyperplasia of synovium, pannus formation and destruction of joint space.

2. X-ray radiography

Rats were anaesthetized by intraperitoneal injection of 50mg/kg pentobarbitone sodium on day 41. Radiographs were taken with X-ray apparatus for lateral and mediolateral projection. The severity of the joint and bone deformation was blindly scored according to the extent of osteoporosis, joint spaces, osteophytes and joint structure [65,66] on a scale of 0-4.

STATISTICAL ANALYSIS

The data was analyzed in terms of Mean ± Standard error of Mean (SEM). For statistical analysis, multiple comparisons of data were made using one and two way analysis of variance (ANOVA) followed by Dunnet‟s test was used for post hoc analysis. Significance was statistically acceptable at a level of $P < 0.05$. Software program GraphPad Prism was used for all data analysis. [67]

RESULTS

Acute toxicity results:

No toxic symptoms were observed after administration of different dose levels of extract up to maximum of 2000mg/kg p.o. according to OECD guideline 423; and in addition, the higher dose of 2000mg/kg dose was administered to a group of animals. No symptoms or

adverse events were identified. Hence, safe tolerable dose was used as therapeutic dose for further pharmacological study. From this experiment, the minimum and maximum therapeutic dose level of ESM extracts were studied as 200mg/kg and 400 mg/kg.

***In vivo* results**

1. Paw edema volume

In FCA induced arthritis model, rats developed a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage, bone destruction and remodeling. These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal[68].

The extract of *Solanum Melongena.* at 400 mg/kg inhibited rat paw edema which is comparable with standard drug methotrexate at 40th day. The results of which are shown in Table 6. The determination of rat paw swelling is apparently simple, sensitive and one of the quick procedures for evaluating the degree of inflammation and the therapeutic effects of drugs. The chronic inflammation involves the release of number of mediators like cytokines, GM-CSF, interferons and PGDF. These mediators are responsible for pain and destruction of bone, cartilage that can leads to severe disability.

2. Arthritic score

Picture 1 depicts the scoring of arthritis by set visual observation of the respective groups of animals after drug treatment and the results are as follows:

3. Locomotor activity and body weight

As the incidence and severity of arthritis increased, there were changes in the body weights of the rats during the course of the experimental period. The loss of body weight during arthritic condition was also supported by earlier observations [69], on alterations in the metabolic activities of diseased rats.

The body weight in standard group almost remained same during 40 days of study. In the low dose and high dose (200 mg/kg and 400 mg/kg) group of animals, body weight declined after 9 days of study and significant loss of weight was observed on 18th and 25th day.

Methotrexate treatment did not produce any significant change in body weight. In non-treated group of rats, no significant change in behaviour was observed. During 9th and 18th day of study, significant decrease in the movement of rats were noted both in plant extract and standard. However, on 40th day of study restoration of the normal movement was observed when compared with non-treated groups. The results of which are shown in table 7.

Table No. 1: Effect of ethanolic extracts of LDSM and HDSM on CFA induced arthritic rats showing changes in body weight and locomotor activity

Group	Physical and behavioural changes									
	0th day		9th day		18th day		25th day		40th day	
	BW (gms)	M (sec)	BW (gms)	M (sec)	BW (gms)	M (sec)	BW (gms)	M (sec)	BW (gms)	M (sec)
Control	180±0.02	20	175±0.01	22	170±0.01	24	173±0.02	22	174±0.01	23
Negative control	170±0.01	20	160±0.01	30	150±0.02	35	100±0.02	50	92±0.02	55
Standard	170±0.01	20	160±0.02	30	140±0.01	32	130±0.01	32	175±0.01	25
LDSM	160±0.02	20	150±0.02	30	140±0.02	30	120±0.01	40	100±0.01	40
HDSM	200±0.02	20	180±0.02	25	160±0.02	25	165±0.01	30	195±0.01*	25

Values are expressed in mean ± SEM, n =6 ,*p < 0.05 are considered significant compared to standard. LDSM (200 mg/kg); HDSM (400 mg/kg); BW – Body weight; M – Movement

***In vitro* results**

1. Effect of *S. Melongena* extracts on Biochemical parameters

The results in Table 8 shows that, in untreated animals (negative control group), serum levels of CRP and R_f significantly increased ($P < 0.001$) and total protein level significantly decreased ($P < 0.001$) compared to the parameters of animals of the healthy group. In animals treated with extracts or methotrexate, all biochemical parameters evaluated tend to return to normal values.

2. Protein Denaturation Inhibition Study

Anti–arthritic effect of ESM extract was studied significantly by testing various *in vitro* parameters. Table 9 depicts the inhibition of protein denaturation of different extracts. In the present investigation, all three extracts inhibited the protein denaturation in a dose dependent manner. However, the ESM has got a higher inhibitory percentage of protein denaturation when compared ($p>0.05$) to the positive control. At the concentrations of 400, 500, 800 and 1000µg/ml, the inhibitory percentage of ESM was significantly comparable to the positive control Acetyl salicylic acid used in this present investigation.

3. Protease Inhibition Study

The proteinase inhibitory activity of the ESM extracts was shown in Table 10. Both the ESM extract and the positive control acetyl salicylic acid exhibited a dose dependent anti–proteinase activity. At concentration of 800 µg/ml the ESM exhibited higher anti–proteinase activity of 75.18% when comparable ($p>0.05$) to the positive control which showed 73.69% of anti–proteinase activity at the same concentration. But at the maximum concentration of 1000µg/ml ESM showed 82.96% protease inhibition against the positive control acetylsalicylic acid which showed the inhibition of 85.90% at the same concentration.

Other methods results:

Analysis of histopathology of ankle joints

Histopathology of the ankle joint of healthy control rats revealed no inflammation, a few lymphocytes infiltration and no bone necrosis. A massive influx of inflammatory cells, cartilage destruction, proliferation of granulation tissue, lymphocytes infiltration and chronic inflammation was detected in arthritic control. In contrast to these pathological changes, animals having received ethanolic extracts of *S. Melongena* or indomethacin showed significant protection against necrosis of bones with low influx of inflammatory cells and minimal bone damage compared.

X-ray radiology of hind paws

Group 1

- Uninjected control group with no degenerative joint changes

Group 2

- Excess soft tissue volume, joint space, sub-chondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes.

Group 3

- Moderate soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes.

Group 4

- Pronounced soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes.

Group 5

- Low to moderate soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes.

Table No. 2: EFFECT OF *SOLANUM MELONGENA* EXTRACTS ON CHANGES IN PAW VOLUME INCFA INDUCED ARTHRITIS IN RATS

Paw edema volume

Group	0th day	9th day	18th day	25th day	40th day
Control	0.65 ± 0.01	0.66 ± 0.01	0.65 ± 0.02	0.67 ± 0.01	0.67 ± 0.02
Negative control	0.66 ± 0.02	0.75 ± 0.01	0.80 ± 0.01	0.86 ± 0.02	0.91 ± 0.02
Standard	0.59 ± 0.01	0.48 ± 0.01	0.43 ± 0.02	0.40 ± 0.01	0.35 ± 0.02
200 mg/kg MP	0.62 ± 0.02	0.51 ± 0.01	0.42 ± 0.02	0.40 ± 0.01	0.38 ± 0.01*
400 mg/kg MP	0.58 ± 0.02	0.42 ± 0.01	0.36 ±0.01*	0.32 ±0.01**	0.27 ± 0.01*

Values are expressed in mean ± SEM, n = 6, *p<0.05 are significant compared to standard, LDSM (200 mg/kg); HDSM (400 mg/kg).

Table No. 3: Effect of ethanolic extracts of LDSM and HDSM on serum parameters in CFA induced arthritic rats

Group	CRP (mg/l)	R_f (IU/ml)	Total Protein (g/dl)
Control	1.65 ± 0.01	-	8.0 ± 0.54
Negative control	6.92 ± 0.22**	58.01 ± 1.50	5.4 ± 0.15*
Standard	3.68 ± 0.30**#	39.15 ± 0.23#	7.6 ± 0.42*
LDSM	4.12 ± 0.18**#	40.03 ± 0.02#	7.1 ± 0.43
HDSM	3.05 ± 0.05*#	36.01 ± 1.51#	7.8 ± 0.25#

Each value represents the mean ± SEM for ANOVA, n=6, *p<0.05, **p<0.001 when compared to healthy control,#p<0.001 when compared to negative control.

Table No. 4: Protein Denaturation Inhibition Study

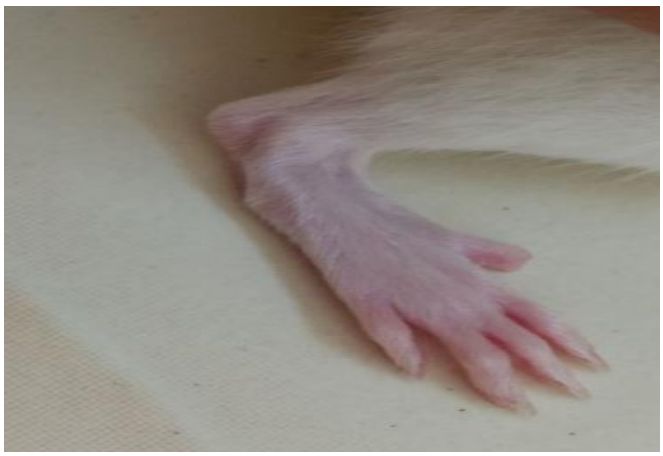
Concentration (mg/ml)	Inhibitory activity of ESM	Inhibitory effect of Acetyl Salicylic Acid (%)
100	24.46 ± 1.12	24.45 ± 1.70
200	33.62 ± 3.08	32.14 ± 2.21
400	45.76 ± 1.98	41.77 ± 1.52
500	54.35 ± 2.37	45.33 ± 2.18
800	76.48 ± 1.92	69.71 ± 2.43*
1000	87.65 ± 3.01	85.17 ± 2.13*

Values are expressed in mean ± SD(n=6), *p<0.05. Statistical significant test for comparison was done by ANOVA followed by Dunnet"s „t" test. Comparison between acetylsalicylic acid vs ESM

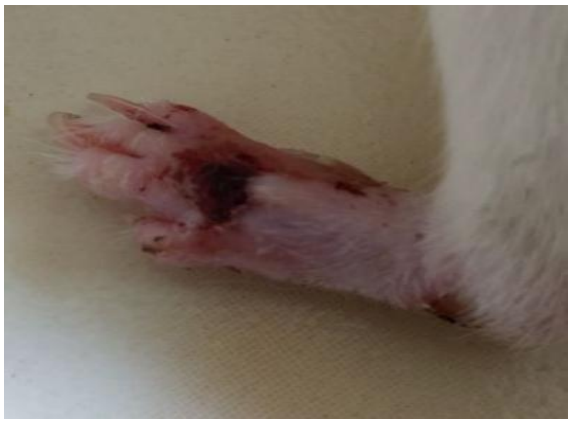
Table No. 5: Protease Inhibition Study

Concentration (mg/ml)	Inhibitory activity of EMP	Inhibitory activity of Acetyl salicylic acid (%)
100	21.33 ± 2.18	21.88 ± 1.90
200	24.21 ± 2.00	27.21 ± 2.31
400	38.75 ± 3.12	41.75 ± 1.59
500	45.28 ± 1.65*	46.70 ± 2.58
800	75.18 ± 1.32*	73.69 ± 1.25
1000	82.96 ± 1.72	85.90 ± 1.10

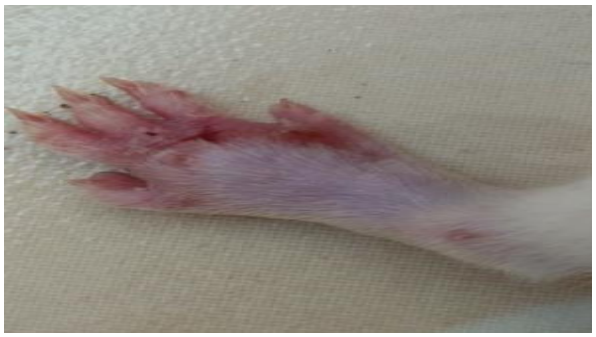
Values are expressed in mean ± SD(n=6), *p<0.05. Statistical significant test for comparison was done by ANOVA followed by Dunnet‟s „t‟ test. Comparison between acetylsalicylic acid vs ESM.



Group 1



Group 2



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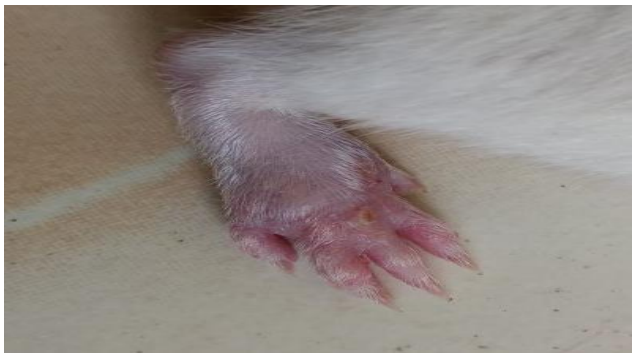

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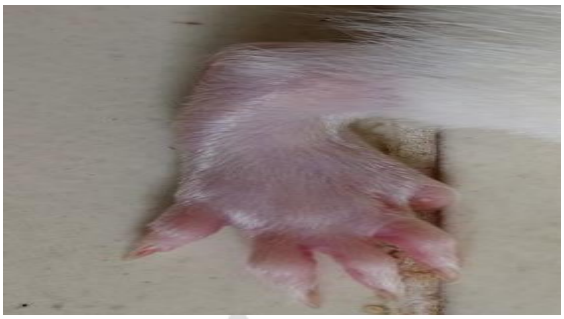
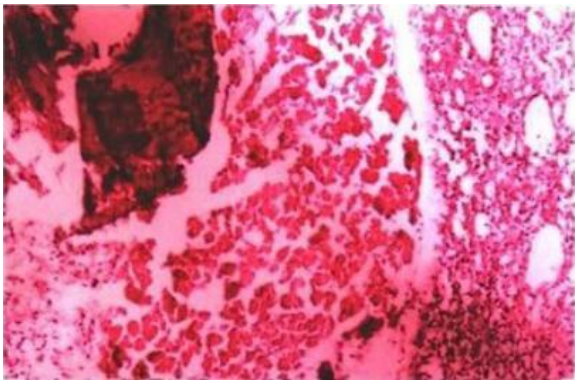


Group 5

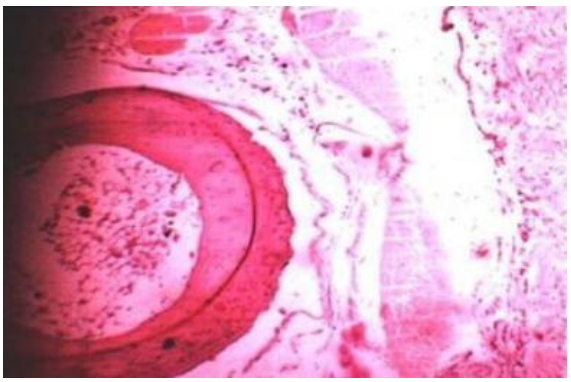
PICTURES

1. **Arthritic score:**

2. **Analysis of histopathology of ankle joints**



Group 1



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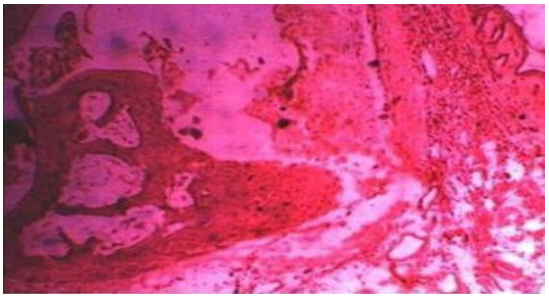

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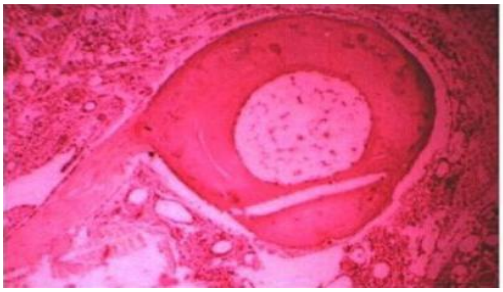

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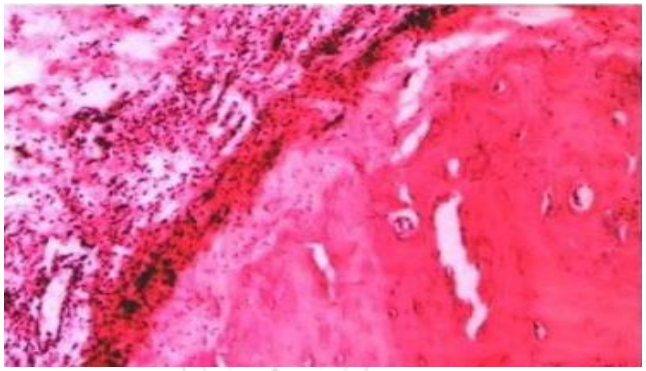
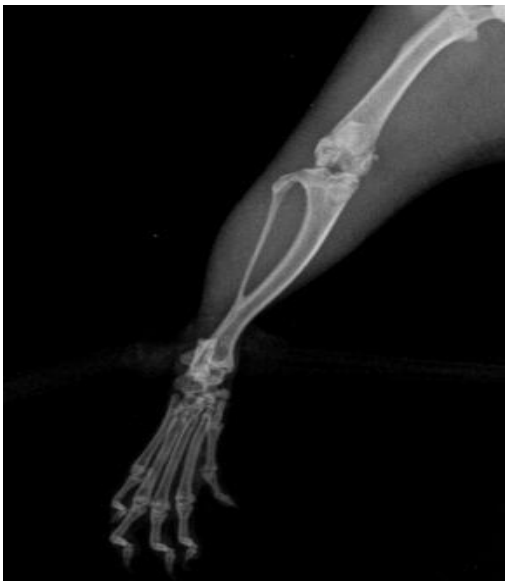
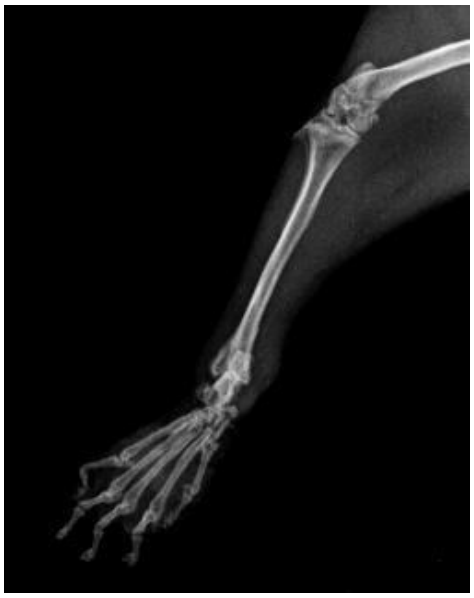


Group 5

3. Radiography of ankle joints



Group 1



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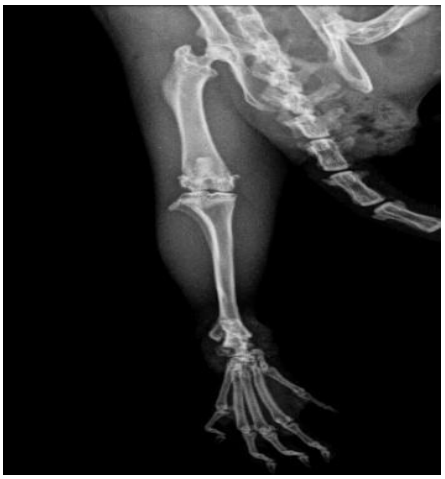

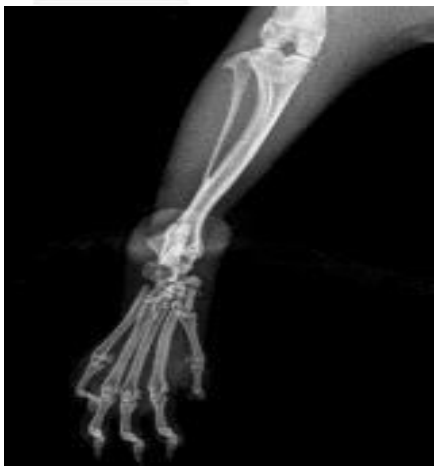

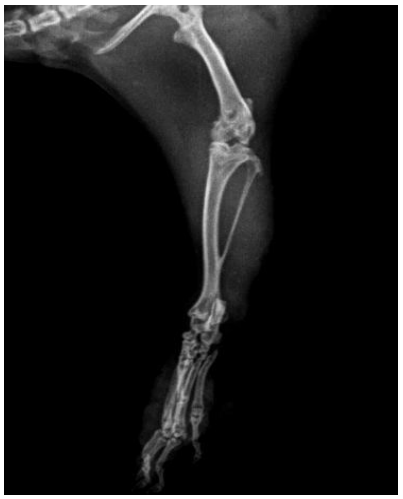

Group 3

DISCUSSION

Rheumatoid arthritis is an inflammatory, autoimmune disorder which destroys its own immune system. The immunologically mediated Complete Freund's adjuvant induced arthritic model of chronic inflammation is considered as the best available experimental

model of rheumatoid arthritis[70]. Complete Freund's adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble rheumatoid arthritis.

In Complete freund's adjuvant-induced arthritis model, rats developed a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling which have close similarities to human rheumatoid disease. These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal. Also, Complete Freund's adjuvant administered rats showed soft tissue swelling around the ankle joints during the development of arthritis, which was considered as edema of the particular tissues.

Paw swelling is an index of measuring the anti-arthritic activity of *Solanum melongena* at the dose level 200&400 mg/kg, p.o. *Solanum melongena* administered groups showed marked reduction in paw volume when compared with the Negative control group (Group II). It was also found that there was significant weight loss when compared to standard[71]. The result of the present study also indicates that there is a close relationship between the extent of inflammation, loss of body weight and arthritic index. The arthritic scoring was done on the basis of visual observation where it can be seen that there is a marked reduction in the swelling and joint damage of the drug treated groups[72]. It was also noted that the high dose *Solanum melongena*. Extract proved its efficacy to reduce the inflammation of the paws. The locomotor activity of the animals was improved in Group 5 animals (HDSM) when compared to the standard animals.

Assessment of the levels of serum parameters provides an excellent and simple tool to measure the anti-arthritic activity of the target drug. The total protein such as albumin and globulin was comparatively equal in all the three groups such as control, plant extract and standard[73]. The C-Reactive protein levels of the plant extract and standard was marginally equal but higher than the control values. Rheumatoid factors are proteins produced by our immune system that can attack our own healthy cells of the body. When the levels rise, it might relate to some form of auto immune diseases. The R_f value of the high dose plant extract was excellent when compared to that standard.

Histopathology provides a noticeable morphological distinctiveness as a practical and unambiguous pathognomonic sign of Rheumatoid arthritis. The histopathological analysis identified the ability of the bones to re-form upon treatment with *Solanum melongena*. Bone structures re-calcified upon treatment with the *Solanum melongena* dose dependently. The high dose of the plant extract exhibited good therapeutic potential from the study results and is therefore consistent with earlier findings that the ability of a drug to suppress inflammation, synovitis and protect a joint is desired in rheumatoid arthritis therapy.

Radiographic changes in Rheumatoid arthritis conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis[74]. The radiographic features of the rat joints in adjuvant induced arthritic model are shown in **Picture 3**. In Freund's adjuvant induced arthritic rat (group II), soft tissue swelling along with narrowing of the joint spaces was severe which implies the bony destruction in arthritic condition. The standard drug Methotrexate (0.75 mg/kg) treated groups have prevented this bony destruction and also there is moderate swelling of the joint. Similarly, according to histopathological studies, extracts of *Solanum melongena* have shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces in the 40 days of treatment when compared with Complete Freund's adjuvant (Negative control group).

CONCLUSION

The given plant *Solanum melongena* provides essential compounds with active principles, having no or minimum side effects holds prospect in future rheumatoid arthritis treatment. From the above review, it should be manifest that there are many medicinal plants which exert anti-arthritic activity at a particular dose.

The preliminary phytochemical studies discovered the presence of various phytoconstituents. *In vivo* study was performed with parameters such as paw edema volume, physical and behavioural changes and arthritic index and the extract possessed a significant effect on inflammation and joint destruction. The biochemical analysis was assessed by estimating the serum values which provided favourable effects. Invitro

study showed the effect of the plant extract on the percentage inhibition of protein denaturation and protease enzymes which gave marked responses. Other methods such as histopathology and the radiographic X-ray analysis of the groups showed good results.

In conclusion, this study has verified that constituents of the plant suppressed the joint inflammation and destruction in adjuvant arthritic rats. We are confident that our data provide mechanistic evidence for anti-arthritic appliance of the plant as a promising candidate for novel therapeutic agent of Rheumatoid arthritis.

REFERENCES

1. Akanitapichat, P., Phraibung, K., Nuchklang, K., & Prompitakkul, S. (2010). Antioxidant and hepatoprotective activities of five eggplant varieties. *Food Chemical Toxicology*, 48(10), 3017–3021. doi: 10.1016/j.fct.2010.07.045 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
2. Azevedo, L., Alves de Lima, P. L., Gomes, J. C., Stringheta, P. C., Ribeiro, D. A., & Salvadori, D. M. (2007). Differential response related to genotoxicity between eggplant (*Solanum melanogena*) skin aqueous extract and its main purified anthocyanin (delphinidin) in vivo. *Food and Chemical Toxicology*, 45(5), 852–858. doi: 10.1016/j.fct.2006.11.004 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
3. Azuma, K., Ohyama, A., Ippoushi, K., Ichiyanagi, T., Takeuchi, A., Saito, T., & Fukuoka, H. (2008). Structures and antioxidant activity of anthocyanins in many accessions of eggplant and its related species. *Journal of Agricultural and Food Chemistry*, 56(21), 10154–10159. doi: 10.1021/jf801322m [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
4. Berridge, M. V., Herst, P. M., & Tan, A. S. (2005). Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnology Annual Review*, 11, 127–152. doi: 10.1016/S1387-2656(05)11004-7 [Crossref], [PubMed], [Google Scholar]
5. Bogdan, C. (2001). Nitric oxide and the immune response. *Nature Immunology*, 2(10), 907–916. doi: 10.1038/ni1001-907 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
6. Bray, H. G., & Thorpe, W. V. (1954). Analysis of phenolic compounds of interest in metabolism. *Methods of Biochemical Analysis*, 1, 27–52. doi: 10.1002/9780470110171.ch2 [Crossref], [PubMed], [Google Scholar]
7. Chien, H. Y., Lu, C. S., Chuang, K. H., Kao, P. H., & Wu, Y. L. (2015). Attenuation of LPS-induced cyclooxygenase-2 and inducible NO synthase expression by lysophosphatidic acid in macrophages. *Innate Immunity*, 21(6), 635–646. doi: 10.1177/1753425915576345 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
8. Cho, B. O., Ryu, H. W., So, Y., Lee, C. W., Jin, C. H., Yook, H. S., … Jeong, I. Y. (2014). Anti-Inflammatory Effect of Mangostenone F in Lipopolysaccharide-Stimulated RAW264.7 Macrophages by Suppressing NF-kappaB and MAPK Activation. *Biomolecules and Therapeutics*, 22(4), 288–294. doi: 10.4062/biomolther.2014.052 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
9. Das, S., Raychaudhuri, U., Falchi, M., Bertelli, A., Braga, P. C., & Das, D. K. (2011). Cardioprotective properties of raw and cooked eggplant (*Solanum melongena L*). *Food and Function*, 2(7), 395–399. doi: 10.1039/c1fo10048c [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
10. Emmanuel, S., Ignacimuthu, S., Perumalsamy, R. & Amalraj, T. (2006). Antiinflammatory activity of *Solanum trilobatum*. *Fitoterapia*, 77(7–8), 611–612. doi: 10.1016/j.fitote.2006.09.009 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
11. Fang, L., Chang, H. M., Cheng, J. C., Leung, P. C., & Sun, Y. P. (2015). Nitric oxide and cGMP

induce COX-2 expression and PGE2 production in human granulosa cells through CREB signaling pathway. *Journal of Clinical Endocrinology and Metabolism*, 100(2), E262–E269. doi: 10.1210/jc.2014-2886 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

12. Han, S. W., Tae, J., Kim, J. A., Kim, D. K., Seo, G. S., Yun, K. J., … Lee, Y. M. (2003). The aqueous extract of *Solanum melongena* inhibits PAR2 agonist-induced inflammation. *Clinica Chimica Acta*, 328(12), 39–44. doi: 10.1016/S0009-8981(02)00377-7 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

13. Helmja, K., Vaher, M., Pussa, T., & Kaljurand, M. (2009). Analysis of the stable free radical scavenging capability of artificial polyphenol mixtures and plant extracts by capillary electrophoresis and liquid chromatography-diode array detection-tandem mass spectrometry. *Journal of Chromatography A*, 1216(12), 2417–2423. doi: 10.1016/j.chroma.2009.01.040 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

14. Jing, P., Qian, B., Zhao, S., Qi, X., Ye, L., Monica Giusti, M., & Wang, X. (2015). Effect of glycosylation patterns of Chinese eggplant anthocyanins and other derivatives on antioxidant effectiveness in human colon cell lines. *Food Chemistry*, 172, 183–189. doi: 10.1016/j.foodchem.2014.08.100 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

15. Kim, B., Kim, J. E., Choi, B. K., & Kim, H. S. (2015). Anti-inflammatory effects of water chestnut extract on cytokine responses via nuclear factor-kappaB-signaling pathway. *Biomolecules and Therapeutics*, 23(1), 90–97. doi: 10.4062/biomolther.2014.080 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

16. Lamy, S., Moldovan, P. L., Ben Saad, A., & Annabi, B. (2015). Biphasic effects of luteolin on interleukin-1beta-induced cyclooxygenase-2 expression in glioblastoma cells. *Biochimica et Biophysica Acta*, 1853(1), 126–135. doi: 10.1016/j.bbamcr.2014.10.010 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

17. Lee, Y. M., Jeong, H. J., Na, H. J., Ku, J. Y., Kim, D. K., Moon, G., … Kim, H. M. (2001). Inhibition of immunologic and nonimmunologic stimulation-mediated anaphylactic reactions by water extract of white eggplant (*Solanum melongena*). *Pharmacological Research*, 43(4), 405–409. doi: 10.1006/phrs.2001.0807 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

18. Liu, X., Luo, J., & Kong, L. (2011). Phenylethyl cinnamides as potential alpha-glucosidase inhibitors from the roots of *Solanum melongena*. *Nature Product Communication*, 6(6), 851–853. [PubMed], [Web of Science ®], [Google Scholar]

19. Lou, T., Jiang, W., Xu, D., Chen, T., & Fu, Y. (2015). Inhibitory Effects of Polydatin on Lipopolysaccharide-Stimulated RAW 264.7 Cells. *Inflammation*, 38(3), 1213–1220. doi: 10.1007/s10753-014-0087-8 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

20. Matsubara, K., Kaneyuki, T., Miyake, T., & Mori, M. (2005). Antiangiogenic activity of nasunin, an antioxidant anthocyanin, in eggplant peels. *Journal of Agricultural and Food Chemistry*, 53(16), 6272–6275. doi: 10.1021/jf050796r [Crossref], [PubMed], [Web of Science ®], [Google Scholar]