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THE ANTI-ARTHRITIC POTENTIAL OF BARBADOS NUT *JATROPHA CURCAS* LINN. (1753) LEAF EXTRACT IN BOVINE TYPE II COLLAGEN-INDUCED ARTHRITIS IN MALE ALBINO MICE *MUS MUSCULUS*

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Keywords:

Arthritis, *Jatropha curcas*, Collagen-induced arthritis, Male albino mice, Inflammation

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ABSTRACT: In the Philippines, about 11 million suffer from arthritis. *Jatropha curcas* is being widely used by traditional users for treating arthritis. The study evaluated the anti-arthritic claims of *J. curcas* leaf extract in male albino mice with collagen type II-induced arthritis by assessing two parameters; the reduction of paw thickness due to inflammation and analysis of histopathological parameters (*e.g.*, inflammatory cell invasion, cartilage destruction, bone erosion, synovial hyperplasia, and pannus formation). Three doses of the leaf extract were used: 250mg, 500mg and 750mg per kilogram body weight while Indomethacin and water were used as positive and negative controls respectively. Post hoc analysis showed significant reduction in the paw thickness and histopathological parameters in comparison to the positive, untreated and negative controls. The median effective dose (ED_{50}) is 963.86 mg. The anti-arthritic activity was dose dependent. The *J. curcas* leaf extract is an anti-arthritic agent in male albino mice.

INTRODUCTION: Shrubs belong to the family Euphorbiaceae are widely distributed in the tropical regions. Although used and recommended by healers for centuries, without rigorous studies they remain lumped under the title 'folk medicine'. The latex of Jatropha, which is a shrub, contains an alkaloid known as "jatrophine" which is believed to have anti-cancer properties. It is also used as an external application for skin diseases, rheumatism, and arthritis [1 - 3]. However, no scientific data has established its anti - arthritic activity yet.

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The emergence of chronic diseases, especially rheumatoid arthritis (RA), nowadays is very alarming. Nearly 50 million Americans is reported to have some form of arthritis as diagnosed by a doctor, one in 20 U.S. workers face limitations due to arthritis, and by 2040, an estimated 78 million (26%) US adults ages 18 years or older are projected to have doctor-diagnosed arthritis [4].

Moreover, RA is one of the leading causes of significant debility among Filipinos, apart from asthma and cancer and about four times as many women as men have this disease. The prevalence is currently estimated to be approximately 0.06 percent National Nutrition & Health Survey (NNHeS) and is expected to increase to 0.07 percent by 2012. The treatment options are limited to conventional drugs such as disease-modifying

antirheumatic drugs (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs) as newer medications such as biologics are well beyond the affordability of the patients [5].

Research has indicated that people suffering from chronic pain, as in RA, and those dissatisfied with current treatment are very likely to seek alternative treatments, and an estimated 60–90% of persons with arthritis use complementary and alternative medicine. Among the most widely used treatments are chiropractic and herbal therapies. This growing interest in alternative medical practices clearly indicates the need to search for better and effective anti-arthritic drugs from plants [6].

Today, a much more aggressive treatment approach is advocated for people with Rheumatoid Arthritis, with prescription of non-biologic disease modifying anti-rheumatic drugs (DMARDs) within three months of diagnosis to reduce disease activity and prevent joint deformity. These treatment regimens however, are limited in their efficacy and are quite toxic. At present, research is still ongoing to discover a more cost-effective cure for all types of arthritis.

This study aimed to determine the anti-arthritic potential of the extract of barbados nut leaves (*Jatropha curcas L.* 1753) on bovine type II collagen-induced arthritis (CIA) in male albino mice (*Mus musculus*). Specifically, to find out if the extract of Barbados nut has anti-arthritic properties based on: Physiological Parameters (determine the percent reduction of the test plant through reduction of paw thickness); and Histological Parameters (infiltration of inflammatory cells, cartilage destruction, pannus formation, and synovial hyperplasia); determine the median effective dose of the test plant.

MATERIALS AND METHODS:

Plant Material: The leaves were collected from the Department of Agriculture, Regional Field Office Estancia Mandaue City and were certified by Mr. Antonio Tambuli, Department of Biology, College of Arts and Sciences, University of San Carlos, Cebu City, Philippines.

Preparation of Plant Extract: The air dried leaves were weighed accurately using a top loading balance. The leaves were placed in a glass jar and were submerged in 95% ethyl alcohol. The submerged leaves in the glass jar were allowed to stand for 72 hours and were agitated from time to time. After which, the content was filtered through a Buchner funnel with gentle suction using reduced pressure. The marc was discarded and the menstruum was concentrated to one-third its original volume using the rotary evaporator at 70°C at 60-70 rpm. The concentrated solution was then placed in the vacufuge and after, was evaporated to dryness in a water bath and powderized using a mortar and pestle. A total of 14.32g of the powdered extract was obtained. The powdered ethanolic extract of Barbados Nut leaves was properly labeled and stored in Sanplatec Dessicator Cabinet at 25°C 65 RH until it was further used.

Solubility Testing: A solubility test was conducted and it was determined that the dry powdered plant extract was practically insoluble in distilled water. The test was done by mixing one gram of the dry powdered plant extract to 1mL, 2mL, 3mL, 4mL and 5mL of distilled water respectively. Due to this problem in solubility, the researchers opted to make a suspension of the plant extract instead of a solution.

Preparation of Test/Stock Solution: Since the plant extract was found to be only practically insoluble in distilled water, a suspension was prepared using Tween 80 as the suspending agent. In a mortar and pestle, 7g of the powdered plant extract was triturated with 1mL of Tween 80. This was transferred to a 200mL beaker and 100mL of distilled water was added. A 7% stock solution was prepared and this was placed in a stoppered Erlenmeyer flask and stored in room temperature.

Test Animals: Eighteen male Albino mice, 8-10 weeks old, each weighed 20-30g were used as test animals. The mice were purchased from petshop in Nasipit Talamban, Cebu City. The test animals were kept by two's in cages that conformed to the specified requirements of the animal house head. The mice were acclimatized for one week before the experimentation began. The test animals were divided into six groups with three mice per group. Each of the groups received a measured amount of feeds. The experiments were conducted in accordance to the Guide for the Care and Use of Laboratory Animals (Animal Rights Committee,

Department of Pharmacy, University of San Carlos, Sitio Nasipit, Talamban, Cebu City, Philippines).

Classification of Treatment and Control Groups: The mice were divided into six groups and labeled as: Group A – Induced and Treated with Test Solution 1 (250mg/kg body weight), Group B – Induced and Treated with Test Solution 2 (500mg/ kg body weight), Group C – Induced and Treated with Test Solution 3 (750mg/ kg body weight), Group D - Induced and Treated with Positive Control (2mg/mL), Group E – Induced and Treated with Negative Control, and Group F – Induced and Untreated Control.

Administration of the Test Solutions. The different arbitrary doses of the extract namely 250mg, 500mg, and 750mg per kilogram of body weight, obtained from the stock solution, were orally administered once daily. The treatment began 2 weeks after the induction of arthritis. The Barbados Nut leaf extract was administered in three different doses: 250 mg /kg body weight for ITw-TS1, 500mg/kg body weight for ITw-TS2, and 750mg/kg body weight for ITw-TS3 groups.

Preparation of the Positive Control: Indomethacin served as the positive control for this study. It was purchased from a licensed drugstore in Cebu City. A 2mg/mL solution was made by emptying the contents of the 100mg capsule in a 100mL beaker and dissolved it in 50mL of distilled water. The weights of the mice were the basis for the amount of drug each mouse received. The 0.26mg/20g mouse dose derived from the 100mg/70kg man standard drug Indomethacin was given twice daily in two divided doses 2 weeks after the induction of arthritis.

Preparation of Negative Control: Distilled water was used as the negative control. The amount of distilled water administered to the test animals was based on an arbitrary dose of 1mL per 25g mouse. If the test animal weighed 22g, the amount of distilled water administered:

Arbitrary dose: 1ml/25g mouse
Weight of test animal : 22g

$$\text{Amount to be administered} = \frac{1\text{ml}}{25\text{g}} = \frac{x}{22\text{g}}$$

$$X = \frac{(1\text{ml})\ (22\text{g})}{25\text{g}}$$

The amount of distilled water for the 22g mouse is 0.88ml.

Induction of Arthritis and Treatment of Samples:

Induction of CIA in Mice: (Hooke Laboratories Inc. 2012)**:** The mouse was immobilized using an improvised restrainer. The tail was cleaned with 70% ethyl alcohol and wiped with sterile gauze. The syringe containing the collagen/CFA emulsion was positioned parallel with the tail, the tip of the needle pointed towards the body of the mouse, over the space between the dorsal and lateral vein of the tail The site of needle entry was be pressed firmly and injected with 0.05 ml or 5 µl of the emulsion using 1mL syringe. During the administration, the white emulsion was seen which ensured proper intradermal administration. The needle was inserted for 10 to 15 seconds after the injection, and then the mouse was released back into the cage. The same procedure was repeated to all of the mice. Administration of the booster dose of collagen/IFA emulsion was done two weeks after the initial immunization and it had the same procedure as the initial immunization of the CII/CFA emulsion.

Measurement of Body Weight and Paw Thickness: The body weight and the paw thickness of the animals were measured before the induction of arthritis and once every week thereafter until the end of the treatment period. The paw thickness was measured using a digital caliper and the body weight was measured using a top loading balance.

Histological Analysis: The mice were euthanized on the 50th day after first injection of collagen. After sacrificing the representative mice, their right hind paws were amputated and was devoid of skin and muscle. The paws were submerged in 10% formalin in preparation for transportation. After placing six amputated paws into their individual beakers with 40mL of 10% Formalin solution, they were brought to Perpetual Soccour Hospital for tissue processing and histological analysis. The tissue processing followed the following procedure:

The paws were submerged into 1st 10% neutral buffered formalin for 1 hour, 2nd 10% neutral buffered formalin for 1 hour, 3rd 65% Ethanol for 1

hour, 4th 85% Ethanol for 1 hour, 5th 95% Ethanol for 1 hour, 6th 95% Ethanol for 1 hour, 7th 100% Ethanol for 1 hour, 8th 100% Ethanol for 1 hour, 9th Xylene for 1 hour, 10th Xylene for 1 hour, 11th Paraffin for 1 hour, 12th Paraffin for 1 hour, 13th Paraffin for 1 hour and 14th Paraffin for 1 hour. For the Hematoxylin and Eosin Staining (H&E Staining) the following procedure was used:

The paws were submerged in 1st Xylene for 5 minutes, 2nd Xylene for 5 minutes, 3rd Dipped in Xylene 10 times, 4th Dipped in Absolute alcohol 10 times, 5th Dipped in Absolute alcohol 10times, 6th Dipped in Absolute alcohol 20 times, 7th Dipped in Distilled Water 10 times, 8th Submerged in Harris Hematoxylin for 5 minutes, 9th Dipped in Tap Water 10 times changing the tap water every tenth dip, 10th Dipped in Distilled Water 10 times, 11th Dipped in Absolute alcohol 10 times, 12th Dipped in Absolute alcohol 10 times, 13th Dipped in Absolute alcohol 20 times, 14th Submerged in Eosin for 1 minute, 15th Dipped in Absolute alcohol 10 times, 16th Dipped in Absolute alcohol 10 times, 17th Dipped in Absolute alcohol 10 times and 18th the slides were dried and cleaned carefully avoiding not to decolorize the tissue sections with 1% Acid Alcohol, dried and prepared for mounting.

TABLE 1: HISTOPATHOLOGICAL CHANGES SCORING SYSTEM FOR EVALUATING ARTHRITIS SEVERITY IN CIA MOUSE MODELS

Severity Score	Histopathology
0	Normal
1	to infiltration of inflammatory cells
2	to mild inflammation and pannus formation
3	to moderate inflammation and pannus formation
4	to marked infiltration
5	to severe infiltration and severe diffuse cartilage

Phytochemical Screening Using the Test Tube Method: The following tests were conducted using the test tube method: Keller Killiani's test for the presence of Deoxysugars, test for the presence of Tannins, Mayer's test for the presence of Alkaloids, Leibermann-Burchard test for the presence of Sterol, test for the presence of Flavonoids, Kedde's test for the presence of Unsaturated Lactones, Salkowski's test for the presence of Terpenes and Fehling's test for the presence of Reducing sugars.

Determination of Median Effective Dose (ED_{50}): A linear regression analysis was used to obtain a linear equation which was used to calculate the ED_{50}. It was calculated using the formula below:

$$X = \frac{y - a}{b}$$

Legends:
x = Endpoint (LC_{50})
y = % Population Affected
a = Slope
b= Vertical Intercept

Statistical Analysis: The data collected from the results were tabulated and expressed as means ± S.E. The significance level of the treatment effects was determined using One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc analysis; p-values lower than 0.05 were considered as statistically significant so as to examine more carefully where the exact significant differences were, after doing the One-way analysis of Variance (ANOVA).

RESULTS:

Percent Anti-arthritic Activity: The percent anti-arthritic activity relative to the positive control of each test dose was calculated. Based on the average percent activity of the test doses the anti-arthritic effect increases with increasing dose.

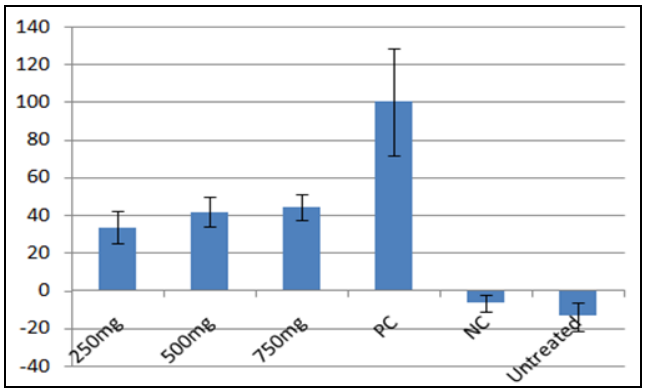

FIG. 1: PERCENT ANTI-ARTHRITIC ACTIVITY

One-way Analysis of Variance was used to compare the means of the anti-arthritic activity of *Jatropha curcas L. 1753* leaf extract. The confidence level was set at 99% with one percent chance of error which means that the probability level (p-level) is 0.01. The probability level obtained was less than 0.01, particularly 0.00000294 (See **Table 2**).

TABLE 2: ONE-WAY ANALYSIS OF VARIANCE

Source of Variation	d.f.	SS	MS	F	p-level
Between Groups	5	25,314.1805	5,062.8361	28.4192	2.94×10^{-6}
Within Groups	12	2,137.7831	178.1486		
Total	17	27,451.9636			

The one-way ANOVA of the percent reduction of inflammation showed that the 250 vs. 500, 250 vs. 750, 500 vs. 750, and NC vs. Untx are considered insignificant since their values exceeded the requirement of 0.01 p-level. The other values considered to be significant have a p-level of ≤ 0.001.

Tukey HSD Test for Differences between Means: A post-hoc test is needed after the completion of an ANOVA in order to determine which groups differ from each other. The post-hoc analysis shows the variables that have a significant difference. A significant difference means that there is a marked difference of the effects between test doses whereas an insignificant difference entails negligible difference between the effects of the test doses. The test doses yielded p values of >0.01 which indicated that there was no significant difference in the effect of the test doses, this entails that the differences in their anti-arthritic effects are negligible.

The NC vs. The Untreated group yielded a p value of >0.01 which was expected since the NC is not expected to cause any significant effect. If all of the test doses were to be individually compared with PC, NC and Untreated it yielded a p values ≤ 0.001 which is considered to be significant same also applies to the p values yielded by PC vs NC vs Untreated which is ≤ 0.001 (See **Table 3**).

TABLE 3: TUKEY'S POST Hoc ANALYSIS

Groups	p-level	Interpretation
250 v 500	0.9711	Insignificant
250 v 750	0.9083	Insignificant
250 v PC	0.0007	Significant
250 v NC	0.00292	Significant
250 v Untx	0.0098	Significant
500 v 750	0.9998	Insignificant
500 v PC	0.0019	Significant
500 v NC	0.0083	Significant
500 v Untx	0.0029	Significant
750 v PC	0.0028	Significant
750 v NC	0.0055	Significant
750 v Untx	0.002	Significant
PC v NC	0.0001	Significant
PC v Untx	0.0001	Significant
NC v Untx	0.9843	Insignificant

Histopathological analysis for anti-arthritic activity:

TABLE 4: SEVERITY SCORE FOR HISTOLOGICAL ANALYSIS

Treatment Groups	Severity Score
250mg	3
500mg	3
750mg	2
Positive	1
Negative	4
Untreated	5

The table shows that the 250mg and 500mg dose representatives indicate normal to moderate inflammation. The 750mg representative indicates normal to mild inflammation; positive representative indicates normal to infiltration of inflammatory cells and the negative and untreated representatives indicates normal to marked infiltration and normal to severe infiltration and severe diffuse cartilage.

Images from Histopathological Examination:

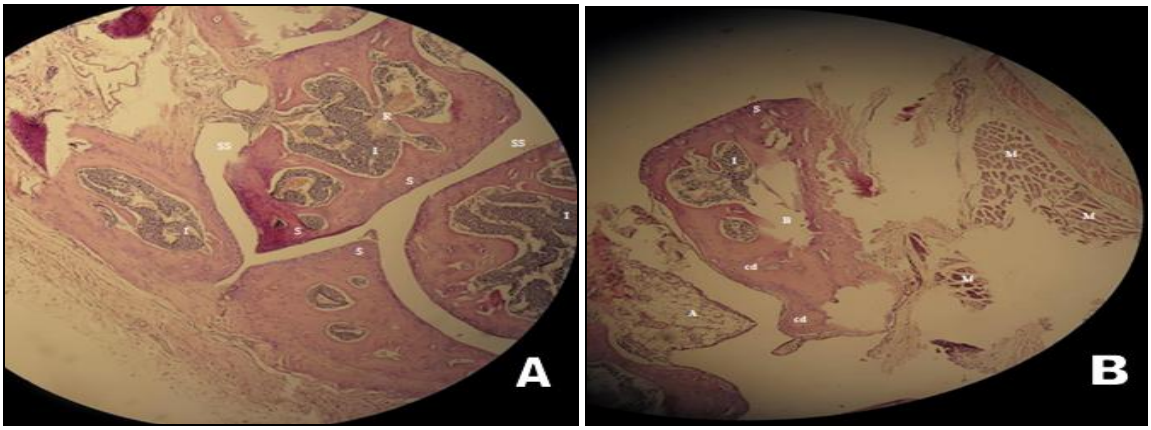

FIG. 2: HISTOPATHOLOGY RESULT FOR THE UNTREATED GROUP A: right hind paw [narrowing of the synovial space (SS), severe infiltration of inflammatory cells (I) and marked synovial hyperplasia (S). Congested accumulations of red blood cells (R) are noted indicating erythema or redness as one of the external signs of inflammation]; B: right hind paw [infiltration of inflammatory cells (I) and marked synovial hyperplasia (S), cartilage destruction (cd) and bone erosion (B). Adipocytes (A) are noted at the lower part of the picture. Presence of muscle fibers (M) is also noted at the upper right portion of the picture with a distinct shape due to the cutting of the ribbons for the preparation of the slides]

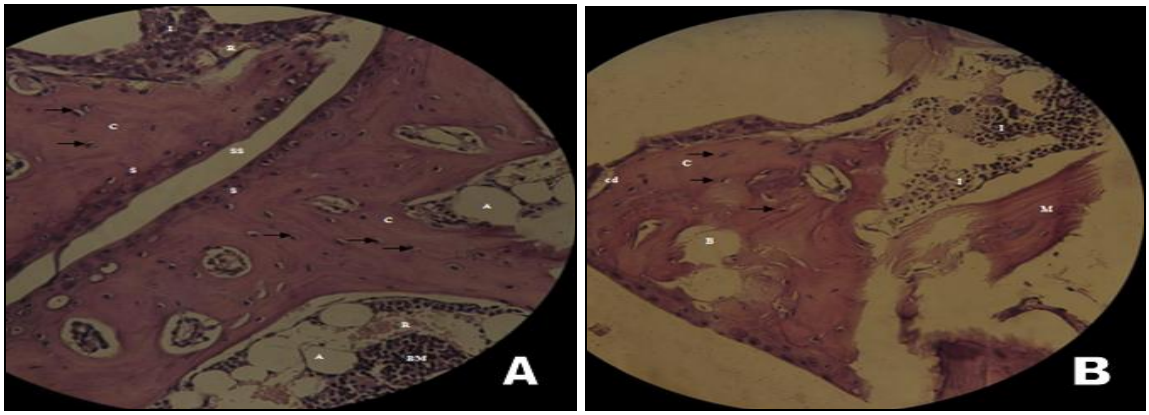

FIG. 3: HISTOPATHOLOGY RESULT FOR THE NEGATIVE CONTROL A: right hind paw [infiltration of inflammatory cells (I) and marked synovial hyperplasia (S). Bone marrow cells (BM) are noted alongside with adipocytes (A). Congested accumulations of red blood cells (R) are noted indicating erythema or redness as one of the external signs of inflammation. Chondrocytes (C) are clearly seen in this picture marked with arrows pointing at them]; B: right hind paw [infiltration of inflammatory cells (I). There were also marked cartilage destruction (cd) and bone erosion (B) noted. Chondrocytes (C) are clearly seen in this picture marked with arrows pointing at them and muscle (M) tissue]

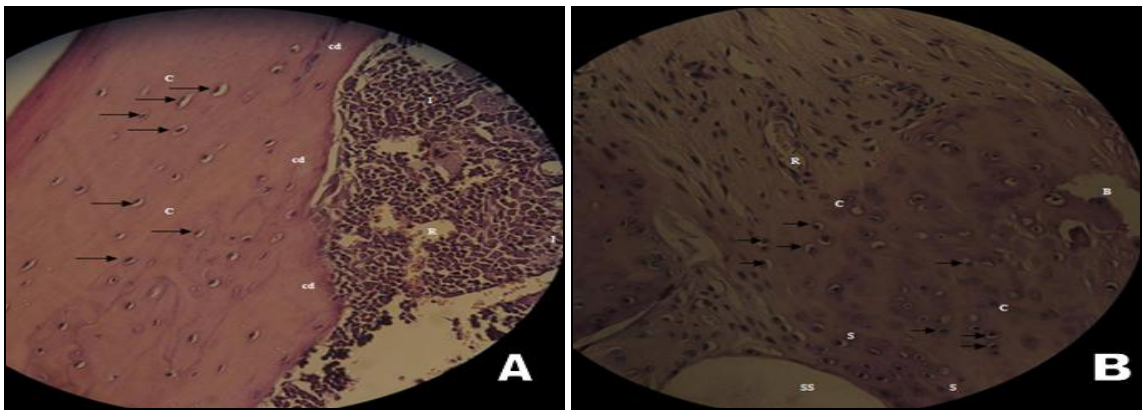

FIG. 4: HISTOPATHOLOGY RESULT FOR 250mg/kbw GROUP A: right hind paw [infiltration of inflammatory cells (I) and marked cartilage destruction (cd) are noted. Chondrocytes (C) are clearly seen in this picture marked with arrows pointing at them and Congested accumulations of red blood cells (R) are noted indicating erythema or redness as one of the external signs of inflammation]; B: right hind paw[synovial hyperplasia (S) and congested accumulations of red blood cells (R) are noted also, indicating erythema or redness as one of the external signs of inflammation. Chondrocytes (C) are clearly seen in this picture marked with arrows pointing at them]

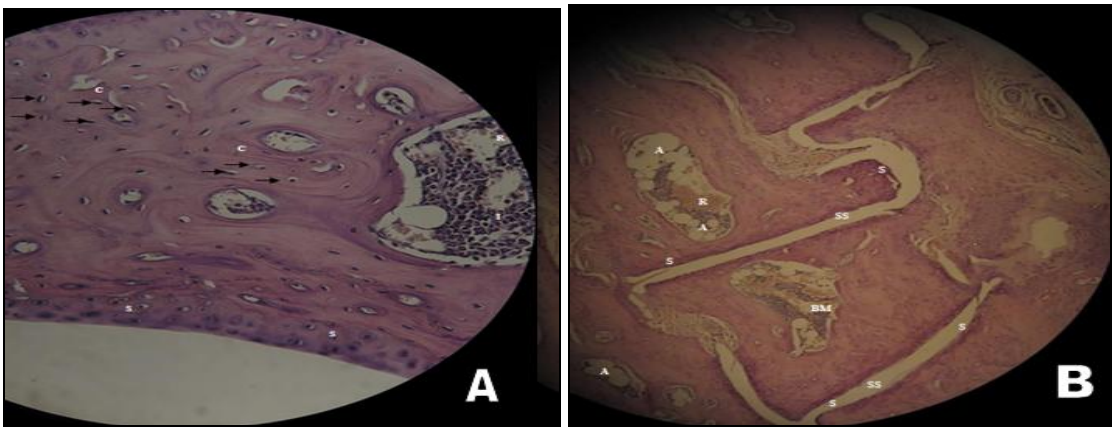

FIG. 5: HISTOPATHOLOGY RESULT FOR THE 500mg/Kbw (A) and 750mg/Kbw (B). A: right hind paw: [synovial hyperplasia (S). Narrowing of the synovial space (SS) was seen but no inflammatory cell (I) invasion was present. Congested accumulations of red blood cells (R) were also noted, indicating erythema or redness as one of the external signs of inflammation. Bone marrow (BM) cells were seen along with adipocytes (A) or fat cells]

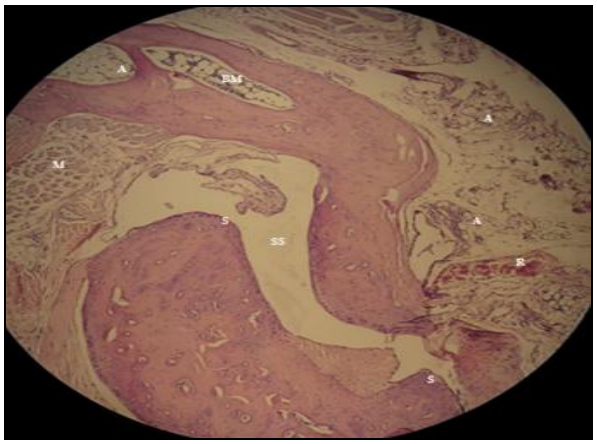

FIG. 6: HISTOPATHOLOGY RESULT FOR THE POSITIVE CONTROL. focal synovial hyperplasia (S). Wider synovial space (SS) was seen but no inflammatory cell (I) invasion was present. Congested accumulations of red blood cells (R) were also noted, indicating erythema or redness as one of the external signs of inflammation. Bone marrow (BM) cells were seen along with numerous adipocytes (A) all throughout the specimen. Muscle (M) tissue was also noted at the left portion of the picture

Median effective dose:

$$ED_{50} = \frac{y - a}{b} \qquad ED_{50} = 963.86mg$$

$$ED_{50} = \frac{50-28.93}{0.02186}$$

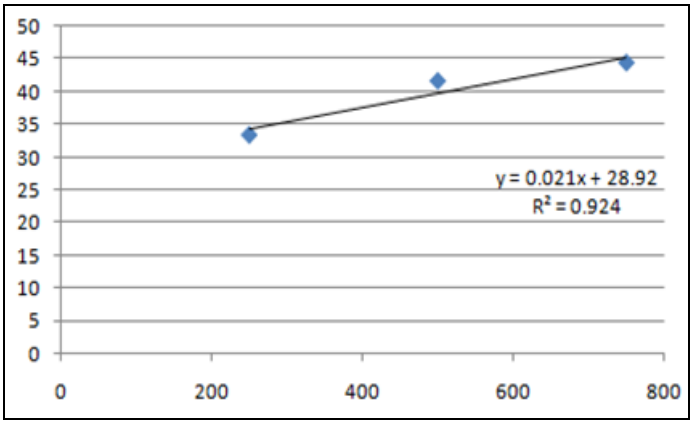

FIG. 7: MEDIAN EFFECTIVE DOSE

TABLE 5: PHYTOCHEMICAL SCREENING RESULT

Test	Constituent present	Expected Result	Actual Result	Interpretation
Keller Killiani's Test	Deoxysugars	Reddish brown color with purple ring	Reddish brown color without purple ring	Positive
Test for Presence of Tannins	Tannins	Brownish green or Blue black color	Brownish green color	Positive
Mayer's Test	Alkaloid	Orange Precipitate	Brownish Orange Precipitate	Positive
Liebermann-Burchard Test	Sterol	Green color turns to red or blue tones	Red Color/ Tone	Positive
Test for the Presence of Flavonoids	Flavonoids	Strong red violet color	Red Color/ Tone	Positive
Kedde Test	Unsaturated Lactones	Blue violet color	Brown Solution	Negative
Salkowski Test	Terpenes	Red brown color at the interface	Reddish brown color at the interface	Positive
Fehling's Test	Reducing sugars	Red to pink precipitate	No Precipitate was formed	Negative
Froth Test	Saponins	Froth formation that will last for 10 minutes	Froth was formed which lasted more than 10 minutes	Positive

DISCUSSION: Herbal medicines have been widely used in oriental clinics to treat bone diseases for thousands of years; these medicines function by strengthening bone and healing bone fractures and will undoubtedly continue to be used as cost-effective alternatives to commercial pharmaceutical products by traditional users [7]. *Jatropha curcas* is a nut belonging to the *Euphorbiaceae* family. It is cultivated in central and south America, Southeast Asia, India and Africa and grow well under adverse climatic conditions [8].

The positive control used was Indomethacin since it is an established treatment of Rheumatoid arthritis. It is a potent nonselective inhibitor of the COXs pathway and inhibits leukocytes, and a direct vasoconstrictor effect [9] which makes it a good drug for RA. Rheumatoid arthritis is a chronic systemic inflammatory disorder that principally attacks the joints, producing a proliferative and inflammatory synovitis that often progress to destruction of the articular cartilage and ankylosis of the joint [10].

Treatment studies for rheumatoid arthritis usually use the mouse model of collagen-induced arthritis. The latter is an experimental autoimmune disease that can be elicited in susceptible strains in rodents and nonhuman primates by immunization with type II collagen, the major constituent protein of articular cartilage. Because of the pathological similarities between CIA and RA, the CIA model has been subject of extensive investigation [11].

In the results obtained from the experimentation, there is an anti-arthritic activity observed in the groups given with the test solutions, that is group A (250mg/Kbw), group B (500mg/Kbw), and group C (750mg/Kbw). The said anti-arthritic activity exhibited by the three test groups are of similar effect. As seen in the mean percent activity of the test doses their percent activity is very near, group A = 33.49%, group B = 41.67%, and group C = 44.42%. The close values signify that the same effect will be produced even with the varied predetermined doses and their difference can be negligible. This might be due to the fact that the extract obtained was crude; there were no specific isolation of compound to be determined of its anti-arthritic activity.

The other parameter to determine anti-arthritic effect is the histological analysis. Characterization of inflammatory cells from bone marrow cells is that inflammatory cells are not accompanied by adipocytes while bone marrow cells are accompanied by fat cells. Adipose tissue is a specialized type of connective tissue in which adipocytes or fat cells predominate. Fat cells are generally spherical when isolated but are polyhedral when closely packed together, its size vary between 50 to 159 micrometer in diameter band contains one huge droplet of lipid and the nuclei of the cell is pushed on to the sides of the cell [12].

All the results coincided with the descriptions stipulated by Mescher, 2010. Cartilage and bone destruction was characterized by loss of cartilage and bone in places where they were usually seen. The untreated group showed the severe inflammatory invasion, synovial hyperplasia, cartilage destruction and bone destruction there was no panuus formation. The negative control showed marked inflammatory cell invasion, bone and cartilage destruction. Test groups 250mg/Kbw and 500mg/Kbw showed comparable results moderate inflammatory cell invasion, cartilage destruction and synovial hyperplasia. Test group 750mg/Kbw showed better results having minimal synovial hyperplasia, less inflammatory cells and distinguishable narrowing of the synovial space. The positive control showed only focal hyperplasia meaning its location is compartmentalized not found everywhere compared to the results shown by the untreated and negative controls.

In reference to the histological examination the test plant did not cause any healing of the cartilage or bone destruction caused by the arthritis but the test solution helped in the reduction of the inflammatory cell invasion and further damaged caused by it such as the cartilage, bone destruction and synovial hyperplasia.

When the percent reduction was calculated it was the positive control which exhibited the largest increase in average percent reduction which is 288.89%, the next was 750mg/Kbw = 128.33, 500mgKbw = 120.37, 250mg/Kbw = 96.76mg/ Kbw and the negative and untreated controls yielded - 18.98% and -39.47%. The percent reduction served as a way to confirm the data given in the statistical analysis. Among the three test doses it showed comparable effects, consequently if it were compared to its percent activity the same results is seen. If the test doses were to be compared to the positive, negative, and untreated controls it showed comparable increase in percent reduction.

The phytochemical screening yielded various amounts of plant constituents that contributed to the plant's anti-arthritic activity. The constituents found in *Jatropha curcus* Linn. 1753 were: deoxysugars, tannins, alkaloid, sterol, flavonoid, terpenes, and saponins. Studies have shown that these phytochemicals have anti-arthritic activities. Through various animal models of arthritis these constituents were proven to have anti-arthritic activities. Of all the phytochemicals that rendered positive results, it was tannins, alkaloids, flavonoids, terpenes, and saponins which are responsible for the anti-arthritic effect of *Jatropha curcas* Linn. 1753. According to the study of the evaluation of the anti-arthritic activity of the ethanol extract of the wood of *Premna serratifolia* Linn., using adjuvant induced arthritis it was tannins, alkaloids, flavonoids, phenolic compounds and glycosides present in the extract that gave its anti-arthritic activity [13].

In study of the determination of the alkaloid-free prenylated flavonoid enriched fraction of the rhizomes of *Sophora flavescens*, it exhibited a potential for the treatment of chronic inflammatory disorders such as rheumatoid arthritis owing its effect from the flavonoid used [14]. In the study of the amelioration of CIA in mice by the saponin fraction of *Gleditsia sinensi* it proved that saponins ameliorated the inflammation and joint destruction in CIA mice which makes it as an potential treatment agent for RA [15]. Rheumatoid arthritis is chararcterized by the presence of activated CD4+ T cells, plasma cells, macrophages, and synovial cells.

Synovial macrophages secrete proinflammatory cytokines- tumor necrosis factor α and IL-1 and IL-6 to induce proliferation of synovial cells, which then release collagenase, extracellular matrix metalloproteases, prostaglandins, and nitric oxide targeted to the destruction of the articular cartilage and subjacent bone. With the total alkaloids of *Tripterygium wilfordii* it exhibited reduced paw swelling, suppressed articular cartilage degeneration and also inhibition of IL-6, IL-8, and TNF-α in the synovial tissue proving its anti-arthritic effects [16]. In the phytochemical screening of the roots of *Jatropha curcas* it was found that it contains diterpenes which may help in its anti-arthritic activity [17].

CONCLUSION: *Jatropha curcas* leaf extract did not exhibit healing of the cartilage or bone destruction caused by the arthritis. However, it exhibited significant effect in the reduction of the inflammatory cell invasion and further damages caused by arthritis.

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CONFLICT OF INTEREST: The authors declare no known conflict of interest.

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