Synergistic activities of ethanolic extracts of ***Jatropha tanjorensis*** leaves in conventional management of rheumatoid arthritis in the ankles of Wistar Rats

ABSTRACT

Background: Rheumatoid arthritis (RA) is a common cause of chronic inflammatory joint disease. Plant extracts contain several bioactive factors which can re-establish the homeostasis of joints and joint cartilages.

Objectives: This study aim to evaluate the synergism of ethanolic extracts of *Jatropha tanjorensis* leaves with Ibuprofen and Sulfasalazine as an antirheumatic agent.

Methodology: 45 adult male Wistar rats were subjected to different types of treatment; A: Positive control, B: Low dose of extract only, C: Medium dose of extract only, D: High dose of extract only, E: Ibuprofen and Sulfasalazine only, F: Ibuprofen, Sulfasalazine and Low dose of extract, G: Ibuprofen, Sulfasalazine and Medium dose of extract, H: Ibuprofen, Sulfasalazine and High dose of extract, I: Normal control. Groups A-H were collagen-induced arthritic (CIA) models. One week before sacrifice, anterior-posterior and lateral diameters of both ankles, physical appearance and weight were assessed. After sacrifice, histological analysis of ankles using modified Mankin scoring system was done.

Results: Groups F-H exhibited significant results in direct proportion to the dosages of J. tanjorensis extract administered and the combination of drugs. Increased doses slowed down the progression of cartilage destruction evidenced by prevention of joint swellings and preservation of chondrocytes and its histological features, while treatement with different dosages of the extract only was found to be unremarkable.

Conclusion: The result indicate that the plant extract could enhance current treatment options of rheumatoid arthritis. The physical appearance, assessment of weight, anterior-posterior and lateral diameters, and histological examination of both ankles of the animal confirms the assumptions of *Jatropha tanjorensis* in management of rheumatoid arthritis.

Keywords: *J. tanjorensis,* Rheumatoid Arthritis, Ibuprofen, Sulfasalazine, Antirheumatic Agents

Summary
What is already known on this topic – The current therapeutic regimen for RA has some disadvantageous side effects.

What this study adds – An alternative to the conventional management of RA with lower side effects.

How this study might affect research, practice or policy – There is need to standardize dosages of newer herbs identified to be therapeutic.

INTRODUCTION

The cultivation of herbs has been ascribed to the discovery of plants rich in pharmacologically active constituents with an edge that is proven against well-known disease entities and medical conditions [1]. Hundreds of species of tropical angiosperms grown have medicinal properties, thereby making traditional medicine more available to the general populace in comparison to modern medicine [2]. The use of traditional herbs in the treatment of diseases is largely due to their nutraceutical components which is attributed to plant secondary metabolites [3, 4]. In Nigeria, some claims have been laid on the efficiency of *Jatropha tanjorensis* in ameliorating oxidative stress [3]; a perennial herb that belongs to the Euphorbiaceae family. It is commonly used in Nigeria, as a blood building vegetable, as an anti-plasmodial agent, for its anti-diabetic, and hypo-glycemic properties, and as an anti-oxidant against oxidative stress [5, 6, 7, 8]. Granted that many studies have been pursued to a conclusion on *Jatropha tanjorensis,* howbeit, none of these numerous studies addressed the therapeutic potentials of ethanolic extract of *Jatropha tanjorensis* leaves on autoimmune diseases.

Rheumatoid arthritis (RA) is the most common cause of chronic inflammatory joint disease; an autoimmune systemic disease with slow but progressive destruction of joints and joint cartilages [9]. There is no known cure to RA presently, thus the adoption of supportive and palliative approaches in its management with aim to slow down disease progression, alleviate symptoms and reduce functional limitations [9]. These approaches include the use of a combination of disease modifying anti-rheumatic drugs (DMARDs) such as sulfasalazine and non-steroidal anti-inflammatory drugs (NSAIDs), like Ibuprofen [9]. Sulfasalazine slows down disease progression by inhibiting neutrophil function, interfering in the functions of T cells via blockade of NF- κB activation and reducing immunoglobulin levels [10]. However, due to its slow time to onset of action, bridge-therapies such as the inclusion of rapid acting NSAIDs as a first-line option to control mild inflammation, pain and joint stiffness [11]. Prolonged use of NSAID in management of RA sometimes lead to gastrointestinal complications, renal toxicity, and cardiovascular accidents, thus limiting its efficiency [9, 12, 13, 14,]

In view of the anabolic actions and synergistic effects of therapeutic options on damaged cartilage from RA, ethanolic extract of *Jatropha tanjorensis* is a compelling candidate to investigate as a therapeutic option, because it contains several bioactive factors from its secondary metabolites which can reestablish the homeostasis of the synovial fluid [15]. It

is also a rich source of antioxidant nutrients like phosphorus, selenium, zinc, vitamin C and vitamin E. These micronutrients has been established as inflammation modulators and cell migration inducers [3]. *Jatropha tanjorensis* phytochemical components contain secondary metabolites including but not limited to; phytates, phenols, alkaloids, terpenoids, saponins, flavonoids and tannins which contributes to the inhibition of progressive damage of joint capsules in the management of rheumatoid arthritis disease [16-22]. *Jatropha tanjorensis* has been reported to exhibit anti-arthritic potentials through inhibition of protein denaturation, controlling production of auto-antigens, stabilizing lysosomal membrane and inhibition of proteinases [2, 16]. Also, with the prevalence of RA mostly involving geriatrics, immune-suppressants such as infliximab and its class of drugs is not totally the best of option in the approach to combat rheumatoid arthritis due to their side effects [11].

In this study, we used the Collage-induced arthritis (CIA) model which represents the gold standard RA animal model because its pathological features closely resemble those in human; synovium lymphocyte infiltration and hyperplasia with cell swelling [23].

We report in this study with intent to evaluate the use of *Jatropha tanjorensis* in the management of RA for these aforementioned reasons; being cost-effective, readily available and ease of preparation. We also evaluated the potentials of ameliorating the side effects of both NSAIDs and DMARDs, which are currently used in the management of RA while enhancing therapeutic efficacy using *J. tanjorensis*. The evaluation concerned mainly; physical appearance, weight, anthropometry which included measuring the anterior-posterior and lateral diameters of both ankles, and histological examination of both ankle joints of the animals.

MATERIALS AND METHODOLOGY

Plant Material and Extract

Jatropha tanjorensis leaves were obtained from a farm settlement in Mbanugo, Coal Camp, Enugu state of Nigeria, between May 2021 and October, 2021. The air dried and ground leaves (2500g) was cold-macerated and extracted in 6 volumes of absolute alcohol to give the extract which was concentrated in a hot water bath at 80°c (mean yield: 38.26±0.89g w/w), and stored in a sample bottle at -4°c until ready for use. Phytochemical analysis gave positive tests for phytates, phenols, terpenoids, saponins, flavonoids and tannins.

Experimental Animals and induction of rheumatoid arthritis

A total of 50 adult male Wistar rats weighing 180-250g were housed in cross-ventilated cages in the animal house of Department of Anatomy, Enugu State University of Science and Technology, Enugu. Conditions of the facility were 20-28°c with 12 hour light/dark cycle. Animals had access to commercial growers mash and tap water ad libitum. All animals were fed and acclimatized for three weeks before using them for the study. The 50 rats were utilized accordingly as shown in Table 1 below;

Table 1: Categorization of Wistar rats utilized in this study

5 rats	For pilot dose-response trial of Bovine CII-CFA emulsion by injection at the base of the tail before determining appropriate dose due to variations in dose requirements in various strains of rodents.
5 rats	Normal control group. (Normal)
40 rats	For construction of rheumatoid arthritis models using Bovine CII-CFA emulsion by injection at the base of the tail. (Model)

The rats injected with Bovine CII-CFA emulsion were anaesthetized using 5% isoflurane in an induction-bell jar. During model construction, 200µl of Bovine CII-CFA emulsion (0.2mg/ml) was injected into the base of the tail using a 26F-guage needle. And 9 days later, booster dosage was administered by injecting 100µl of bovine CII-CFA emulsion (0.1mg/ml) into the base of the tail. Rats in the normal control group received equal volume of normal saline orally.

Evaluating rheumatoid arthritis in the rats

On day 1 and day 16 (1 week after booster dose was given), the rats' physical appearances and body weights were assessed. Their limbs were measured using Vernier callipers to check for possible limb swellings. Arthritis index (AI) was determined to be positive and models considered successful when limb swellings involved an entire limb, affecting multiple joints symmetrically. The anterior-posterior diameter (between the posterior strip of the ankle and heel) and lateral diameter of the ankle of both hindlimbs were measured on days 0 and on day 16, to confirm limb swellings. Also on day 16, blood samples from 5 rats with positive limb swellings, chosen randomly from the model category were investigated for anti-cyclic citrullinated peptide antibody (ACCPA). They were all positive with average value of 49 units.

Grouping and Treatment of animals

At day 16, the 40 successful models were randomized into eight treatment groups (A-H) with five animals each. All groups were subjected to different types of treatment orally for 12 weeks as demonstrated in Table 2 below:

Table 2: Grouping and Treatment of animals

Groups	Type of treatment
A – Positive control	Distilled water only.
B – JT Low dose	*J. tanjorensis* (JT) extract at a dose of 0.5g/kg/day corresponding to 1ml/kg body weight only.
C – JT Medium dose	*J. tanjorensis* (JT) extract at a dose of 1g/kg/day corresponding to 2ml/kg body weight only.
D – JT High dose	*J. tanjorensis* (JT) extract at a dose of 1.5g/kg/day corresponding to 3ml/kg body weight only.
E – Ibuprofen & Sulfasalazine (IS)	Ibuprofen (5mg/kg/day), and Sulfasalazine (5mg/kg/day) only. corresponding to 1ml/kg body weight.
F – IS + JT Low dose	

G – IS + JT Medium dose	Ibuprofen (5mg/kg/day), Sulfasalazine (5mg/kg/day), and *J. tanjorensis* (JT) extract at a dose of 1g/kg/daycorresponding to 2ml/kg body weight.
H – IS + JT High dose	Ibuprofen (5mg/kg/day), Sulfasalazine (5mg/kg/day), and *J. tanjorensis* (JT) extract at a dose of 1.5g/kg/day corresponding to 3ml/kg body weight.
I – Normal control	Distilled water only.

At day 93, the anterior-posterior and lateral diameters of both hindlimbs of the nine groups were measured. All rats were sacrificed by cervical dislocation one week later. Both ankles were rapidly dissected and processed for histological study.

Histological study

Tissues of the ankles were fixed in 10% buffered formalin and post-fixed in zenker's fluid for 18 hours, and then processed for paraffin sectioning. Sections were cut at 5µ in thickness and stained using Hematoxylin and Eosin (H&E) dyes. The slides were analyzed and scored using the Modified Mankin scoring system.

RESULTS

General observations (day 0 to day 16)

The observation in the physical appearance of the rats in the model group on day 0 few hours after injection of Bovine CII-CFA emulsion were yellowish discoloration of furs noted to be in patches (2cm by 2cm). Few days later, some rats had ulceration and scab formation at the injection site measuring about 0.5cm by 1 cm on average. On day 16, the yellowish fur patches were markedly increased in size to about 4cm by 5cm. At day 93, there was no notable difference in the fur discoloration when compared to earlier observations.

Changes observed in body weight (day 0 to day 16)

As shown on Table 3 below, on day 0, the body weight between the rats in the normal control group and models had no significant difference ($P > 0.05$). However, by day 16, the body weights of the rats in the model group had a significant decrease in comparison to the normal control group ($P \leq 0.05$), confirming limb swelling in the rats of the model category.

Table 3: Changes observed in the body weight of the rats between day 0 and 16

Category	Body weight (grams)	
	Day 0	Day 16
Model	212.4 ±7.9	184.6 ±13.2[a]
Normal	211.9 ±6.2	223.4 ± 9.8

The values are expressed as mean ± SD. [a]$P \leq 0.05$, statistically significantly different in comparison to the normal group.

Changes observed in body weight (day 93)

By day 93, the body weights of rats in groups A-F were significantly decreased in comparison to the normal control [group I]. While groups G and H showed significant improvement in weight and were significantly different in comparison to the positive control [group A] ($P \leq 0.05$) [Table 4].

Table 4: Changes observed in the body weight of the rats on day 93

Groups	Body weight (grams) on day 93
A – Positive control	175.9 ± 8.8[a]
B – JT Low dose	179.7 ±4.6[a]
C – JT Medium dose	187.2 ± 4.1[a]
D – JT High dose	192.8 ±3.7[a]
E – Ibuprofen & Sulfasalazine (IS)	186.1 ±4.6[a]
F – IS + JT Low dose	187.3 ±12.2[a]
G – IS + JT Medium dose	199.5 ± 5.9[b]
H – IS + JT High dose	210.6 ± 8.2[b]
I – Normal control	227.6 ± 7.3[b]

The values are expressed as mean ± SD. [a]$P \leq 0.05$, statistically significantly different from normal control group (I). [b]$P \leq 0.05$, statistically significantly different from Positive control group (A).

Changes observed in the limbs between day 0 and 16

At day 0, there was no significant difference in the anterior-posterior and lateral diameters of both ankles of the hind-limbs between the rats in the normal control group and models ($P > 0.05$). However, by day 16, the anterior-posterior and lateral diameters of both ankles of the hind-limbs in the model groups were significantly larger than that of the normal control group ($P \leq 0.05$).

Table 5: Changes observed in the limbs of the rats between day 0 and 16

Ankles	Category	Anterior-posterior diameter (mm)		Lateral diameter (mm)	
		Day 0	Day 16	Day 0	Day 16
Right ankles	Model	7.54 ± 0.65	11.94 ± 0.38[a]	6.32 ± 0.56	8.92 ± 0.46[a]
	Normal	7.49 ± 0.43	7.66 ± 0.29	6.42 ± 0.42	6.62 ± 0.77
Left ankles	Model	7.62 ± 0.57	12.05 ± 0.71[a]	6.29 ±0.74	9.19 ± 0.54[a]
	Normal	7.58 ± 0.66	7.75 ± 0.36	6.32 ± 0.43	6.58 ± 0.27

The values are expressed as mean ± SD. [a]$P \leq 0.05$, statistically significantly different from normal control group

Changes observed in the limbs (day 93)

By day 93, the anterior-posterior and lateral diameters of both ankles measured in groups A-F were significantly larger in comparison to the normal control group ($P \leq 0.05$). Groups H demonstrated significant improvement, exhibiting a significant difference when compared to the positive control group (A). However, Group G was significantly

different in comparison to both group A and E (positive and normal control groups) ($P \leq 0.05$) [Table6].

Table 6: Changes observed in the limbs of the rats on day 93

Group	Anterior-posterior diameter (mm)		Lateral diameter(mm)	
	Right ankles	Left ankles	Right ankles	Left ankles
A – Positive control	14.23 ± 0.41[a]	13.97 ± 0.11[a]	13.89 ± 0.71[a]	13.71 ± 0.48[a]
B – JT Low dose	13.72 ± 0.72[a]	12.91 ± 0.37[a]	12.61 ± 0.24[a]	12.73 ± 0.63[a]
C – JT Medium dose	11.72 ± 0.24[a]	11.81 ±0.83[a]	11.21 ± 0.33[a]	10.96 ± 0.31[a]
D – JT High dose	10.32 ± 0.51[a]	10.61 ± 0.44[a]	9.48 ± 0.21[a]	9.11 ± 0.73[a]
E – Ibuprofen & Sulfasalazine (IS)	10.12 ± 0.87[a]	10.74 ± 0.17[a]	8.79 ± 0.81 [a]	9.37 ± 0.62 [a]
F – IS + JT Low dose	12.18 ± 0.67[a]	12.43 ± 0.71[a]	11.91 ± 0.74[a]	11.76±0.81[a]
G – IS + JT Medium dose	9.72 ± 0.61[a,b]	9.61 ± 0.32[a,b]	7.91 ± 0.32[a,b]	7.69 ± 0.17[a,b]
H – IS + JT High dose	8.21 ± 0.85[b]	7.91 ± 0.83[b]	7.1 ± 0.68[b]	6.82 ± 0.31[b]
I – Normal control	7.73 ± 0.46[b]	7.64 ±0.52[b]	6.54 ± 0.49[b]	6.45 ± 0.36[b]

The values are expressed as mean ± SD. [a]$P \leq 0.05$, statistically significantly different from normal control group (I). [b]$P \leq 0.05$, statistically significantly different from Positive control group (A). [a,b]$P \leq 0.05$, statistically significantly different from Positive control group (A) and Normal control group (E).

Light microscopic analysis with modified Mankin scoring system

Paraffin sections of the ankle joints of ethanolic *J. tanjorensis* leaf extract-treated rats showed that the extract produced a significant dose-dependent improvement in the cartilage erosion, peripheral staining of chondrocytes, spatial arrangement of chondrocytes and background staining intensity of the matrix. Micrographs of group A demonstrated features suggestive of massive cartilage destruction which included cartilage erosion extending into calcified cartilages, intensely enhanced chondrocyte periphery staining, sparsely distributed chondrocytes and no background staining; giving a total score of 14/15 (figure 1). With an extract dose of 0.5g/kgbw only, group B demonstrated erosion of uncalcified cartilages only, with an intensely enhanced chondrocyte periphery staining, clustered distribution of chondrocyte arrangement and a poor background staining intensity; giving a total Mankin score of 11/15 (figure 2). In the group administerd a moderate dosage of 1g/kgbw only, group C, the histopathological features noted were limited to separation of uncalcified cartilage from the calcified cartilages, slightly enhanced periphery staining, clustered distribution of chondrocyte arrangement and a poor background staining intensity; giving a total Mankin score of 9/15 (figure 3). The groups treated with high dosage of extract (1.5g/kgbw) only (group D), with 5mg/kg/day of Ibuprofen & 5mg/kg/day of sulfasalazine only (group E), and with a combination of 5mg/kg/day of Ibuprofen, 5mg/kg/day of sulfasalazine and Low dose (0.5g/kgbw) of extract (group F), demonstrated similarities with regards to the extent of cartilage damage. The features included superficial fibrillation of cartilages only, clustered spatial distribution of the chondrocytes, and moderately decreased background staining intensity. The only observed difference was in the high dose only (D) group which exhibited mostly slightly enhancement of chondrocyte periphery staining,

with a total Mankin score of 7/15, whereas groups E and F demonstrated intensely enhanced chondrocyte periphery staining, both with total Mankin scores of 8/15 (figure 4, 5, & 6). Doubling the dosage of the extract (1.0g/kgbw) in combination with 5mg/kg/day of Ibuprofen & 5mg/kg/day of sulfasalazine, demonstrated a remarkable decrease in the extent of cartilage damage (group G) with a Mankin score of 5/15. The cartilages showed superficial fibrillation, however the chondrocyte periphery staining was slightly enhanced with diffuse hyper-cellular spatial arrangement of the chondrocytes and slightly reduced background staining intensity (figure 7). In group H, the combination of 5mg/kg/day of Ibuprofen & 5mg/kg/day of sulfasalazine, with a dosage of 1.5g/kgbw improved the Mankin score to 3/15. The cartilages appeared rough but non-eroded with a normal chondrocyte periphery staining, diffuse hyper-cellular spatial arrangement of chondrocytes and slightly reduced background staining intensity (figure 8). There was no remarkable feature noted in the micrograph of group I (normal control) (figure 9).

Figure 1: Micrograph of joint cartilage in group A (positive control) showing features suggestive of massive cartilage destruction. CE = cartilage erosion extending into calcified cartilage, with clustering chondrocytes spatial arrangement. CP = intensely enhanced chondrocyte periphery staining. Background intensity staining showing no dye absorption. [H & E Stain x 400].

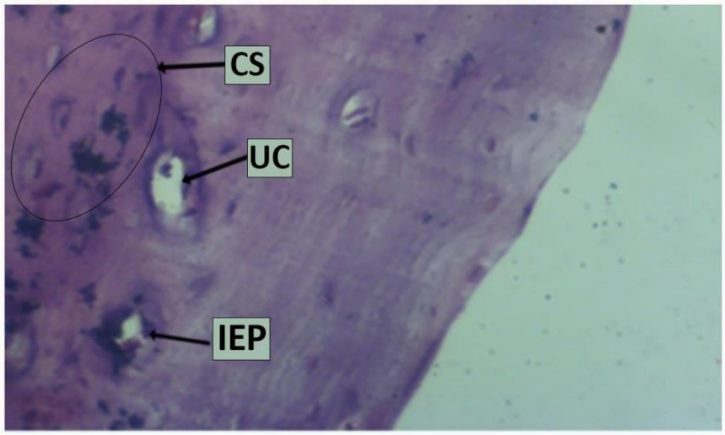

Figure 2: Micrograph of joint cartilage in group B showing features suggestive of gross cartilage destruction. UC = slight erosion of uncalcified cartilages, CS = clustering chondrocytes spatial arrangement, IEP = intensely enhanced chondrocyte peripheral staining, with severely reduced background staining intensity. [H & E stain x 400]

Figure 3: Micrograph of joint cartilage in group C (1.0g/kg of *J. tanjorensis* leaf extract), showing cartilage destruction evidenced by separated uncalcified cartilage from calcified cartilage (UC), slightly enhanced chondrocyte periphery staining (PS), clustering spatial arrangement of chondrocytes (CS), and severely reduced background staining intensity. [H & E stain x 400].

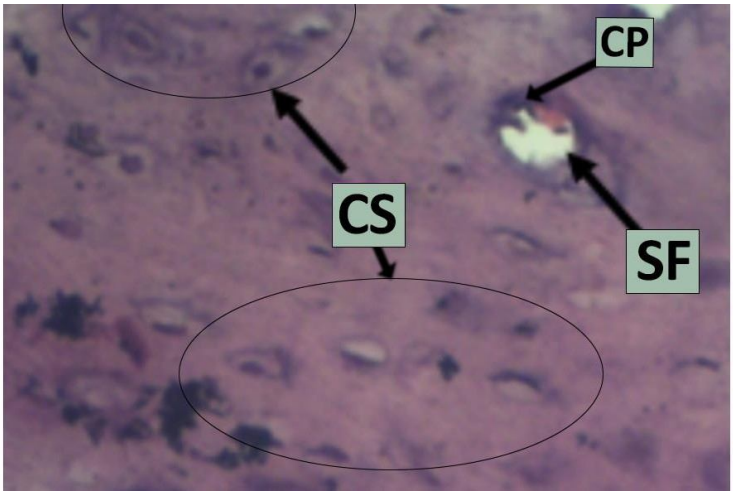

Figure 4: Micrograph of joint cartilage in group D (1.5g/kg of *J. tanjorensis* leaf extract), showing superficial fibrillation of cartilages (SF), slightly enhanced chondrocyte periphery staining (CP), clustering spatial arrangement of chondrocytes (CS), and moderately reduced background staining intensity [H & E stain x 400].

Figure 5: Micrograph of joint cartilage in group E (5mg/kg/day of Ibuprofen, and 5mg/kg/day of sulfasalazine), showing superficial fibrillation of cartilages (SF), intensely enhanced chondrocyte periphery staining (IEP), clustering spatial arrangement of chondrocytes with moderately reduced background staining intensity. [H & E stain x 400]

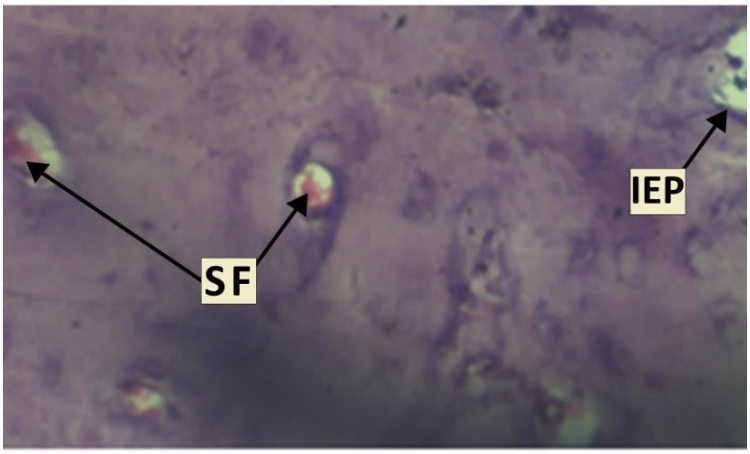

Figure 6: Micrograph of joint cartilage in group F (5mg/kg/day of Ibuprofen, 5mg/kg/day of sulfasalazine and 0.5g/kg of *J. tanjorensis* leaf extract) showing cartilage destruction; SF = superficial fibrillation of cartilage, IEP = intensely enhanced chondrocyte periphery staining, clustering arrangement of chondrocytes and moderately enhanced background staining intensity [H & E Stain x 400].

Figure 7: Micrograph of joint cartilage in group G (5mg/kg/day of Ibuprofen, 5mg/kg/day of sulfasalazine and 1.0g/kg of *J. tanjorensis* leaf extract) showing moderate cartilage destruction; SF = superficial fibrillation of cartilage, CL = chondrocyte lacunae with slightly enhanced periphery staining, diffuse hypercellular spatial arrangement of chondrocytes and slightly reduced background staining intensity [H & E Stain x400].

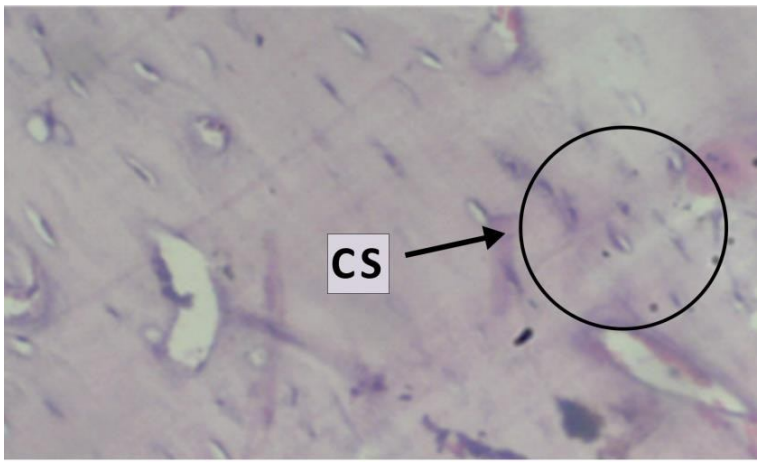

Figure 8: Micrograph of joint cartilage in group H (5mg/kg/day of Ibuprofen, 5mg/kg/day of sulfasalazine and 1.5g/kg of *J. tanjorensis* leaf extract), showing no cartilage destruction, evidenced by rough non-eroded cartilages, normal chondrocyte periphery staining, CS = diffuse hyper-cellular spatial arrangement of chondrocytes and slightly reduced background staining intensity [H & E Stain x 400].

Figure 9: Micrograph of joint cartilage in group I (normal control) showing no remarkable changes. C = normal chondrocytes with normal periphery staining, spatial distribution and background staining intensity [H & E Stain x 400].

DISCUSSION

In this study, the marked changes observed in the physical appearances of the rats in the model group is an important indication of RA in the research animals, which was also assessed and monitored through the notable progression of symmetrical swelling of multiple joints, limited mobility, fur discoloration and weight loss of research animals. The skin ulceration and scab formation identified was not an indication of RA, however they can be attributed to have come from possible errors in the animal handling technique adopted. The disparity in the body weight of rats in the normal control group and model group at day 16 can be attributed to the onset of RA disease.[23]

After treatment commenced, further disparities in the body weight of experimental animals were noted on day 93. Groups G and H were consistent with the range of optimal weight of Wistar rats between 7 to 11 weeks [24, 25]. However, the body weight of rats in groups A to F were suggestive of poor appetite and poor weight gain. This can be interpreted as a positive response due to the effect of increased dosage of the plant extract with potential of increased efficiency in alleviating gastrointestinal disorders due to the synergy of Ibuprofen and sulfasalazine in alleviating rheumatoid arthritis symptoms. The weight loss were more pronounced in groups A and B, which may suggest gastrointestinal (GI) disorder. Here, manifestation of GI disorder could be inferred as extra-articular symptoms of rheumatoid arthritis [9, 11]. While in groups C and D appreciable weight gain were noted. The reduction observed in the body weight of the rats in group E can be attributed to side effects of prolonged usage of NSAID (Ibuprofen) [12, 13]. The remarkable weight loss exhibited by group F could be as a result of the extract dosage, due to the difference seen in groups G and H. Again, group H had better results when compared to group G and group F, thus reiterating the stance of the effect of high extract dosage. This suggests potential synergistic effect of the *Jatropha tanjorensis* plant extract in achieving sustained clinical remission in extra-articular manifestation of rheumatoid arthritis [26]. It could also be attributed to the antioxidant properties of *J. tanjorensis* or its ameliorative effect on gastrointestinal disorders caused by NSAIDs [2, 5, 16].

The anterior-posterior and lateral diameters of the ankles measured served as an analytical tool to assess the treatment phase and administration of the ethanolic *J. tanjorensis* leaf extract [23]. The difference noted on day 16 between the normal control group and model group was a clear indication of onset of RA, however on day 93, long after treatment commenced, group B-F exhibited results suggestive of poor response to the treatment. In group G, effective response to treatment was observed and this was more pronounced in group H, indicating a better treatment response to higher dosage of extract in combination with Ibuprofen and Sulfasalazine. Group C, D and E responded to treatment, however, the observation was not as efficient as the response observed in groups G and H. This also strongly suggests a potential synergistic effect of *Jatropha tanjorensis* plant extract in combination with Ibuprofen and Sulfasalazine in the management of rheumatoid arthritis.

The results from the Modified Mankin scoring system analysis of the micrograph on *J. tanjorensis* extract effect on rheumatoid arthritis suggest that the extract may not have synergistic effect at low dose levels. This was evident in the marked level of cartilage destruction demonstrated by superficial fibrillation of chondrocytes and clustering, with

increased intensity of periphery and background staining of the cartilages and matrix respectively in group F. However, increasing the extract dose level to 1.0g/kg in group G improved the features observed in the cartilage, and instead, apart from superficial fibrillation of the chondrocytes, it demonstrated diffuse hyper-cellular spatial arrangement of the chondrocytes with slightly enhanced chondrocyte periphery and matrix background staining. The improved features observed in the cartilages of ankles of rats treated with increased doses of *J. tanjorensis* leaf extracts suggests that the extract could cause the stimulation of the actions of the Ibuprofen and Sulfasalazine earlier than normal due to the anti-inflammatory and antioxidant activities which suppresses oxidative reactions [27]. However, the ameliorative effect of the leaf extracts on rheumatoid arthritis could be attributed to constituent phytochemicals such as terpenoids, saponins or flavonoids and tannins with known antioxidant and anti-inflammatory properties [18, 19, 28]. The observations in the groups treated with increasing dosages of *Jatropha tanjorensis* extracts only is suggestive of poor response to treatment of rheumatoid arthritis when using the plant extract as a stand-alone drug. It also demonstrates that the anti-arthritic activities of *Jatropha tanjorensis* is most efficient only in combination with non-steroidal anti-inflammatory drugs and disease modifying anti-rheumatic drugs, and is equally dose dependent.

CONCLUSION

In conclusion, it would be conceivable to suggest that the ethanolic extract of *Jatropha tanjorensis* could serve as a synergistic potential in the management of rheumatoid arthritis, possibly through achieving sustained clinical remission in extra-articular manifestations, which in turn is predicated on its anti-oxidant properties. Secondly, the extract is implicated in the attenuation of iatrogenic effects of NSAIDs and DMARDs, and could enhance the duration of NSAID usage without detrimental side effects. Thirdly, and lastly by acting as a stimulant to enhance the activities of Sulfasalazine, the ethanolic extract serves as a bridge-therapy due to the slow time to onset of action of DMARDs. However, the growing interest in alternative medicine clearly indicates a need for research to go beyond identifying herbs but as well searching for a better and effective anti-rheumatic and anti-inflammatory drugs from plants. There is equally a very important need to standardize dosages of newer herbs identified to be therapeutic. It is also recommended that the side effects of such herbs be finely elucidated especially with regards their effects on certain organs in the body such as the kidneys and liver for obvious reasons.

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Ethical approval

Animal experiment protocol was approved by the Research and Ethical Clearance Committee, Animal Research Ethics Committee, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology (ESUCOM/FBMS/ETR/2021/007).

COMPETITING INTEREST

None declared.

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