

Full length Research Article

Anti-arthritis Activity of Methanol Extract of *Peperomia pellucida* in Complete Freund's Adjuvant Induced Arthritis in Wistar Rats

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Summary: Plants in the Piperaceae family include *Peperomia pellucida*. It has been eaten and used to treat illnesses. This study tested the anti-arthritis activity of a 70% methanol extract of *Peperomia pellucida*. A complete Freund's adjuvant-induced arthritis animal model was used. Oral distilled water (10 ml/kg) did not cause arthritis in Group 1. In groups 2-4, arthritis was induced and treated with 100, 200, and 400 mg/kg of a 70% methanol extract of *Peperomia pellucida*. Group 5 received oral methotrexate (0.7 mg/kg) for arthritis pain. Group 6 received distilled water (10 ml/kg) for arthritis and was untreated. All inflamed animals' paw diameters, organ-to-body weight ratio, percentage change in body weight, erythrocyte sedimentation rate, haematological indices, biochemical parameters, and superoxide dismutase enzyme activity were measured. Additionally, joint and organ histopathology were examined. The 70% methanol extract of *Peperomia pellucida* significantly reduced arthritis inflammation diameter (** $p < 0.001$) compared to arthritis control. The weight change in extract-treated animals was not significant ($p > 0.5$) compared to healthy controls. Compared to the healthy control group, animals treated with the extract had no significant change ($p > 0.5$) in hematological and biochemical parameters. The extract and standard drug significantly reduced the erythrocyte sedimentation rate (** $p < 0.01$) compared to the arthritis control group. The extract increased superoxide dismutase enzyme activity in arthritis animals (** $p < 0.01$) compared to controls. Bone damage was significantly reduced in extract-treated animals compared to arthritis controls. Methanol extract of *Peperomia pellucida* increased endogenous superoxide dismutase and possessed anti-arthritis activity.

Keywords: *Peperomia pellucida*, superoxide dismutase, arthritis, aspartate aminotransferase.

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INTRODUCTION

Phytomedicine has been useful in the treatment and management of diseases in humans. The use of herbal medicine dates back to the origin of the human race (Petrovska, 2012). Widely used herbal medicines have been synthesized from the therapeutic experience of generations of practicing physicians of ancient systems of medicine (Khopade and Vispute, 2011). Plant parts, as well as root, stem, and leaf extracts and polyherbal preparations, are administered to treat and manage numerous disorders (Choudhary *et al.*, 2015). Research into herbal drugs is of growing interest in order to develop alternatives to orthodox medicines that may produce fewer adverse effects and be more cost effective (Badami *et al.*, 2004). *Peperomia pellucida* is a common annual weed native to tropical North, Central, and South America, where, till date, the largest diversity has been encountered. Many species are endemic to the Andes and Amazon regions. It is now pantropical in distribution and is abundantly available in tropical and subtropical regions worldwide, including Angola, Zambia,

and Nigeria. The plant flourishes in loose and humid soil in moist and shady areas. *P. pellucida* is characterized by its smooth, fleshy, and heart-shaped leaves, succulent stems, shallow roots, and minute flowers, which eventually grow into numerous tiny seeds attached to the cord-like spikes (Frenzke *et al.*, 2015; Mosango, 2008). The current study evaluates the anti-arthritis activity of *Peperomia pellucida* using complete Freund's adjuvant induced arthritis in an animal model.

MATERIALS AND METHODS

Collection of Plant Material: The plant, *Peperomia pellucida*, was collected from humid farmland in Ogbeson, Ikpoba Hill, Benin City, from November to December, 2020. It was identified by Dr. H. A. Akinnibosun, a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

Extraction of Plant: The root of the plant was isolated from the whole plant and discarded. 95 g of plant was weighed and thoroughly washed using distilled water. The plant was then blended with 300 ml of distilled water using a blender. Afterwards, the mixture was dissolved in 700 ml of methanol and left to stand for three days. At the completion of the duration, the plant was sieved using cheesecloth, and the filtrate was concentrated over a water bath. The extract was stored in a refrigerator before use.

Animal Design: Thirty healthy Wistar rats of both sexes weighing between 205-300 g were purchased from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State. The animals were housed within the animal house facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City. They were acclimatized for one week amid ideal environmental conditions. All animals were adequately fed with grower pellets and given potable water. The animals were handled according to standard protocol for laboratory animals (National Institute of Health, USA; Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002) and the ethical committee of the Department of Science and Laboratory Technology, Faculty of Life Sciences, University of Benin.

Anti-Arthritis Activity Of 70% Methanol Extract of *Peperomia pellucida* in Complete Freund Adjuvant (CFA) Induced Arthritis: Thirty (30) Wistar rats of both sexes weighing between 205-300 g were divided into six groups of four animals per group. In Group 1, the healthy control group, distilled water (10 ml/kg) was administered orally daily, and there was no induction of arthritis. Groups 2-4 received 100 mg/kg, 200 mg/kg, and 400 mg/kg of a 70% methanol extract of *Peperomia pellucida*, respectively, with arthritis induced. In Group 5, the standard group, methotrexate (0.7 mg/kg) was administered orally with arthritis-induced pain. In Group 6, the arthritis control group, arthritis was induced, and animals were left untreated. Before treatment, arthritis was induced in the animals in groups 2-6 by injecting a single dose of 0.1 ml of complete Freund's adjuvant (CFA) into the foot pad of the left hind paw of the animals and observed for 9 days. Complete Freund's adjuvant contains 1 mg/ml of dry, heat-killed *Mycobacterium tuberculosis* dissolved in sterile paraffin oil. Treatment was administered orally for 14 days after day 9 (Uwaya *et al.*, 2022; Fatima and Fatima, 2016).

Arthritis Assessment: The paw size reading of left hind limb, 2cm below ankle, was taken on days 0, 2, 6, 10 and 14. This was done using a Digital Vernier Caliper (Mitutoyo, Japan) on all animals. Paw diameter was compared to baseline values obtained before induction of CFA.

Body Weight Assessment and Measurement of Organ Weights: The weights of the animals were taken on days 1 and 14 of the experiment before sacrifice. After sacrifice, the liver, kidney, lungs, heart, and spleen were discreetly removed and weighed using an analytical weighing balance. Percentage weight change and organ to body weight ratio were calculated using the formulas below:

Percentage weight change (%) = [(final weight - initial weight) / final weight] x 100
Organ-to-body weight ratio = weight of internal organ / final body weight

Determination of Erythrocyte Sedimentation Rate Using Wistar Green Method: On day 14, animals were sacrificed by anesthetizing with chloroform and dissected. Blood was collected from the abdominal aorta. 1 ml of blood was transferred into a Wistar green disposable ESR tube, 1 ml into an EDTA container, and the remaining volume was transferred into a plain container. The blood in the disposable ESR tube was allowed to stand for 1 hour, and the reading was taken.

Determination of Haematological Parameters: The blood in the EDTA bottle was used to analyze haematological parameters using an automated machine model (PCE-2100, Japan).

Determination of Biochemical Parameters: Blood collected in plain sample bottles without anticoagulant was centrifuged for 5 minutes (4900 rpm), and the serum was collected as a sample for assessment of biochemical parameters.

Determination of Alkaline Phosphatase (ALP): Alkaline phosphate substrate (0.5 ml) was pipetted into test tubes labeled blank, standard, and sample, respectively. 50 ul of distilled water, a standard, and a sample were added to the test tubes, respectively, and incubated for 10 minutes at 37 °C. Then, 2.5 ml of alkaline phosphatase colour developer was added to labeled test tubes. The spectrophotometer was zeroed with reagent blank, and the absorbencies of all tubes were measured at a wavelength of 590 nm. The concentration of alkaline phosphate was calculated using the following formula:

Alkaline Phosphatase Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

Determination of Alanine Aminotransferase (ALT): To 5 ml of each blood sample, 0.1 ml of solution R1 (buffer) was added and mixed. It was incubated for 30 minutes at 37 °C. 0.5 ml of solution R2 (2,4-dinitrophenylhydrazine) was added, and the mixture was allowed to stand for 20 minutes at room temperature, after which 5.0 ml of sodium hydroxide was added. The process was repeated for all samples. The samples were read using a spectrophotometer at a wavelength of 546 nm. The samples were read against a blank.

Determination of Aspartate aminotransferase (AST): To 5 ml of each of the blood samples, 0.1 ml of solution R1 (buffer) was added, mixed, and incubated for 30 minutes at 37 °C. Solution R2; 2,4-dinitrophenylhydrazine (0.5 ml) was added, and the mixture was allowed to stand for 20 minutes at room temperature, after which 5.0 ml of sodium hydroxide was added. The process was repeated for all samples. The samples were read using a spectrophotometer at a wavelength of 546 nm.

Determination of Total Protein: To 0.02 ml of the blood samples, distilled water and standard were pipetted into separate test tubes and labeled sample, blank, and standard,

respectively. To the test tube containing the sample and standard, 0.5 ml of solution R1 was added, and 0.5 ml of solution R2 was added to the test tube containing the blank. The solution in the test tube was then mixed and incubated for 30 minutes at 25 °C. The samples were read using a spectrophotometer at a wavelength of 546 nm. The samples were read against the blank, which contained both solutions and sodium hydroxide and water for absorbance. The concentration of total protein was calculated using the following formula:

Total Protein Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

Determination of Superoxide Dismutase (SOD):

Carbonate buffer (2.5 ml) was measured into labelled test tubes. 0.2 ml of tissue homogenate was added to the test tubes. 0.2 ml of distilled water was also measured in the reference test tube. 0.3 ml of epinephrine solution was added to each of the test tubes and to the reference tube. They were properly mixed and read at an absorbance of 420 nm every 30 – 120 seconds with a UV spectrophotometer (Model T80+UV spectrophotometer, PG Instruments Ltd.). Distilled water was used to zero the machine (Misra and Fridouich, 1972).

Histopathological Assessment: After sacrifice, the left hind paws were severed discreetly. Tissue specimens were dehydrated in alcohol, processed in xylene, and then embedded in paraffin wax before sectioning was done using a microton. Sections of the tissues 5 µm thick were stained with hematoxylin and eosin (H & E) for viewing. All sections were viewed under a Leica R light microscope (Model DM500) at x100 and x400 magnification by a

histopathologist who was oblivious to the experimental grouping and protocol.

Statistical Analysis: Data are expressed as mean ± standard error of mean (SEM) in this study. Multiple comparisons of data were done using one-way ANOVA. $P > 0.5$ was consistent with non-significant values. The analysis of all the data was done using the software program GraphPad Prism.

RESULTS

Anti-arthritis effect of 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund adjuvant induced arthritis in Wistar rats

Table 1 shows the effect of 14-day daily oral administration of 70 % methanol extract of *Peperonia pellucidain* paw diameter in complete Freund's adjuvant induced arthritis in Wistar rats. Seventy percent methanol extract of *Peperonia pellucidain* significantly reduced (** $p < 0.001$) the diameter of inflammation of arthritis compared to arthritis control rats.

Organ-to-body weight ratio and percentage change in body weight following 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund's adjuvant induced arthritis in Wistar rats: Table 2 shows the organ to body weight ratio and percentage change in body weight following 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund's adjuvant induced arthritis in wistar rats. In the animals treated with the extract, change in weight was not significant ($p > 0.05$) compared to animals belonging to the healthy control group.

Table 1:

The anti-arthritis effect of 14-day daily oral administration of 70 % methanol extract of *Peperonia pellucidain* complete Freund adjuvant induced arthritis in Wistar rats

Dose (mg/kg)	Diameter (mm)				Inhibition (%)
	1 st	2 nd	3 rd	4 th	
Control	0.76 ± 0.15	0.66 ± 0.19***	0.91 ± 0.13***	0.72 ± 0.14****	73.43
100	2.20 ± 0.25*	2.00 ± 0.06**	1.51 ± 0.08*	1.32 ± 0.08**	51.29
200	2.51 ± 0.21**	1.89 ± 0.12**	1.48 ± 0.12**	1.22 ± 0.16***	54.98
400	2.82 ± 0.21**	1.77 ± 0.20***	1.45 ± 0.21**	1.11 ± 0.24***	59.04
STD	2.41 ± 0.51*	2.18 ± 0.14*	1.19 ± 0.21**	1.17 ± 0.38***	56.83
Arthritis alone	3.04 ± 0.34***	3.13 ± 0.27	2.78 ± 0.45	2.71 ± 0.07	-

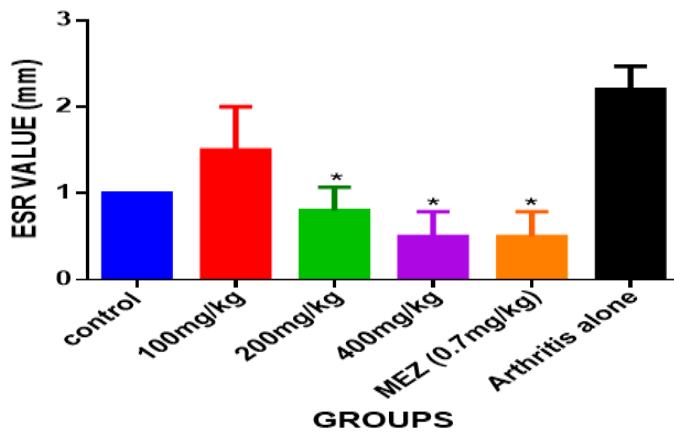
The 70% methanol extract of *Peperonia pellucidain* significantly decreased the diameter of inflammation of arthritis at 100 mg/kg ($p > 0.01$) and all other dose levels ($p > 0.001$) as compared to the arthritis alone control group. Values are represented as mean ± SEM, $n = 4$ per group.

Table 2:

Organ-to-body weight ratio following 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund adjuvant induced arthritis in Wistar rats.

Dose (mg/kg)	H:BW (10^{-3})	Lu:BW (10^{-3})	S:BW (10^{-3})	Li:BW (10^{-3})	K:BW (10^{-3})	% Body Weight Change (%)
Control	3.2±0.48	6.5±0.96	3.3±0.48	3.3±0.46	4.3±0.48	0.69 ± 0.43
100	4.0±0.00	7.0±0.58	3.5±0.29	3.3±0.17	5.8±0.25	9.75 ± 4.96
200	3.3±0.25	6.3±0.25	3.3±0.25	3.2±0.70	5.0±0.00	3.75 ± 1.65
400	3.3±0.25	6.3±0.25	3.3±0.25	3.1±0.96	5.3±0.25	2.65 ± 2.27
Standard	3.5±0.50	8.5±0.25	2.8±0.48	2.4±0.65	6.5±0.87	6.35 ± 1.39
Arthritis alone	4.0±0.41	1.1±2.60	3.5±0.29	2.8±0.75	5.8±0.25	6.13 ± 2.20

Test groups show no significant difference ($p > 0.5$) in organ-to-body weight ratio and no significant difference ($p > 0.05$) in percentage weight change compared to the arthritis-only control group. Values are represented as mean ± SEM, $n = 4$ per group.

**Figure 1**

The effect of 14 days daily oral administration of a 70% methanol extract of *Peperomia pellucida* on erythrocyte sedimentation rate in complete Freund adjuvant induced arthritis in Wistar rats.

There was a significant decrease (** $p > 0.01$) in erythrocyte sedimentation rate at 200 mg/kg and 400 mg/kg dose levels and in the standard group as compared to the arthritis-only control group. Values are represented as mean \pm SEM, $n = 4$ per group

Table 3:

The effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on haematological parameters in complete Freund adjuvant induced arthritis in Wistar rats

DOSE(mg/kg)	WBC ($10^3/uL$)	LYM ($10^3/uL$)	MID ($10^3/uL$)	GRAN ($10^3/uL$)	RBC ($10^6/uL$)	HGB (g/dL)	HCT (%)	PLT ($10^3/uL$)	PCT (%)
Control	9.2 \pm 0.27	83.4 \pm 1.10	12.1 \pm 0.82	4.5 \pm 0.29	7.5 \pm 0.10	14.3 \pm 0.22	44.0 \pm 0.53	629.3 \pm 66.2	0.46 \pm 0.06
100	12.6 \pm 0.94	77.6 \pm 8.95	16.4 \pm 6.14	6.0 \pm 2.82	6.7 \pm 0.73	12.9 \pm 1.47	40.6 \pm 3.47	839.0 \pm 202.10	0.6 \pm 0.17
200	13.4 \pm 0.90	82.9 \pm 3.32	11.3 \pm 2.17	5.8 \pm 1.17	7.7 \pm 0.10	15.0 \pm 0.26	45.0 \pm 0.68	650.0 \pm 12.33	0.5 \pm 0.01
400	13.2 \pm 1.49	80.6 \pm 2.87	13.5 \pm 1.86	5.9 \pm 1.17	7.8 \pm 0.07	15.1 \pm 0.25	44.9 \pm 1.34	879.0 \pm 160.30	0.8 \pm 0.18
Standard	12.9 \pm 0.51	72.9 \pm 6.93	17.4 \pm 3.52	9.7 \pm 3.45	7.0 \pm 0.10	14.0 \pm 0.28	41.3 \pm 1.05	759.80 \pm 55.47	0.62 \pm 0.08
Arthritis alone	10.63 \pm 1.37	83.6 \pm 3.97	10.2 \pm 2.72	6.23 \pm 1.73	7.3 \pm 0.12	13.6 \pm 0.31	43.7 \pm 1.58	642.5 \pm 59.20	0.45 \pm 0.042

There was no significant difference ($p > 0.5$) in haematological parameters at all dose levels compared to the arthritis-only control group. Values are represented as mean \pm SEM, $n = 4$ per group.

The effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on erythrocyte sedimentation rate and haematology parameter in complete Freund's adjuvant induced arthritis in wistar rats: Figure 1 and Table 3 shows the effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on erythrocyte sedimentation rate and haematology parameters in complete Freund's adjuvant induced arthritis in Wistar rats. The extract and standard drug reduced (** $p < 0.01$) the erythrocyte sedimentary rate compared to arthritis control. There was no significant alteration ($p > 0.05$) in haematological parameters in animals treated with the extract compared to animals in the healthy control group (Table 3).

The effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on biochemical parameters and superoxide dismutase in complete Freund's adjuvant induced arthritis in Wistar rats: Table 4 shows the effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on biochemical parameters in complete Freund's adjuvant induced arthritis in Wistar rats. There was no significant alteration ($p > 0.5$) in biochemical parameters in animals treated with the extract compared to animals in the healthy control group. Figure 2 shows the effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on superoxide dismutase enzyme activity in complete Freund's adjuvant-induced arthritis in Wistar rats. The plant extract increased (** $p < 0.01$, * $p < 0.05$) the superoxide Table 4: The effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on biochemical parameters in complete Freund's

adjuvant induced arthritis in Wistar rats. There was no significant difference ($p > 0.5$) in biochemical parameters in animals treated with the extract compared to animals in the healthy control group.

Table 4:

The effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on biochemical parameters in complete Freund's adjuvant induced arthritis in wistar rats.

Dose (mg/kg)	AST (UL)	ALT (UL)	ALP (UI/L)	TP (mg/dl)
Control	163.8 \pm 6.8	16.0 \pm 1.47	15.3 \pm 1.48	5.0 \pm 0.36
100	207.6 \pm 21.4	9.7 \pm 3.49	21.6 \pm 2.12	3.8 \pm 0.48
200	165.4 \pm 42.67	13.0 \pm 3.19	11.7 \pm 0.24	1.4 \pm 0.78
400	95.9 \pm 21.24	6.9 \pm 2.10	15.9 \pm 2.03	3.7 \pm 1.18
Standard	129.2 \pm 7.51	6.4 \pm 2.04	15.2 \pm 3.79	5.1 \pm 0.11
Arthritis alone	115.7 \pm 13.09	18.1 \pm 8.53	16.4 \pm 0.88	6.0 \pm 0.57

TP = Total protein

At the 200 mg/kg dose level, there was a significant reduction (** $p < 0.01$) in total protein as compared to the arthritis-only control group. Values are represented as mean \pm SEM, $n = 4$ per group.

The histopathology of the liver, kidney, knee joint, and stomach following 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* in complete Freund's adjuvant induced arthritis in Wistar rats was compared to arthritis control: Plates 1 (slides A - F) 2 (slides G - L), 3 (slides M - Q) and 4 (slide R - X) show the histopathology of the liver, kidney, knee joint, and stomach respectively, following 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* in complete Freund's adjuvant induced arthritis in Wistar rats compared to arthritis control. Animals treated with extract showed significant protection from bone damage compared to the arthritis control group.

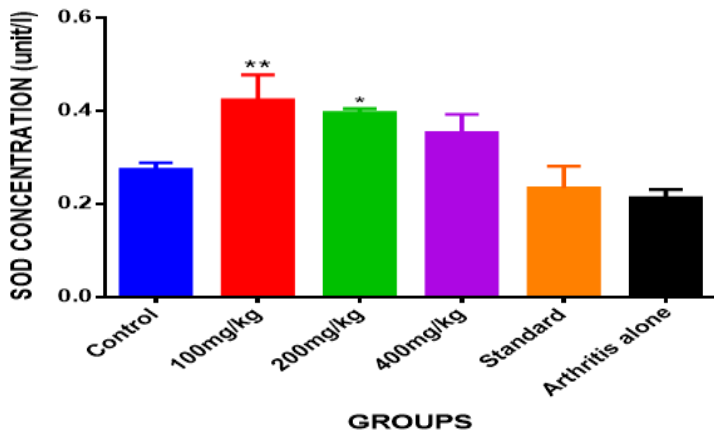


Figure 2:

The effect of 14 days of daily oral administration of a 70% methanol extract of *Peperonia pellucida* on superoxide dismutase enzyme activity in complete Freund adjuvant induced arthritis in Wistar rats. There was a significant increase (** $p < 0.01$, * $p < 0.05$) in the level of superoxide dismutase enzyme activity in 100 mg/kg and 200 mg/kg as compared to the arthritis-only control group. Values are represented as mean \pm SEM, $n = 4$ per group.

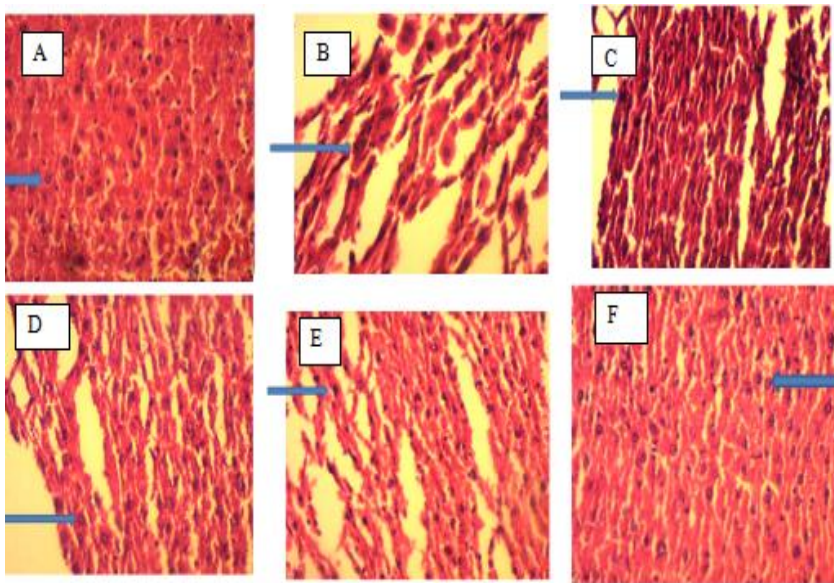


Plate 1

A: histological slide of the liver showing normal hepatocytes in the control group (X400).
B: histological slide of the liver showing normal hepatocytes in the standard group (X400).
C: histological slide of the liver showing a normal hepatocyte-arthritis control group (X400).
D: Histological slide of liver showing normal hepatocytes in the 100 mg/kg group (X400).
E: Histological slide of liver showing normal hepatocytes in the 200 mg/kg group (X400).
F: histological slide of the liver showing pyknotic hepatocytes in the 400 mg/kg group (X400).

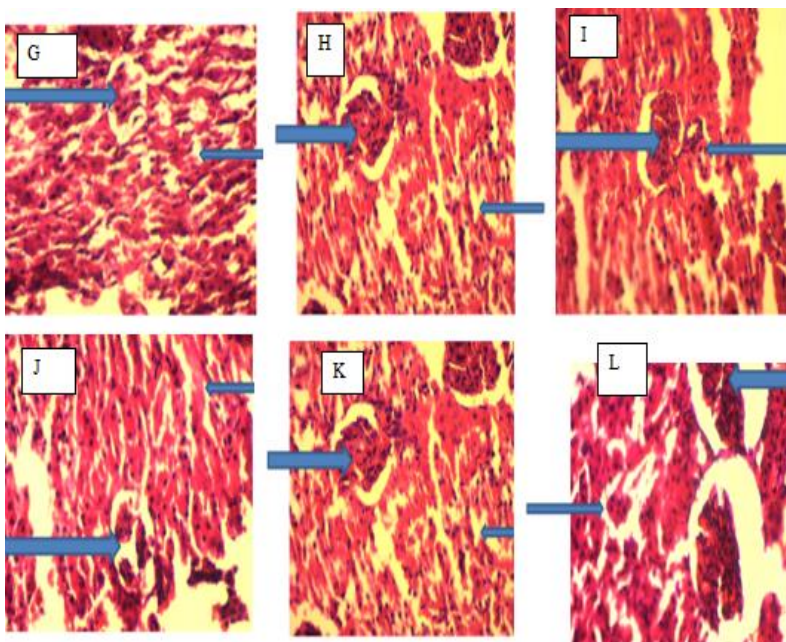
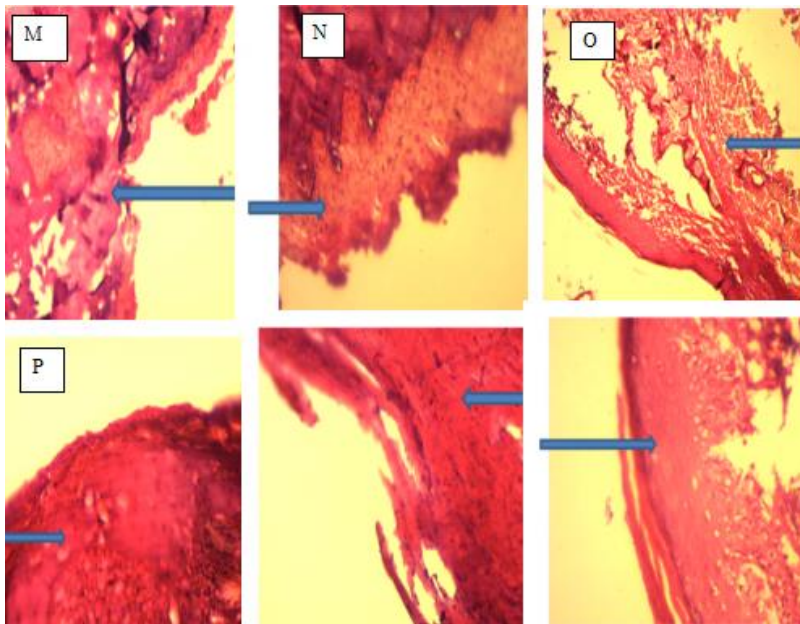


Plate 2

G: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the control group (X400).
H: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow)—standard group (X400).
I: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow)—arthritis control group (X400).
J: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the 100 mg/kg group (X400).
K: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the 200 mg/kg group (X400).
L: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the 400 mg/kg group (X400).

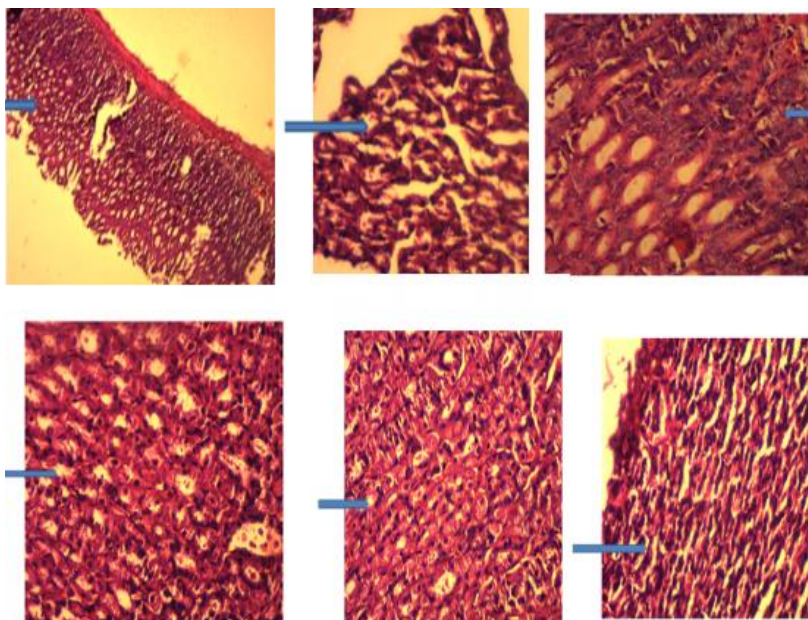
**Plate 3**

M: histological view of the knee joint showing normal fibroconnective tissue in the control group (X100).

N: histological view of knee joint showing normal fibroconnective tissue, standard group (X100). Plate **O:** histological view of knee joint showing fragmentation of connective tissue—arthritis alone group (X100).

P: histological view of the knee joint showing normal fibroconnective tissue in the 100 mg/kg group (X100).

Q: Histological view of knee joint showing normal fibroconnective tissue, 200 mg/kg group (X100).

**Plate 4**

R: Histological view of the knee joint showing normal fibroconnective tissue in the 400 mg/kg group (X100).

S: Histological view of stomach showing normal gastric epithelium - control group (X400).

T: Histological view of the stomach showing normal gastric epithelium, Standard Group (X400).

U: Histological view of the stomach showing necrosis of the gastric epithelium—arthritis alone group (X400).

V: Histological view of the stomach showing necrosis of the gastric epithelium, 100 mg/kg group (X400).

W: Histological view of the stomach showing necrosis of the gastric epithelium, 200 mg/kg group (X400).

X: Histological view of the stomach showing necrosis of the gastric epithelium, 400 mg/kg group (X400).

DISCUSSION

Arthritis is a chronic inflammatory and systemic disease associated with inflammation of the joints, joint damage, synovial inflammation, cartilage damage, and bone damage (Chunxia *et al.*, 2011). The primary symptoms include pain, swelling, and destruction of cartilage and bones, as a result of which permanent disability may occur (Babushetty and Sultanpur, 2012). In most patients, prolonged inflammation rapidly results in bone erosion (Van der Heijde, 1995).

Treatment forms are mainly targeted at eliminating symptoms, stopping the progress of the disease, inhibiting bone destruction, and protecting bone and muscle function (Ichikawa *et al.*, 2012). This study shows the result of 14-day oral administration of a 70% methanol extract of *Peperomia pellucida* in complete Freund's adjuvant induced arthritis in rats. Complete Freund's adjuvant is among the oldest experimental models used for antiarthritis studies (Colpaert *et al.*, 1982). This mimics rheumatoid

arthritis and osteoarthritis in humans (Pathak *et al.*, 2009). In this study, the 70% methanol extract of *Peperomia pellucida* reduced the diameter of inflammation of arthritis in animals treated with the extract when compared with the arthritis control group. The percentage inhibition increased as the dose increased. This means that the anti-arthritis activity of a 70% methanol extract of *Peperomia pellucida* is dose dependent. The percentage inhibitions of the extract at 400 mg/kg (59.04%) and that of the standard drug, methotrexate (56.83%) are comparable (Table 1). Methotrexate is a first-choice drug in the treatment of rheumatoid arthritis. It is a disease modifying antirheumatic drug with cytotoxic and immunosuppressant activity believed to act by blocking adenosine uptake (Chan and Cronstein, 2010). The suppression of inflammation by a 70% methanol extract of *Peperomia pellucida* may be related to this mechanism.

The extract and standard, methotrexate, significantly reduced the erythrocyte sedimentation rate compared to

arthritis control. Erythrocyte sedimentation rate is not specific in diagnosis but a marker related to certain allergies, inflammatory diseases, and infections. It is influenced by the number and size of red blood cells and is involved in the increased formation of endogenous proteins such as fibrinogen and globulins. Clumping of red blood cells is associated with certain inflammatory and autoimmune diseases, and this increases the rate at which cells sink towards the bottom of the test tube. In this study, there was no significant weight change in animals treated with the extract of *Peperomia pellucida*. Weight loss is a potent predictor of health, especially in pathological conditions (Higgins *et al.*, 1993). In this study, the organ-to-body weight ratio was not significantly altered in animals treated with a 70% methanol extract of *Peperomia pellucida* compared to the healthy control group. An increase or decrease in the average organ-to-body weight ratio suggests underlying conditions that may either cause inflammation or shrinkage of internal organs. As a result of the study, the extract of *Peperomia pellucida* supported normal haematological parameters compared to arthritis control.

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are endogenous enzymes that indicate the state and function of the liver, and these values are commonly used to assess the presence of underlying cellular injury (Faqi, 2017). Levels in the blood are increased in certain pathological conditions, including hepatitis, liver damage, and bone and muscle damage (Lala *et al.*, 2021). According to the results of this study, there was no significant alteration in levels of ALP, AST, and ALT in animals treated with 70% methanol *Peperomia pellucida* extract compared to arthritis control.

In this study, the level of endogenous superoxide dismutase enzyme (SOD) was increased in animals treated with an extract of *Peperomia pellucida*. The superoxide dismutase enzyme plays a role in defense against oxidative stress (Younus, 2018). It is an endogenous antioxidant that catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko *et al.*, 2002). It can serve as an anti-inflammatory agent and can also prevent precancerous cell changes (Inal *et al.*, 2001). It has been found to inhibit neutrophil-mediated inflammatory responses (Yasui and Baba, 2006).

In the histopathological study, the knee joints of animals that were given the extract at all doses and standards had healthy fibroconnective tissues. This is contrary to the connective tissue fragmentation obtained in the arthritis control group. Thus, the extract protected the knee joints of the experimental animals from destruction by arthritis induced by complete Freund's adjuvant. There were normal glomeruli and tubules in the kidneys. However, there was necrosis of the gastric epithelium of the stomach in the animals treated with a 70% methanol extract of *Peperomia pellucida* and in the arthritis control group. This suggests that exercising caution is necessary when utilizing an extract of *Peperomia pellucida*.

In conclusion, this study showed that *Peperomia pellucida* possesses anti-arthritis activity. The anti-arthritis activity of *Peperomia pellucida* could be a result of an increase in endogenous superoxide dismutase enzyme activity.

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