

Anti-Arthritic Activity of Ethanolic Extract of Lawsonia Inermis in Freund S Adjuvant Induced Arthritic Rats

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Abstract: The aim of the present study was to investigate anti-arthritic activity of Lawsonia Inermis Linn in wistar rats. The anti-arthritic activity of ethanolic extract of Lawsonia Inermis Linn (EELI) was studied against Freund's Complete Adjuvant (FCA) induced arthritis in rats. The (EELI) was administered at the doses of 200mg/kg, 400mg/kg body weight orally for 21 days after the injection of (FCA) in the rats right hind paw. The parameters assessed were paw diameter, body weight changes, haematological parameters like (RBC, WBC, Hb, ESR), biochemical parameters like (SGPT, SGOT, ALP, TP), histopathology and radiology of hind legs. Diclofenac sodium drug was taken as standard. The result of this study revealed that administration of EELI produced significant ($P < 0.05$) reduction of paw diameter and gain in body weight. The altered haematological parameters and biochemical parameters in the arthritic control rats were significantly brought back to near normal by the EELI treatment at the dose level of 200mg/kg and 400mg/kg. Further the histopathological and radiological studies revealed the significant anti-arthritic activity of EELI as indicated by fewer abnormalities in these groups when compared to the arthritic control group. From this study it has been concluded that the ethanolic extract of Lawsonia Inermis having good anti-arthritic activity, which is compared to Diclofenac sodium.

I. Introduction

Arthritis is one of the most common medical problems in the world. Which is a generic term that describes inflammation of joint. Inflammation is the general response of tissue to injury of any type (1). The most commonly prescribed medication of arthritis treatment is steroidal, non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressant drugs (2). The aim of these drugs has been to relieve pain, decrease joint inflammation, and prevent joint destruction and to restore function of disabled joints (3), however, the side effects of currently available drugs include gastric ulcer, renal damage, bronchospasm, and cardiac abnormalities have limited their use (4). With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies that have been considered safe and effective in all elevating chronic pain associated with arthritis (5). Agents derived from plants include flavonoids, steroids, polyphenols, coumarins, terpenes, alkaloids due to their wide range of pharmacological significance including analgesic, anti-inflammatory and anti-arthritic activities with lesser side effects (6),(7).

Lawsonia Inermis Linn belonging to family Lythraceae commonly known as Henna. It is a much branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. The plant is reported to contain carbohydrates, proteins, flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthenes and fatty acids. The plant has been reported to have analgesic (8), hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antifungal (9), antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, tuberculostatic, and anticancer properties (10). The arthritis is induced by a sub-cutaneous injection of Freund's Complete Adjuvant. The denatured *Mycobacterium butyricum* suspended in mineral oil can be injected sub-cutaneously at the base of the rat's tail or in the paw's planter surface, or by intra-joint via. Freund's adjuvant-induced arthritis have been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance for the study of pathophysiological and pharmacological control of inflammatory processes, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs (11),(12). One of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agents in this model and in rheumatoid arthritis in human. The present study made an attempt to evaluate the anti-arthritic of ethanolic extract of Lawsonia Inermis against Freund's Complete Adjuvant induced arthritis in experimental rats.

II. Material And Methods

Collection Of Plant Materials And Extraction :

The leaves of Lawsonia Inermis Linn was purchased from the local market at Basra-Iraq. The dried leaves was finely powdered in chemical mixer, 50gm of powder were put in the round bottle flask, 200ml of

ethanol (70%) were added to flask and extracted for 12hours at 70 °C . the extract was filtered by using whatmann filter paper, then the extract were put in the petridish and left at room temperature under the shade, the collection extracts were kept in tight closed container and stored until using (13).

Experimental Animals:

Healthy adult albino rats of wistar strain 150-250 gm. were used, the animals kept in suitable cages in the animal house of veterinary medicine college / Basra university , and were feeder standard diet and water *ad libitum*.

Induction Of Arthritis:

On day 0 rats were injected with 0.1ml of Freund 's Complete Adjuvant (FCA) into the right hind paw of all animals, administration of EELI and standard drug was started on the next day and continued for 21 days.

Experimental Set Up:

Total animals were randomly divided into five groups, each group containing 6 animals and treated for 21 days according to the following schedule :

Group 1: normal rats received distilled water.

Group 2: adjuvants induced arthritic rats.

Group 3: arthritis induced rats administered with extract of *Lawsonia Inermis* (200mg/kg body weight /rat/day for 21 day p.o).

Group 4: arthritis induced rats administered with extract of *Lawsonia Inermis* (400mg/kg body weight /rat/day for 21 day p.o).

Group 5: arthritis induced rats administered with Diclofenac sodium (1mg/kg for 21day p.o).

Paw thickness was measured on 1th, 7th, 14th and 21st by using verneir caliper (14).

The body weight of animals were measured by digitalis balance to access the course of the disease at the initial day before induction and at the end of 21st day. At the end of the study, blood samples were withdrawn from all groups through cardiac puncture, then the blood samples were put in two different screw tubes, the first one contains EDTA anticoagulant then centrifuged at 5000 rpm for 15 minutes to get plasma for haematological parameters as the red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) level and the erythrocyte sedimentation rate (ESR) were estimated manually (15), (16), (17). While the second part of collected blood was put in a screw tube without anticoagulant then centrifuged at 5000 rpm for 15 minutes to get serum for Biochemical parameters like glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) marker for bone destruction and total protein (TP) were estimated by SGOT, SGPT, ALP and TP by using various diagnostic kits (18).

Radiographical Analysis (X-Ray): of all the experimental animals were taken X-ray , before they were subjected to sacrifice (19).

Histological Analysis: rats were sacrificed on 21day, hind limbs were removed and fixed in 10% buffered formalin. The limbs were decalcified in 15% formic acid, processed for paraffin embedding, sectioned at 5µm thickness, and subsequently stained with haematoxylin –eosin for examination under a light microscope with 10x magnification.

Statistical Analysis: Data obtained from experiments was expressed as mean ±SD , the results were analyzed statistically using (ANOVA) by SPSS programming difference were considered significant at P< 0.05 (20).

Results: Effect of ethanolic extract of *Lawsonia Inermis* and Diclofenac sodium on joint diameter:

The result obtained from Table (1) showed that the paw diameter was increased up to 21st day of adjuvant induction. Diclofenac sodium treated group shows significant inhibition of paw diameter on day 7th, 14th, 21st (P< 0.05). EELI (200mg/kg) shows significant inhibition of paw diameter on day 21st with at P< 0.05. also rats treated with EELI (400mg/kg) shows significant inhibition of paw diameter on day 7th, 14th, and 21st with at P< 0.05.

Table 1: Anti-arthritic activity of EELI and Diclofenac sodium drug on rat paw diameter.

Groups	Day 1	Day 7	Day 14	Day 21
I	4.50±0.632	4.53±0.816	4.48±0.408	4.50±0.00
E		E	E	E
II	5.53±0.121	6.75±0.137	6.98±0.132	7.03±0.377
A		A	A	A

III	5.33±0.121 B	5.90±0.104 B	5.52±0.123 B	5.22±0.125 B
IV	5.16±0.103 C	5.52±0.113 C	5.03±0.121 C	4.82±0.114 C
V	5.03±0.894 D	4.916±0.116 D	4.72±0.117 D	4.62±0.116 D

Values are expressed as mean± SD of 6 rats in each group. Significantly different from Group II (P< 0.005).

Effect Of Ethanolic Extract Of Lawsonia Inermis And Diclofenac Drug On Body Weight :

The results obtained from Table (2) exhibited that the body weight of the animals in the control group increased significantly till day 21, whereas body weight of all the animals in the arthritic control group significantly decrease till day 21 as compared to the normal control group. Further body weight of all the animals in ethanolic extract and diclofenac sodium treated groups increased significantly (P<0.005) as compared to the arthritic control group.

Table 2: Effect of EELI and Diclofenac drug on body weight (gm.).

Groups	Day 0	Day 7	Day 14	Day 21
I	205±0.816	213±1.214 B	216±0.519 B	219±0.901 B
II	206±0.408	201±0.400 E	199±0.516 E	196±0.993 E
III	206±0.632	208±0.516 D	211±0.592 D	216±0.392 D
IV	206±1.211	210±0.204 C	215±0.491 C	218±0.418 C
V	205±1.632	214±0.783 A	217±0.408 A	220±0.895 A

Values are expressed as mean± SD of 6 rats in each group. Significantly different from Group II (P< 0.005).

Effect of ethanolic extract of *Lawsonia Inermis* and Diclofenac sodium drug on haematological parameters:

The results obtained from Table (3) showed a significant reduces in the levels of RBC and Hb and increases in the level of ESR and WBC was observed in arthritic control, when compared to the normal control group. Groups treated with diclofenac sodium, EELI 400mg/kg show increase significantly in RBC whereas the group treated with EELI 200mg/kg showed insignificant result, Groups treated with diclofenac sodium, EELI 200mg/kg, EELI 400mg/kg show increase significantly in Hb and reduces the WBC and ESR with P<0.05 when compared with arthritic control.

Table 3: Effect of EELI and Diclofenac drug on haematological parameters:

Parameters	Group I	Group II	Group III	Group IV	Group V
RBC $10^6/mm^3$	6.48±0.381 A	5.25±0.524 D	5.61±0.563 D	5.93±0.550 C	6.13±0.513 B
WBC $10^3/mm^3$	6.80±0.357 E	11.75±2.091 A	9.83±1.471 B	8.29±0.82 C	7.91±0.861 D
Hb (gm/dL)	12.58±0.917 A	9.05±1.059 E	10.96±0.755 D	11.75±0.75 C	12.00±0.70 B
ESR(mm/hr.)	1.26±0.250 E	15.00±3.178 A	7.50±0.894 B	6.33±0.816 C	5.50±1.048 D

Values are expressed as mean± SD of 6 rats in each group. Significantly different from Group II (P< 0.005)

Effect of the ethanolic extract of *Lawsonia Inermis* and Diclofenac sodium drug biochemical parameters:

The challenge with FCA (0.1ml) showed significant (P<0.05) elevation of the serum SGOT, SGPT, ALP level and decrease in the TP level, when compared to normal control. On treatment with EELI 200mg/kg, EELI 400mg/kg and diclofenac sodium all these changes were brought back to near normal (Table 4).

Table 4: Effect of EELI and Diclofenac sodium drug on biochemical parameters.

Parameters	Group I	Group II	Group III	Group IV	Group V
SGOT(U/L)	37.50±2.428 E	108±7.968 A	81.33±4.546 B	61.60±4.226 C	50.50±2.428 D
SGPT(U/L)	42.16±5.631 E	104±2.868 A	64.66±10.424 B	48.33±6.250 C	48.50±2.428 D

ALP(U/L)	55.1±3.656 E	258±6.831 A	206±11.273 B	173±3.777 C	138±1.169 D
TP(g/dL)	6.26±0.258 A	4.63±0.408 E	5.21±0.222 D	5.98±0.306 C	6.18±0.511 B

Values are expressed as mean± SD of 6 rats in each group. Significantly different from Group II (P< 0.005).

Histopathological Studies:

Histopathology of ankle joint of normal rats showed normal cartilage with normal articular joint surface with normal synovial fluid , No infiltration of inflammatory cells was observed (figure1), arthritic control rats showed accumulation of mononuclear cells with fibrin and excessive vacuolated where increase in the synovial fluid, severe cartilage destruction , (figure 2), treatment with EELI 200mg/kg showed oedematous fluid, moderate influx of inflammatory cells and cartilage destruction (figure 3), EELI 400mg/kg showed very low inflammatory cells in synovial space and present oedematous fluid with precipitation of fibrin on synovial surface, reduced destruction of cartilage (figure 4), treatment with diclofenac sodium drug showed very low influx of inflammatory cells, fibrosis in the synovial fluid in the joint, reduced destruction of cartilage (figure 5).

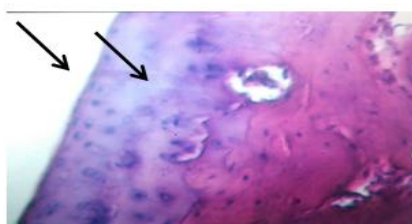


Figure (1): Histopathological section of joint of normal rats showed normal cartilage with normal articular joint surface , normal synovial fluid . (H&E stain, 40X).

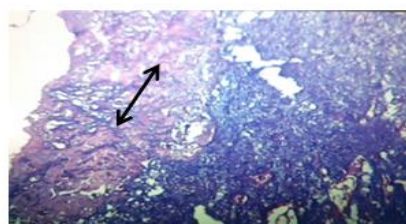


Figure (2): Histopathological section of joint of Arthritic control showed accumulation of mononuclear cells with fibrin in the synovial fluid of the joint. (H&E stain, 40X)

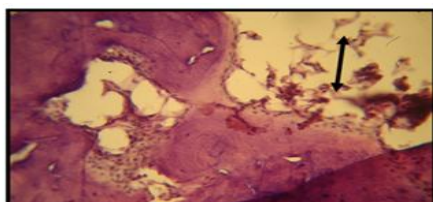


Figure (3): Histopathological section of joint of rats treated with 200 mg/kg showed oedematous fluid and moderate inflammatory cell infiltration in the synovial fluid of the joint. (H&E stain, 40X).

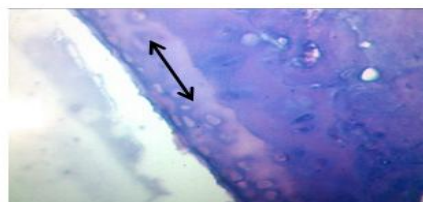


Figure (4): Histopathological section of joint of rats treated with 400 mg/kg showed very few inflammatory cells and present oedematous fluid with fibrin in the synovial fluid of the joint. (H&E stain, 40X).

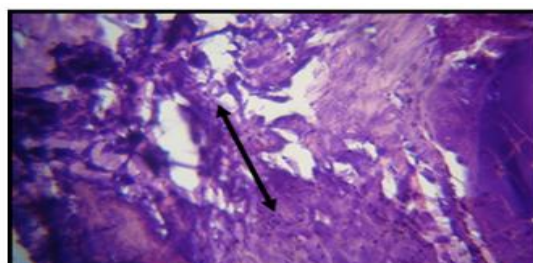


Figure (5): Histopathological section of joint of rats treated with Diclofenac sodium drug showed low influx of inflammatory cells, fibrosis in the synovial fluid of the joint (H&E stain, 40X).

Radiographic Analysis (X-Ray):

The result were observed from x-ray was the normal group animals showed absence of soft tissue swelling and bony destruction, the arthritis control group animals was found with soft tissue swelling and sign

of bony destruction. The treatment groups have shown prevention against bony destruction and showing less soft tissue swelling (figure 6).

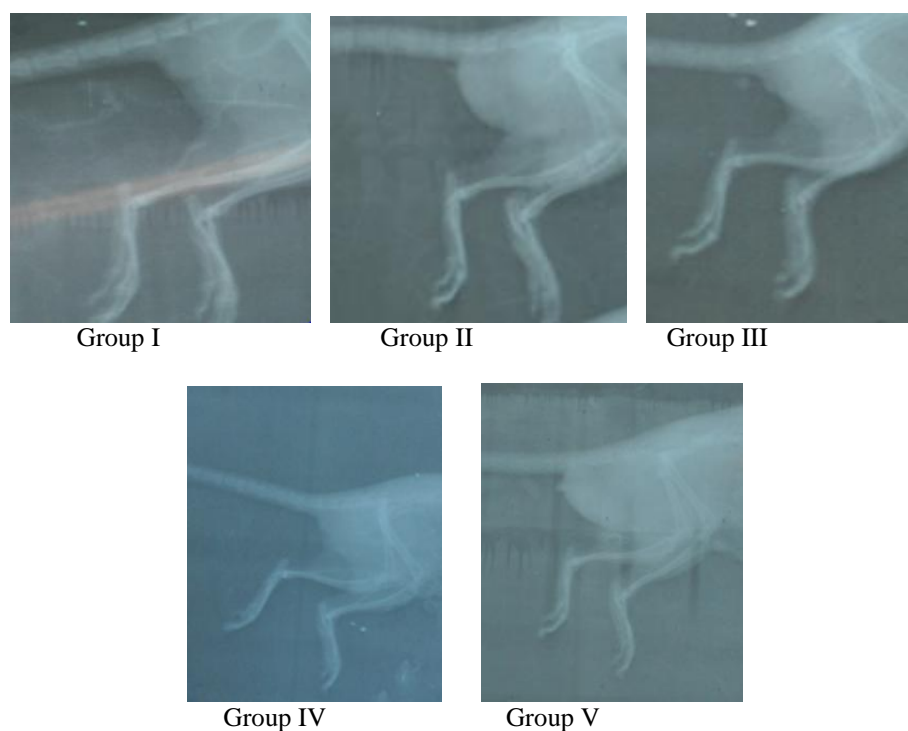


Figure (6) : radiography study

III. Discussion

Evaluation of anti-arthritic activity of EELI was studied on Freund's Complete Adjuvant (FCA) induced arthritis in wistar strain albino rats. The choice of the animal strain has been found to be very important for the performance of this test. Wistar- strain rats have been proven to be very suitable in contrast to other sub strains(21). Paw swelling is an index of measuring the anti-arthritic activity of various drugs and it is employed here to determine the activity of *Lawsonia Inermis* extract, diclofenac sodium. *Lawsonia Inermis* extract administered at different doses (200mg/kg, 400mg/kg) showed marked reduction in paw diameter when compared with the arthritic control group by inhibiting the release of inflammatory mediators (22), because of presence of flavonoids and alkaloids may be responsible for the mechanism of action in suppressing the inflammation and antioxidant activity. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and pause in the body weight gain. During the acute period hind paw joint diameter increases. In the later acute stages of the disease (7day) rats with adjuvant arthritis are often relatively immobile due to the severity of paw swelling, body weight, food intake and metabolism are affected by immunity and inflammation and they are regulated by a cytokine -like hormone known as leptin in CFA induced arthritis, with in 24hrs of administration of CFA the plasma leptin level were rapidly increased which lead to anorexia and body weight loss. From the results it is clear that the decrease in RBC count and haemoglobin level represents the anemic condition in arthritic rats. The more important causes are the abnormal storage of iron in the reticulo endothelial system and the failure of bone marrow to respond to anemia (23). The significant increase in leukocyte count in adjuvant induced arthritic rats may be due to the stimulation of immune system against the invading antigens or and the respective decrease in extract and standard drug treated groups showed it is immune-modulation effect (24). The Erythrocyte Sedimentation Rate (ESR) count which significantly increased in arthritic control group which is attributed to the accelerated formation of endogenous protein such as fibrinogen and α , β globulin, and such arise in the ESR indicates an active but obscure disease process (25). Thus the reduction in ESR brought about by *Lawsonia Inermis* extract treatment further supports anti-arthritic effect. Assessment of the levels of GOT,GPT and ALP provides an excellent and simple tool to measure the anti-arthritic activity of the target drug (26). The activities of GOT, GPT and ALP were significantly increased in arthritic rats, since these are good indices of liver impairment, which are also considered as the features of adjuvant arthritis (27). Serum GOT and GPT have been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process confirmed a positive correlation between the increased activity of serum ALP and the disease activity in arthritic (28). Elevated levels of serum ALP in arthritic rats can be due to increase in the liver and bone fraction or due to an increase of both

isoenzymes (29, 30). The decreased GOT, GPT enzymes levels in *Lawsonia Inermis* extract treated rats indicated decreased bone loss and organ protective role of *Lawsonia Inermis* extract in arthritis rats.

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