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Anti-arthritic effects of a hydroethanolic extract of *Justicia flava* (Forsk) Vahl. (Acanthaceae) in rats

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Abstract

Justicia flava (Acanthaceae) is a perennial herbaceous plant, erect or creeping, reaching 120 cm in height and pubescent. *Justicia flava* is a species widespread in tropical Africa and southern Africa. The aim of this study was to evaluate the chronic anti-inflammatory potential of a hydroethanolic extract of *Justicia flava* (HEJF) in rats. The non-immunological arthritis model was used. The experiments lasted 14 days. Six groups of six rats were formed and received various treatments daily for 10 days. Arthritis was induced by injecting 0.1 mL of 2.5% formaldehyde into the right hind paw of rats in all groups except group 1 (negative control group) on day 1 and day 3. The initial

thickness of the rats' paws was measured using a caliper before any treatment. HEJF was prepared by macerating 100 g of *Justicia flava* powder in one liter of a mixture of 30% water and 70% ethanol for 24 hours. The rats were force-fed 9‰ NaCl (10 mL/kg bw) (Group 1 and group 2 or arthritic control). The rats in groups 3, 4, and 5 were force-fed HEJF (125, 250, and 500 mg/kg bw). The rats in group 6 were force-fed aspirin (100 mg/kg bw). The progression of arthritis was assessed by measuring the thickness of the rats' paws. The percentage of edema inhibition was calculated for each group of treated rats relative to the arthritic control group. The results showed that HEJF caused a significant, dose-dependent inhibition of the average paw thickness of rats, with reductions ranging from 34.5% to 51.71% compared to the arthritic control group on the 10th day of treatment. The number of white blood cells, which had previously increased, was also reduced in the presence of HEJF. HEJF also corrected the decreases in red blood cell and platelet counts recorded on the third day of the experiment. The hydroethanolic extract of *Justicia flava* has remarkable anti-arthritic properties similar to aspirin, inhibiting edema of the paw induced by 2.5% formaldehyde**.** These results could therefore justify the traditional use of *Justicia flava* in the treatment of pain associated with gastric and abdominal ulcers.

Keywords: Hydroethanolic extract, *Justicia flava*, anti-arthritic, formaldehyde, rat.

Introduction

Inflammation is classified into two types: acute and chronic inflammation (O'Connor and Nichol, 2015). Nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics are widely prescribed in modern medicine due to their effectiveness in managing pain, fever, inflammation, and rheumatic disorders (Russo *et al*., 1998; Miner *et al*., 2007). Although inflammation is a protective immune response, excessive inflammatory response can cause serious diseases (arthritis, diabetes, asthma, allergies, and cancer) (Viladomiu *et al*., 2016). Rheumatoid arthritis is an autoimmune disease characterized by chronic and destructive inflammation of the joints (Calop *et al*., 2012). It is the most common form of chronic inflammatory rheumatism and accounts for 5 to 29% of all inflammatory arthritis cases. Its global prevalence is estimated at 1% (Silman, 1998; Sibilia, 2000). In Cameroon, Burundi, and Côte d'Ivoire, its prevalence is estimated to be between 0.8% and 2.3%. However, it remains significantly lower than that found in European countries (Jeandel *et al*., 1991). In addition, this disease is accompanied by inflammation of the synovium, hyperplasia, cartilage destruction, bone deformation with weakening, and systemic complications such as cardiovascular, pulmonary, psychological, and skeletal disorders (McInnes and Schett, 2011). Rheumatoid arthritis cannot be cured, but several combinations of anti-inflammatory and analgesic drugs are used during treatment to inhibit the process (Zhao *et al*., 2006; Makinen *et al*., 2007). As a result, there is a need to search for new anti-inflammatory substances that are more effective in reducing inflammation. The use of medicinal plants is becoming an important avenue to explore in the search for effective drugs with fewer side effects (Lardry and Haberkorn, 2007). In Côte d'Ivoire, several species of medicinal plants and medicinal recipes have been identified (Aké Assi, 1991;Tra Bi *et al*., 2008).It is therefore appropriate to continue exploring herbal medicine for its therapeutic potential. It is with this in mind that the species *Justicia flava* (Forsk) Vahl, commonly used in traditional medicine, was selected for this study. This plant is used in Africa to treat various conditions. In Sudan, for example, the seeds are used to treat toothache (El Kamali, 2009). It is well known in Côte d'Ivoire as a hemostatic agent for cuts, vaginal bleeding, and hemoptysis. The leaf pulp is used to rub babies who have convulsions or suffer from feverish aches and pains (Bouquet and Debray, 1974). *Justicia flava* could therefore have interesting anti-arthritic properties that are in line with the research focus of the Laboratory of Physiology, Pharmacology, and Pharmacopoeia on the effects of plant extracts on various types of inflammation and pain. This plant was therefore chosen to evaluate its effects on a model of chronic inflammation. The objective of this study is to evaluate the chronic anti-inflammatory potential of a hydroethanolic extract of *Justicia flava*. Specifically, the aim is to

evaluate chronic anti-inflammatory activity using a model of inflammatory arthritis induced in rats.

Materials and Methods

Materials

Plant

This material consists of the aerial parts (leaves, flowers, inflorescence, and stem) of *Justicia flava* harvested in Petit Yapo in the department of Agboville in southern Côte d'Ivoire in November 2023.The species was identified at the botany laboratory of Nangui ABROGOUA University in Abidjan and authenticated by the laboratory's teaching staff. This species was subsequently confirmed with a herbarium specimen preserved under number 17511 dated July 29, 1986, at the National Center for Floristics at Félix Houphouët Boigny University.

Animal

Wistar strain *Rattus norvegicus* albino rats were used to evaluate chronic anti-inflammatory activity. These rats, aged 8 to 12 weeks, had a body weight between 100 and 125 g. All these animals were supplied and raised at the animal facility of the Physiology, Pharmacology, and Pharmacopoeia Laboratory of the Natural Sciences Training and Research Unit (UFR-SN) at Nangui ABROGOUA University (Côte d'Ivoire). They had free access to water and food with a photoperiod of 12 hours of light and 12 hours of darkness in accordance with good laboratory practice standards. The various experimental protocols were developed in accordance with the European Council's protocols for the protection of laboratory animals 87/609/EEC.

Laboratory equipment

The technical equipment consists of a magnetic stirrer (OVAN, USA) to homogenize the various substances, a caliper to measure the thickness of the rats' paws, an electronic scale (Neo-Tech SA, Belgium) for small weighings, an automated device (MINDRAY, France) for hematological parameters, an oven (Heto, CD 52-I, France) for drying the extract, an electric grinder (RETSH, SM 100, Haan, Germany) for grinding the species, a scale (Digital scal-SF-400, China) for weighing the rats, a mobile phone (Samsung Galaxy Core Prime, Korea) for taking photos of the species, the equipment, and the rats' paws, a feeding tube and syringes for administering the various substances to the rats, Whatman 3 mm filter paper and cotton wool for extraction, Pasteur pipettes, and EDTA tubes for sampling and performing the complete blood.

Pharmacodynamic and chemical substances

The substances used in this study are hydroethanolic extract of *Justicia flava*, aspirin® (Côte d'Ivoire), and tramadol (trabar®) (Germany) as reference products, 2.5% formaldehyde for the induction of arthritis, 9‰ NaCl as the physiological solution, and ether (Belgium) to euthanize the rats.

Methods

Preparation of the hydroethanolic extract of *Justicia flava*

Hydroethanolic extract of *Justicia flava* was prepared according to the method described by Zirihi (1991). The aerial parts (leaves, flowers, inflorescences, and stems) of *Justicia flava* are washed with distilled water and dried at room temperature (25°C) for 5 days, then finely pulverized with an electric grinder. Thus, 100 grams of powder obtained from *Justicia flava* was cold macerated for 24 hours in one liter of a mixture of 30% water and 70% ethanol (hydroethanolic mixture). The resulting solution is filtered through cotton wool and then through 3 mm Whatman filter paper. Half a liter of the hydroethanolic mixture is added to the residue and another cold maceration is carried out for 24 hours. This solution is also filtered. The filtrates obtained are evaporated and dried in an oven at 45°C for 48 hours. This yields 12.3 g of chlorophyll-green powder, which corresponds to the hydroethanolic extract of *Justicia flava* (HEJF).

Evaluation of chronic anti-inflammatory activity

Induction of arthritis by 2.5% formaldehyde

The non-immunological arthritis model described by Singh *et al*. (2015) was used with a slight modification. The experiments lasted 14 days. The curative model was used. Six groups of six rats received various treatments. Arthritis was induced by injecting 0.1 mL of freshly prepared 2.5% formaldehyde into the right hind paw of rats from all groups except group 1 (negative control) on day 1 and day 3. First, the initial thickness of the rats' right hind legs was measured using a caliper before any treatment. Next, rats in group 1 (negative control) and group 2 (arthritic control) were gavaged with 9‰ NaCl at a dose of 10 mL/kg bw. As for the rats in groups 3, 4, and 5, they were fed hydroethanolic extract of *Justicia flava* at doses of 125, 250, and 500 mg/kg bw, respectively. Finally, the rats in group 6 were force-fed aspirin at a dose of 100 mg/kg bw. These various procedures were performed daily for 10 days on each rat. In order to assess the progression of arthritis, the thickness of the rats' paws is measured. The percentage of INH inhibition (%) of edema was calculated for each group of treated rats relative to the arthritic control group. It is obtained using the formula developed by Alamgeer *et al*. (2017):

$$\mathbf{INH}\ (\%) = \frac{\mathbf{Vc - Vt}}{\mathbf{Vc}}\ \mathbf{X\ 100}$$

Vc = thickness of edema in the right hind paw of rats in the arthritic control group
Vt = thickness of edema in the right hind paw of rats in the treated groups.

Measuring the body weight of rats

In order to assess the body weight of the rats throughout the study period, the rats were weighed using a scale (Digital scal- SF-400, China) on days 0, 3, and 14 of the experiment.

Measuring the amount of food and water consumed by rats

During this study, the amount of food and the volume of water were measured. First, each group of rats was given 200 g of food and 300 mL of water per day. The following day, the amount of food and water remaining was measured. Next, a calculation was made between the amount of food and water given and the amount of food and water remaining. Finally, this calculation made it possible to obtain the average amount of food and water consumed by the rats in each lot.

Blood sampling and hematological parameter measurements

During this study, three blood samples were taken on days 0, 3, and 14 of the experiment. The rats were anesthetized with ether, and then a Pasteur pipette was inserted into the animal's retro-orbital sinus and rotated. Blood was drawn into the pipette. The blood was then placed into EDTA tubes. These blood samples taken from these tubes were used to perform a complete blood count (CBC) using an automatic hematology analyzer, the Coulter (Mindray BC-2800), which is an impedance variance device.

Impedance measurement principle

The impedance measurement technique, also known as the Coulter principle after its inventor Wallace Coulter, is used to count particles and cells and measure their size (Coulter, 1953). The cells pass through an opening at which an electric current is applied. Each time a cell passes through the opening, there is an increase in electrical resistance, which is translated into electrical impulses whose height is directly proportional to the cell volume.

Determination of Complete Blood Count

A 13 µL sample of whole blood collected on EDTA is diluted in an iso-osmotic buffer solution and then sucked through an orifice separating two

chambers, one containing a positive electrode and the other a negative electrode. Each particle passing through the orifice momentarily produces an increase in electrical resistance, which is recorded as a pulse. The analyzer (Mindray BC 2800) counts platelets and red blood cells on the same dilution channel and considers small particles between 2 and 36 fl as platelets and those larger than 36 fl as red blood cells. When two cells pass through the aperture simultaneously (coincidence passage), they generate only a single high-intensity pulse, which is automatically corrected by the analyzer (coincidence correction).The pulses generated are sorted so that only those corresponding to a standard passage are retained, then recorded individually and classified into different channels, enabling erythrocyte and platelet histograms to be constructed. A stream of M-18R RINSE flushes the rear of the orifice, preventing cells from returning to the detection area and disrupting the analysis. Erythrocyte indices are determined from the histogram, whose height is directly proportional to the mean corpuscular volume (MCV).Since the number of red blood cells is determined by the total number of pulses recorded, the hematocrit (Ht) level is then calculated using the formula: Ht = RBCxMCV/10. The red blood cells are then lysed by M-18L LYSE and the released hemoglobin is converted into methemoglobin, the concentration of which is measured using spectrophotometry (525 to 550 nm).After lysing the red blood cells and platelets, the suspension is diluted with M-18D DILUENT to 1/250 and the leukocytes are counted from 35fl. Leukocyte cytolysis is then performed. The cytoplasm is released and the membrane retracts around the nucleus and granules. The size of the nucleus, its lobular state, and granulations allow leukocyte subpopulations to be determined by spectrophotometry. All these reactions are performed simultaneously by the Mindray BC 2800 automated system.

Shooting

To associate the percentage of edema thickness inhibition with the photographs, pictures were taken on days 0, 3, and 14 of the experiment. The instrument used is a mobile phone (Samsung Galaxy Core Prime, Korea) equipped with a 10-megapixel camera.

Statistical analyses

The results obtained are given as a mean followed by the standard error of the mean (M±SEM).Statistical analysis was performed using Graph Pad Prism 5.01 software (San Diego, California, USA). ANOVA1 followed by Tukey's multiple comparison test was used to identify differences between the treated and control groups. The differences are significant for $p < 0.05$.

Results

Effect of HEJF on the body weight of rats

Table 1 shows the change in body weight of rats during the induction of arthritis by 2.5% formaldehyde. The rats' weights are consistent at the start of the experiment. Except for the negative control rats (9‰ NaCl), all rats that received injections of 2.5% formaldehyde on days 1 and 3 showed a non-significant ($p>0.05$) reduction in body weight. These percentages of body weight reduction are estimated at 4.62, 4.94, 4.54, and 1.35%, respectively, for the arthritic control group and the groups treated with HEJF at doses of 125, 250, and 500 mg/kg bw. The body weight of rats treated with HEJF increased significantly at a dose of 500 mg/kg bw, from 123.8 ± 1.19 to 132.3 ± 1.52 g, with a percentage increase of 9. 61% compared to the body weight of rats in the arthritic control group, which was 120.7 ± 1.99 g. This increase is dose-dependent. The same applies to rats that received aspirin (100 mg/kg), the reference product in this study. The body weight of these rats increased significantly from 121.8 ± 0.7 to 130.5 ± 0.56 g compared to the control rats with arthritis. This corresponds to a percentage of 8.11%. The effect of weight normalization by HEJF is slightly greater at a dose of 500 mg/kg bw compared to aspirin.

Effect of HEJF on the amount of food ingested by rats

The amount of food remaining per day during this study in all groups of rats did not exceed 3 g out of 200 g. The average food consumption per day in negative control rats and arthritic control rats was 198.2 ± 0.47 g and 197.2 ± 0.47 g, respectively. For rats treated with HEJF at doses of 125, 250, and 500 mg/kg bw, the average daily food intake was 198.5 ± 0.42, 198.7 ± 0.49, and 198.8 ± 0.30 g, respectively. As for the rats in the aspirin-treated group, the average amount of food consumed per day throughout the experiment was estimated at 198.7 ± 0.42 g. There were no significant differences in the amount of food consumed by rats treated with HEJF and aspirin and control rats (negative control and arthritic control) (Table 2).

Effect of HEJF on the volume of water ingested by rats

The average volume of water consumed per day by rats in the negative control and arthritic groups during this experiment was 165 ± 3.51 and 164.2 ± 3.27 mL, respectively. For rats treated with hydroethanolic extract of *Justicia flava* at doses of 125, 250, and 500 mg/kg bw, the average volume of water consumed per day was 160.00 ± 5.27, 169.2 ± 4.16, and 162.5 ± 4.16 mL, respectively. As for the rats in the aspirin-treated group, the average volume of water consumed per day was estimated at 164.2 ± 2.00 mL. As with the food intake parameter, there were no significant differences between the average volume of water ingested by treated rats (HEJF and aspirin) and untreated rats (negative and arthritic controls) (Table 2).

Table 1: Effect of HEJF on the body weight of rats

Groups	Average body weight of rats (g) / percentage reduction (day 3) or gain (day 14) in body weight in parentheses (%))		
	Day 0	Day 3	Day 14
Negative control	120.8 ± 1.16	125.5 ± 0.95 ^{ns}	138 ± 1.96 ^{ns}
Arthritic control	119.3 ± 2.02	119.7 ± 2.15 ^{ns} (4.62)	120.7 ± 1.99 ^{ns}
HEJF (125 mg/kg bw)	118 ± 2.03	119.3 ± 2.30 ^{ns} (4.94)	121.7 ± 1.90^{ns} (0.82)
HEJF (250 mg/kg bw)	118 ± 2.25	119.8 ± 1.95 ^{ns} (4.54)	125.7 ± 3.02^{ns} (4.14)
HEJF (500 mg/kg bw)	121.3 ± 1.04	123.8 ± 1.19 ^{ns} (1.35)	132.3 ± 1.52** (9.61)
Aspirin (100 mg/kg bw)	119.5 ± 0.92	121.8 ± 0.70 ^{ns} (2.94)	130.5 ± 0.56* (8.11)

*ns = not significant: comparison between negative control group and other groups on day 3 after arthritis induction ($p > 0.05$). **$p < 0.01$; *$p < 0.1$: comparison between arthritic control group and treated groups on day 14 after arthritis induction. n = 6.HEJF : Hydroethanolic Extract of Justicia flava*

Table 2: Effect of HEJF on the amount of food and water ingested by rats during chronic inflammation

Groups	Average amount of food (g/day)	Average water volume (mL/day)
Negative control	198.2 ± 0.47	165 ± 3.51
Arthritic control	197.2 ± 0.47	164.2 ± 3.27
HEJF (125 mg/kg bw)	198.5 ± 0.42	160.00 ± 5.47
HEJF (250 mg/kg bw)	198.7 ± 0.49	169.2 ± 4.16
HEJF (500 mg/kg bw)	198.8 ± 0.30	162.50 ± 4.61
Aspirin (100 mg/kg bw)	198.7 ± 0.42	164.2 ± 2.00

No significant difference in the amount of food and water ingested between control rats and treated rats ($p > 0.05$)

HEJF : Hydroethanolic Extract of Justicia flava

Effect of HEJF on edema induced by 2.5% formaldehyde in rats

Table 3 and Figure 1 show the effect of the hydroethanolic extract of *Justicia flava* on edema caused by arthritis induced by 2.5% formaldehyde in rats. The initial average thickness of the right hind paw measured on day 0 in all groups of rats ranged from 2.48 ± 0.03 to 2.56 ± 0.02 mm with no signs of arthritis or inflammation (edema).After the third day of arthritis induction, the average thickness of the right hind paw of all rats, except for the negative control rats, increased significantly ($p < 0.01$) compared to the negative control rats, whose average right hind paw thickness was 2.51 ± 0.04 mm. The values of these increases in the average thickness of the legs of rats from different batches range from 5.62 ± 0.18 to 5.87 ± 0.18 mm. Signs of arthritis and inflammation (edema) appeared in these rats. There was an increase in edema, which reached its maximum level and then persisted. The presence of redness, swelling, and deformation of the paw are the various symptoms of arthritis observed in these rats during this study. In rats treated with HEJF at doses of 125, 250, and 500 mg/kg bw, there was an extremely significant decrease in the average thickness of the right hind paw compared to rats in the arthritic control group. This decrease varies throughout the treatment from 3.72 ± 0.29 to 2.53 ± 0.04 mm, which corresponds to reductions in the thickness of the right hind leg ranging from 34.5% to 51.71%. This reduction in edema observed is dose-dependent. As for rats treated with aspirin, there was also an extremely significant decrease in the average thickness of the right hind paw of the rats. This decrease ranges from 3.59 ± 0.11 to 2.50 ± 0.04 mm, which corresponds to a 52.29% reduction compared to the arthritic controls on the 10th day of treatment. Photographs of the right hind paw of rats treated with HEJF at different doses and aspirin showed a reduction in redness and swelling associated with signs of arthritis and inflammation. This image clearly shows a reduction in the thickness of the paws of rats treated with HEJF compared to rats in the arthritic control group. It should be noted that the reducing effect of aspirin is slightly greater than that of HEJF at these different doses.

Table 3: Effect of HEJF on arthritis induced by 2.5% formaldehyde in rats

Groups	Average thickness (mm) of the paw / Percentage reduction in edema in parentheses (%)					
	Day 0	Day 3 after induction	Day 1 after treatment	Day 3 after treatment	Day 7 after treatment	Day 10 after treatment
Negative control	2.51 ± 0.04	2.51 ± 0.04	2.51 ± 0.04	2.52 ± 0.06	2.52 ± 0.06	2.54 ± 0.03
Arthritic control	2.52 ± 0.06	5.84 ± 0.23###	6.31 ± 0.37	5.68 ± 0.15	5.50 ± 0.11	5.24 ± 0.15
HEJF (125 mg/kg bw)	2.56 ± 0.02	5.80 ± 0.21###	3.89 ± 0.16*** (38.35)	3.72 ± 0.29*** (34.50)	2.82 ± 0.22** (48.72)	2.66 ± 0.04*** (49.23)
HEJF (250 mg/kg bw)	2.48 ± 0.03	5.70 ± 0.16###	3.83 ± 0.09*** (39.30)	3.51 ± 0.04*** (38.20)	2.75 ± 0.07*** (50.00)	2.57 ± 0.05*** (50.95)
HEJF (500 mg/kg bw)	2.51 ± 0.04	5.87 ± 0.18###	3.79 ± 0.10*** (39.93)	3.34 ± 0.08*** (41.19)	2.66 ± 0.04*** (51.63)	2.53 ± 0.04*** (51.71)
Aspirin (100 mg/kg bw)	2.48 ± 0.04	5.62 ± 0.18###	3.59 ± 0.11*** (43.10)	3.35 ± 0.07*** (41.02)	2.65 ± 0.05*** (51.81)	2.50 ± 0.04*** (52.29)

*###p < 0.001: comparison between negative control and other groups on day 3 after induction of arthritis. *p < 0.1; **p < 0.01; ***p < 0.001: comparison between arthritic group and respective treated groups for each day after treatment, ns = not significant; n = 6.HEJF : Hydroethanolic Extract of Justicia flava*

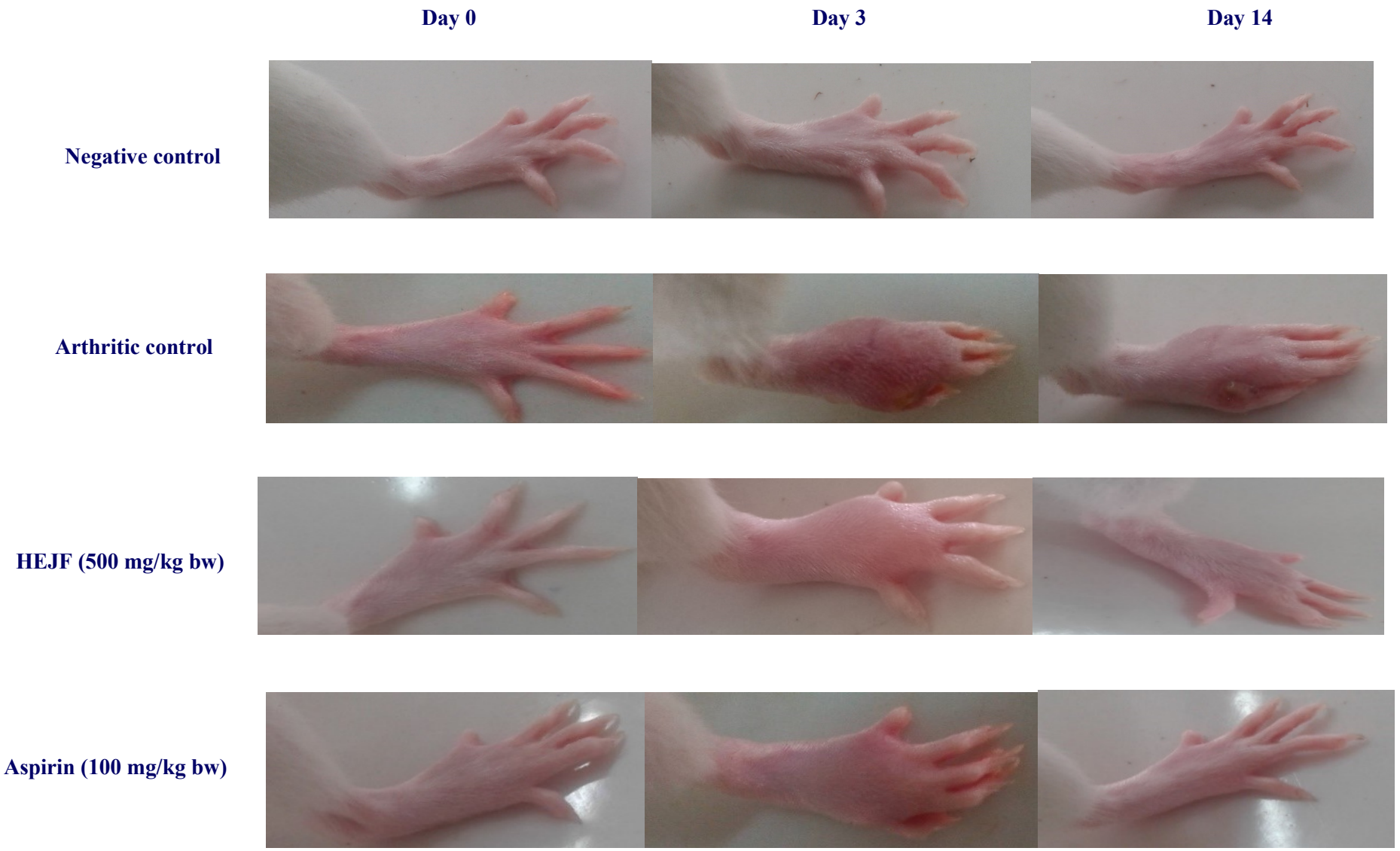

Figure 1: Photograph of the right paw of rats before and after induction of arthritis with 2.5% formaldehyde in rats

Effect of HEJF on white blood cell count in rats

The change in white blood cell count during the induction of arthritis by 2.5% formaldehyde in rats is shown in Table 4. The number of white blood cells measured in all rats on day 0 is consistent. It ranges from 9.93 ± 0.32 to 10.92 ± 0.21 × 10^3/µL. After the third day of arthritis induction, a significant increase in the average white blood cell count was observed in all rats except for the negative controls. This increase ranges from 12.72 ± 0.63 to 14.27 ± 0.53 × 10^3/µL compared to that of the negative control, which is 10.08 ± 0.21 × 10^3/µL. These values correspond to increases ranging from 26.19 to 41.56% compared to negative controls. On the 14thday of the experiment, after using HEJF at doses of 125, 250, and 500 mg/kg bw, a significant decrease in white blood cell count was observed, ranging from 11.78 ± 0.26 to 10.63 ± 0.64. 10^3/µL was recorded compared to the white blood cell count of rats in the arthritic control group, which was 14.63 ± 1.08.10^3/µL, corresponding to reductions ranging from 19.48 to 27.34% compared to the arthritic control. The white blood cell count of rats treated with aspirin also decreased by 11.37 ± 0.29 × 10^3/µL compared to the white blood cell count of arthritic controls, which was 14.63 ± 1.08 × 10^3/µL. This decrease corresponds to a percentage of 22.28%.

Table 4: Effect of HEJF on white blood cell count in rats

Groups	Average white blood cell count (10^3/µL) / percentage increase or decrease in parentheses (%)		
	Day 0	Day 3	Day 14
Negative control	10.02 ± 0.34	10.08 ± 0.21	9.93 ± 0.24
Arthritic control	9.95 ± 0.35	14.27 ± 0.53$^{###}$ (41.56)	14.63 ± 1.08
HEJF (125 mg/kg bw)	9.93 ± 0.32	12.72 ± 0.63$^{\#}$ (26.19)	10.63 ± 0.54*** (27.34)
HEJF(250 mg/kg bw)	10.23 ± 0.23	13.20 ± 0.59$^{\#\#}$ (30.95)	11.30 ± 0.47** (22.76)
HEJF (500 mg/kg bw)	10.92 ± 0.21	14.07 ± 0.4$^{###}$ (39.58)	11.78 ± 0.26* (19.48)
Aspirin (100 mg/kg bw)	10.15 ± 0.27	13.95 ± 0.68$^{###}$ (38.39)	11.37 ± 0.29** (22.28)

*#p < 0.1; ##p < 0.01; ###p < 0.001: comparison of white blood cell counts between the negative control group and the other groups on day 3. *p < 0.1 **p < 0.01; ***p < 0.001: comparison between the arthritic control and the treated groups on day 14; n = 6. HEJF : Hydroethanolic Extract of Justicia flava*

Effect of HEJF on the number of red blood cells and blood platelets

The number of red blood cells and platelets in all rats on day 0 remained constant at 6.85 ± 0.31 to 7.23 ± 0.25 × 10^6/µL and 1015 ± 21.1 to 1042 ± 34.83 × 10^3/µL, respectively (Table 5). On the third day of arthritis induction, the number of red blood cells decreased insignificantly from 5.73 ± 0.4 to 5.97 ± 0.6 × 10^6/µL compared to the negative controls, which was 6.95 ± 0.37 × 10^6/µL, corresponding to a percentage reduction ranging from 14.10 to 17.55%.After treatment (day 14) with HEJF at different doses, there was a non-significant increase in the number of red blood cells from 6.53 ± 0.25 to 6.86 ± 0.26. 10^6/µL was recorded compared to rats in the arthritic control group, whose red blood cell count was 5.92 ± 0.25.10^6/µL. These values correspond to increases ranging from 10.30 to 15.87%. Aspirin also caused a non-significant increase in the number of red blood cells of 6.94 ±0.11

(10^6/µL) compared to the arthritic controls, which was 5.92 ± 0.25 × 10^6/µL, representing a 17.22% increase.

With regard to platelets, a decrease was observed in all rats on the third day of arthritis induction, except for rats in the negative control group. The platelet count decreased from 742 ± 103.4 to 580 ± 176.4 × 10^6/µL compared to the platelet count in the negative control group, which was 1001 ± 25.7 × 10^6/µL. This decrease ranges from 25.87% to 34.66%.After treatment with HEJF at doses of 125, 250, and 500 mg/kg bw, a non-significant increase in platelet count ranging from 901.5 ± 62.39 to 972.3 ± 182.2 × 10^6/µL was observed compared to arthritic controls, whose count was 651.5 ± 95.09 × 10^6/µL. These variations in platelet count correspond to increases of 38.37 to 49.24%.Rats treated with aspirin after 10 days of treatment had a non-significant increase in platelet count of 909.2 ± 52.6 × 10^6/µL compared to arthritic rats, whose platelet count was 651.5 ± 95.09 × 10^6/µL. Aspirin increases platelet count by 39.55% after 10 days of treatment (Table 5).

Table 5: Effect of HEJF on the number of red blood cells and blood platelets

Groups	Red blood cell and platelet counts / percentage reduction or increase in parentheses (%)					
	Day 0		Day 3		Day 14	
	RBC (10^6/µL)	Platelets (10^3/µL)	RBC (10^6/µL)	Platelets (10^3/µL)	RBC (10^6/µL)	Platelets (10^3/µL)
Negative control	6.85 ± 0.31	1015 ± 21.1	6.95 ± 0.37	1001 ± 25.7	6.51 ± 0.19	1029 ± 23.2
Arthritic control	7.08 ± 0.45	1020 ± 26.5	5.73 ± 0.4 (17.55)	742 ± 103.4 (25.87)	5.92 ± 0.25	651.5 ± 95.09
HEJF (125 mg/kg bw)	6.98 ± 0.21	1038 ± 28.2	5.83 ± 0.72 (16.11)	695.3 ± 266.4 (30.53)	6.53 ± 0.25 (10.30)	972.3 ± 182.2 (49.24)
HEJF (250 mg/kg bw)	7.14 ± 0.25	1040 ± 39.1	5.97 ± 0.60 (14.10)	690 ± 198.3 (31,06)	6.61 ± 0.21 (11.65)	901.5 ± 62.39 (38.37)
HEJF (500 mg/kg bw)	7.23 ± 0.25	1042 ± 34.83	5.93 ± 0.69 (14.67)	654.0 ± 157.0 (34.66)	6.86 ± 0.26 (15.87)	952.0 ± 111.0 (46.12)
Aspirin (100mg/kg bw)	6.92 ± 0.27	1027 ± 23.0	5.88 ± 0.32 (15.39)	580 ± 176.4 (42.05)	6.94 ± 0.11 (17.22)	909.2 ± 52.6 (39.55)

No significant difference between the number of red blood cells and platelets in the control and treatment groups ($p > 0.05$). RBC: Number of red blood cells (106/µL)
HEJF : Hydroethanolic Extract of Justicia flava

Discussion

The results obtained at the end of these tests show that during the induction of arthritis by 2.5% formaldehyde, the effect of HEJF at doses of 125, 250, and 500 mg/kg bw on the body weight of rats was determined. Arthritic rats obtained by injecting 2.5% formaldehyde after the third day of the experiment had a non-significant reduction in body weight compared to the negative control. However, the weight of rats treated with HEJF at a dose of 500 mg/kg bw and aspirin increased significantly by 9.61% and 8.11%, respectively, compared to the arthritic controls during the treatment period. In fact, the weight loss observed in the arthritic control group may be due to a change in their metabolic activity because, according to Walz *et al.* (1971), changes in the weight of rats also occur during the experimental arthritic period. Furthermore, in this study, all rats ingested almost all of the food and water provided to them. The weight gain observed in rats treated with HEJF could be due to a beneficial effect on the digestion of the food consumed. These results are similar to those of Shruthi *et al.* (2012), who showed that body weight loss in all experimental

groups during 2.5% formaldehyde-induced arthritis was restored by the various treatments with *Kirganelia reticulata* at the end of treatment. Furthermore, Sarkar *et al*. (2025) showed that the methanolic extract of the whole plant *Equisetum diffusum* restored body weight in rats made arthritic.

The anti-arthritic potential of hydroethanolic extract of *Justicia flava* at doses of 125, 250, and 500 mg/kg bw in rats was evaluated using the 2.5% formaldehyde-induced arthritis model. The edema caused by arthritis was significantly reduced by this extract, with a reduction percentage of 51.71% at a dose of 500 mg/kg bw. The hydroethanolic extract of *Justicia flava* has anti-arthritic properties similar to aspirin in the treatment of this condition. However, its effects are slightly less significant than those of aspirin. In this model, inflammation was induced on the first and third days, leading to chronic inflammation. This chronic inflammation could lead to the presence of cells that destroy the functional integrity of the animal's right hind leg (Carl, 1963).Furthermore, chronic inflammation involves the release of numerous destructive mediators such as cytokines, interferons, histamine, prostaglandins, leukotrienes (Ammon *etal*., 1993), platelet-activating factor, nitric oxide, and tumor necrosis factor (Clarke *et al*., 1996).According to Eric and Lawrence (1996), these mediators are responsible for the presence of edema, persistent pain, destruction of cartilage and bone, and severe disability in animals.

The inhibition of edema observed during this experiment suggests that HEJF has anti-arthritic properties. These results are similar to those obtained by Shruthi *et al*. (2012) with polyphenolic extract from *Kirganelia reticulata* (100 µg/mL and 250 µg/mL) and Udegbunam *et al*. (2014) with the methanolic extract of *Sterculia tragacantha* (100 and 300 mg/kg).These two extracts significantly reduced the edema induced by 2.5% formaldehyde in rats by 58.2% and 51%, respectively, on the 10^{th} day of treatment. In addition, treatment with the methanolic extract of processed *Plumbago zeylanica* roots significantly attenuated rheumatoid arthritis induced by Freud's complete adjuvant, as evidenced by a reduction in paw volume and joint diameter (Das *et al*., 2025).

The number of white blood cells during the induction of arthritis by 2.5% formaldehyde in rats increased significantly in all rats made arthritic compared to rats in the negative control group after the third day of induction. However, after 10 days of treatment with HEJF, a significant reduction in white blood cell count was recorded in the groups of rats treated with HEJF and aspirin compared to the arthritic controls. Indeed, the injection of 2.5% formaldehyde caused inflammation, triggering the active release of leukocytes. This active release of white blood cells is thought to maintain the defensive activity of the host's immune system system (Mahgoub *et al*., 2008). This activity can be attributed to a systemic immune response in rats following inflammation induced by 2.5% formaldehyde in the arthritis model. These results are similar to those of Udegbunam *et al.* (2014), who showed that the number of white blood cells, which had previously increased with the use of 2.5% formaldehyde, was significantly reduced from 7.80 ± 0.10 to $6.80 \pm 0.20 \times 10^3/\mu L$ in the presence of *Sterculia tragacantha* methanolic extract at a dose of 300 mg/kg. Similarly, the work of Zafar *et al*. (2025) showed a decrease in white blood cells in the treated groups compared to the control group.

The number of erythrocytes during the induction of arthritis by 2.5% formaldehyde decreased after the third day of induction in all rats in the experiment except for the rats in the negative control group. However, an increase in the number of erythrocytes was recorded in rats treated with HEJF and aspirin compared to rats in the arthritic control group at the end of treatment. Indeed, 2.5% formaldehyde injected into the right hind paw of rats on days 1 and 3 caused systemic inflammation following a complex enzymatic reaction process (Das *et al*., 2010). However, according to Habibur *et al*. (2015), the survival of a cell depends on the integrity of its membrane. Exposure of red blood cell membranes to a harmful substance such as formaldehyde could

cause reactions that contribute to red blood cell lysis. In the presence of the extract and aspirin during the 10 days of treatment, the increase in red blood cell count is thought to be due to the significant protection of the cell membrane against the effects of 2.5% formaldehyde (Liu *et al*.1992; Perenz *et al*.; 1995; Shinde *et al*. 1999).These results are similar to those obtained by Shruthi *et al*. (2012) and Habibur *et al*. (2015), who showed in their work that the number of red blood cells increased with treatment with polyphenolic extracts of *Kirganelia reticulata* (100 µg/mL and 250 µg/mL) and ethanolic extracts of *Oryza sativa* (var. Joha rice) (50-250 mg/kg bw), respectively. Furthermore, the work of Zafar *et al*. (2025) showed a significant increase in erythrocytes in group VI (AeOB750) compared to those in the control group II with arthritis.

The platelet count of all rats except the negative control rats decreased insignificantly after the third day of induction, but this count increased insignificantly after 10 days of treatment with HEJF and aspirin. In the group of control rats with arthritis, a decrease in platelet count was recorded throughout the entire experimental period. It is known that platelets participate in hemostasis and play a role in local vasoconstriction. The inflammatory reaction caused by formaldehyde could, by inducing vasodilation and edema, lead to a loss of platelets. The anti-inflammatory action of HEJF may contribute to the restoration of platelet levels. These results corroborate those of Alamgeer *et al*. (2015). These authors showed that hydromethanolic extract of *Berberis calliobotrys* in the treatment of arthritis restored platelets by significantly increasing their number compared to untreated rats. Furthermore, the work of Sarkar *et al*. (2025) showed a decrease in platelets in the groups treated with the methanolic extract of the whole plant *Equisetum diffusum* compared to the arthritic control group.

Conclusion

The anti-inflammatory effect of hydroethanolic extract of *Justicia flava* was studied in rats. The model of non-immunological arthritis induced by 2.5% formaldehyde was used. The results showed that the hydroethanolic extract of *Justicia flava* has remarkable anti-arthritic properties similar to aspirin. This extract caused a significant, dose-dependent inhibition of the average paw thickness of rats, with reductions ranging from 34.5% to 51.71% compared to the arthritic control group after 10 days of treatment. The hydroethanolic extract of *Justicia flava* led to a significant reduction in the number of white blood cells, which had previously increased. It also corrected the decreases in erythrocyte and platelet counts recorded on the third day of arthritis induction with 2.5% formaldehyde. These results could therefore justify the traditional use of this plant by traditional healers in the treatment of feverish aches and pains, pain associated with gastric and abdominal ulcers, and fever.

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