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Effect of a hydroethanol extract of *Malacantha alnifolia (*Bak.) Pierre (Sapotaceae) on formaldehyde-induced arthritis in Wistar rats

N'Dia Kouadio Frédéric[1], Kouame Djè Kouamé Wenceslas[2], Diby Yao Bernard[3], Kouakou Kouakou Léandre[1], and Yapo Angoué Paul[1]

[1]Nangui ABROGOUA University, Nature Sciences Training and Research Unit, Laboratory of Physiology, Pharmacology and Pharmacopoeia, PO Box 801 Abidjan 02, Côte d'Ivoire

[2]Université Polytechnique de Man, Unité de Formation et de Recherche-Ingénierie, Agronomique, Forestière et Environnementale (UFR-IAFE), BP 20 Man, Côte d'Ivoire

[3]Jean Lorougnon GUEDE University, Training and Research Unit of Agroforestory, Laboratory of Agrovalorisation, PO Box 150 Daloa, Côte d'Ivoire

ABSTRACT: *Malacantha alnifolia* (Sapotaceae) is a perennial plant found in the tropical rainforests of West Africa. In Côte d'Ivoire, this species usually used rheumatism and diarrhea children treatment. This study evaluated anti-arthritic effect of hydroethanol extract of *Malacantha alnifolia* (HEMa) at doses of 125, 250 and 500 mg/kg bw in rats. HEMa is prepared by cold maceration of 100 g of *M. alnifolia* trunk bark powder in one liter of a 30% water/70% ethanol mixture for 24 h. Thirty-six albino *Rattus norvegicus* rats (130-185 g), aged 8 to 16 weeks, were divided into 6 batches of 6 rats each and treated daily during 10 days. Healthy and arthritic rats control received NaCl 9 ‰ (10 mL/kg bw) by gavage, those from batches 2, 3 and 4 received HEMa at 125, 250 and 500 mg/kg bw, respectively. Positive control rats received methotrexate at 0.75 mg/kg bw by gavage. Non-immunological arthritic model induced by injection of 0.1 mL 2.5% formaldehyde was used. Arthritic was assessed throught paw and knee edema, arthritic index scores, sedimentation rate, and inflammation-related hematological and biochemical parameters. Percentages inhibition of these parameters were calculated for each treated group of rats compared to the control group with arthritis. Edema established for 3 days by formaldehyde was significantly reduced by HEMa at doses of 250 and 500 mg/kg bw with reductions in paw thickness ranging from 23.18 to 28, respectively. 89% and 25.60 to 31.34% compared to paw thickness of arthritic control rats. Hydroethanolic extract of *Malacantha alnifolia* has remarkable anti-arthritic properties similar to that of methotrexate.

KEYWORDS: *Malacantha alnifolia,* anti-arthritic, formaldehyde, rat.

1 INTRODUCTION

Rheumatoid arthritis is the most common autoimmune disease. It is a chronic, progressive, systemic inflammatory disease affecting the joints and associated with long-term disability and premature mortality [1]. Epidemiological study has shown that around 1% of the world's population has rheumatoid arthritis, which has a significant impact on their life quality [2]. In Côte d'Ivoire, a study carried out in the rheumatology department of the CHU de Cocody (Abidjan) by [3] showed that around 2% of rheumatoid arthritis patients were affected. Despite advances in conventional medicine, the cause of the disease is still unknown. HLA-DR genes are the most important genetic risk factor. In addition, this disease inducted inflammation of the synovium, hyperplasia, cartilage destruction, bone deformation and systemic complications (cardiovascular, pulmonary, psychological and skeletal disorders) [4]. In modern medicine, treatments with non-steroidal anti-inflammatory drugs (NSAIDs) and steroids, biological agents such as antagonists of pro-inflammatory cytokines (tumour necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1ß)) and analgesics are prescribed to treat this disease without any real success [5,6]. However, the prolonged using of anti-inflammatory pharmaceutical substances is accompanied by secondary undesirable effects [7] Prolonged using of NSAIDs can lead to gastrointestinal disorders, kidney and skin toxicities, infections and cardiovascular risks [8,9]. Then, research is needed into new anti-inflammatory and analgesic substances that are more effective in reducing inflammation and pain. Medicinal plants are becoming an important sources to explore in the search for effective drugs with fewer side effects [10,11]. Several medicinal plant species and recipes have been identified in Côte d'Ivoire [12,13]. Among these plants *Malacantha alnifolia* (Bak.)

Pierre, commonly used in traditional medicine to treat children diarrhoea, rheumatism, conjunctivitis of the eyes and stomach ache, and as a purgative in the treatment of poisoning and elephantiasis of the scrotum [14]. The aim of this study was to evaluate chronical and anti-inflammatory potential of a hydroethanol extract of *Malacantha alnifolia* trunk bark. Specifically, chronical and anti-inflammatory activity of formaldehyde oedema induction method will be assessed by determining haematological and biochemical parameters associated with inflammation.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Plant material consists of *Malancatha anifolia* trunk bark harvested in *Béoumi* in August 2021. The species was identified at Nangui ABROGOUA University in Abidjan and authenticated by lecturers from the university's botany laboratory.

2.2 ANIMALS

Rats, (*Rattus norvegicus*) aged 8 to 16 weeks and weighing between 130 and 185 g were used in the study. These animals were supplied and bred in the animal house of the Physiology, Pharmacology and Pharmacopoeia laboratory of the Natural Sciences Training and Research Unit (UFR-SN) of Nangui ABROGOUA University (Côte d'Ivoire). They had free access to water and food (Ivograin®) with a photoperiod of 12 h of light and 12 h of darkness in accordance with standards of good laboratory practice. Various experimental protocols were drawn up in accordance with the laboratory animal protection protocols of the European Council of Legislation 2012/707/EU [15].

2.3 LABORATORY EQUIPMENT

Equipment consisted of an electric grinder (Culati, France), electronic digital balance (Digital scal- SF-400, China), magnetic stirrer (OVAN, USA), oven (Heto type CD 52- I, France), digital micrometer or calliper (Hardened Stainless, China), a Coulter haematological analyser (Mindray BC-2800, China), semi-automatic spectrophotometer (Prietest Touch Robonik, India) and a camera (TECNO, China).

2.4 PHARMACODYNAMIC REAGENTS AND SUBSTANCES

Reagents used in this study were hydroethanol extract of *Malancatha anifolia,* methotrexate (Bellon®) (Sanofi, France) as the reference anti-inflammatory, 2.5% formalin solution, ether and 9 ‰ NaCl solution as the reference physiological solution.

2.5 METHODS

2.5.1 PREPARATION OF HYDROETHANOLIC EXTRACT OF MALACANTHA ALNIFOLIA

Hydroethanolic extract of *Malacantha alnifolia* was prepared according to the method described by [16]. *Malacantha alnifolia* trunk bark was washed with distilled water, dried at room temperature for 15 days and then finely pulverised using an electric grinder (Culati, France). Then quantity of 100 g of powder obtained from *Malacantha alnifolia* was cold macerated for 24 h in a litre of a mixture of 30% water and 70% ethanol. Resulting solution was filtered through cotton wool and then Whatman Number 3 mm filter paper. Half a litre of the hydroethanol mixture was added to the pellet and a further cold maceration was carried out for 24 h, followed by filtration. Filtrates substances obtained were evaporated and dried in an oven at 45°C for 48 h. A 6.7 g yellow paste corresponding to the hydroethanol extract of *Malacantha alnifolia* (HEMa) was obtained.

2.5.2 ASSESSMENT OF CHRONIC ANTI-INFLAMMATORY ACTIVITY (ARTHRITIS) INDUCED BY 2.5% FORMALDEHYDE 2.5%

Non-immunological arthritis model described by [17] was used with slight modification. Experiments lasted 14 days and the curative model was used. 36 rats were divided into 6 batches and 6 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 batches except batch 1 (healthy control) on days 1 and 3. Firstly, initial thickness of the rats' right hind leg or knee was measured using a calliper prior to any treatment. Then, after the 3rd day of formaldehyde injection, rats from batch 1 (healthy control) and batch 2 (arthritic control) were given NaCl 9 ‰ at a rate of 10 mL/kg bw by gavage. Rats in batches 3, 4 and 5 were force-fed with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw respectively. Finally, rats in batch 6 received methotrexate at a dose of 0.75 mg/kg bw by gavage. These different substances were administered daily for 10 days to each rat. Progression of arthritis was assessed by measuring the thickness of the rats' leg or knee again. Percentage of INH inhibition (%) of oedema was calculated for each group of treated rats compared to the arthritic control group according to the formula used in the work of [18]:

$$\%\ INH\ (\%) = \frac{Ec - Et}{Ec} \times 100$$

Ec: thickness of oedema in the right hind leg or knee of rats in the arthritic control group
Et: thickness of oedema in the right hind leg or knee of rats from treated batches.

2.5.3 CALCULATION OF ARTHRITIC INDEX SCORES

Determination of clinical symptoms in the formaldehyde-induced arthritis test was assessed by a visual scoring system on a scale from 0 to 4. A score of 0 means that no change is visible. A score of 1 indicates slight swelling of the limb and a score of 2 indicates slight swelling and skin redness of the limb. A score of 3 indicates significant swelling and reddening of the skin of the limb, and a score of 4 indicates significant deformity and incapacity of the limb. Every three days, scores were determined on each rat and then averaged for the group [1].

2.5.4 RAT BODY WEIGHT MEASUREMENT

Body weights of the rats were assessed on days D0, D4 and D14 of the experiment using a balance (Digital scal-SF 400, China).

2.5.5 BLOOD SAMPLING

During this study, three blood samples were taken on D0, D4 and D14 of the experiment. Rats were anesthetized with ether then blood samples were taken by puncture from the orbital sinus of the eye using a Pasteur pipette according to the technique described by [19]. Pasteur pipette was rotated, and once introduced into the animal's retro-orbital sinus, blood was drawn up into the pipette. Blood samples were introduced into EDTA tubes for blood count analysis using an automatic haematological analyser, coulter (Mindray BC-2800, China), which is an impedance variance device, and into dry tubes for determination of biochemical parameters using an automatic spectrophotometer (Prietest Touch Robonick, India).

2.5.6 PRINCIPLE OF IMPEDANCE MEASUREMENT

Impedance measurement technique, also known as the Coulter principle after its inventor Wallace Coulter, is designed to count particles and cells and measure their size. The cells pass through an opening to which an electric current is applied. Each time a cell passes through the aperture, the electrical resistance increases, which is translated into electrical impulses whose height is directly proportional to the cell volume.

2.5.7 DETERMINATION OF BLOOD COUNT

13 µL sample of whole blood taken on EDTA is diluted in an iso-osmotic buffer solution and aspirated through an orifice that separates 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. Coulter Hematology Analyzer (Mindray BC-2800, China) enumerates platelets and red blood cells on the same dilution channel and considers particles of small sizes between 2 and 36 fL as platelets and those larger than 36 fL like red blood cells. Erythrocyte indices are determined from the histogram whose height is directly proportional to the mean corpuscular volume (MCV).

2.5.8 DETERMINATION OF BIOCHEMICAL PARAMETERS

Blood collected in dry tubes was used to determine serum levels of various biochemical parameters using a spectrophotometer (Prietest touch Robonik, India) and various diagnostic kits. Alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the kinetic method [20,21]. Serum concentrations of total protein, calcium, albumin and phosphorus were measured by colorimetric assay [22].

2.5.9 SHOOTING

To associate the percentage inhibition of oedema thickness with the photographs, photos were taken on days 0, 4 and 14 of the experiment. The instrument used was a mobile phone (TECNO, China) fitted with a camera.

2.5.10 STATISTICAL ANALYSIS

Results were given as mean followed by standard error on the mean (M ± SEM) and percentage inhibition. Statistical analysis was carried out using Graph Pad Prism 5.01 software (San Diego, California, USA). Student's t-test and ANOVA1 (one-way analysis of variance) followed by Dunnett's comparison test were used to identify differences between the treated batches and the negative and positive control batches. Differences are significant for $p< 0.05$.

3 RESULTS

3.1 EFFECT OF HYDROETHANOL EXTRACT OF MALACANTHA ALNIFOLIA ON FORMALIN-INDUCED PAW OEDEMA IN RATS

At day 0, initial mean thickness of the legs varied non-significantly and ranged from 2.25 ± 0.14 to 2.31 ± 0.16 mm. There were no signs of arthritis or inflammation (oedema). Three days later of formalin arthritis induction, the mean leg thickness of arthritic rats increased significantly ($p< 0.001$) from 6.38 ± 0.12 to 6.96 ± 0.16 mm compared with the mean leg thickness of healthy control rats (2.34 ± 0.13 mm) (Table 1). Arthritic and inflammatory signs of arthritic rats were apparent in the presence of oedema, redness, swelling and deformation of the paw (figure 1). The mean thickness of the paw of the arthritic control rats increased and reached a maximum of 7.37 ± 0.35 mm on day 3 after treatment, with a slight and non-significant decrease to 6.68 ± 0.16 mm on day 10. In arthritic rats treated with HEMa at 125 mg/kg bw, mean leg thickness was significantly reduced, ranging from 20.62% (day 1 post-treatment) to 26.86% (day 7th post-treatment) compared with the arthritic control. Arthritic rats treated with EHMa at doses of 250 and 500 mg/kg bw, respective decreases in leg thickness ranging from 23.18 to 28.89% and from 25.60 to 31.34% compared with the mean leg thickness of arthritic control rats were recorded. The reduction of leg oedema in methotrexate-treated rats ranged from 26.74% (day 1 post-treatment) to 35.47% (day 10 post-treatment) compared with arthritic control rats (table 1). Photographs of arthritic legs treated with HEMa at 125, 250 and 500 mg/kg bw and methotrexate showed reduction in redness and swelling at the sites of arthritis and inflammation (Figure 1).

Table 1. Effect of hydroethanol extract of Malacantha alnifolia on paw thickness during formaldehyde-induced arthritis in rats

Lots (mg/kg bw)	**Average leg thickness (mm)/Percentage reduction in oedema in brackets (%)**					
	Day 0	**Day 3 after induction**	**Day 1 after treatment**	**Days 3 after treatment**	**Days 7after treatment**	**Days 10 after treatment**
Healthy control (NaCl 9‰)	2.31 ± 0.25	2.34 ± 0.13	2.30 ± 0.19	2.32 ± 0.11	2.27 ± 0.15	2.29 ± 0.15
Arthritic control (NaCl 9‰)	2,25 ± 0,14	6,65 ± 0,25$^{###}$	7,03 ± 0,31$^{###}$	7,37 ± 0,35$^{###}$	6,96 ± 0,27$^{###}$	6,68 ± 0,16$^{###}$
HEMa 125	2.31 ± 0.16	6.43 ± 0.17$^{###}$	5.58 ± 0.15*** (20.62)	5.66 ± 0.23*** (23.20)	5.09 ± 0.26*** (26.86)	5.01 ± 0.19*** (25)
HEMa 250	2.28 ± 0.27	6.52 ± 0.12$^{###}$	5.40 ± 0.22*** (23.18)	5.46 ± 0.22*** (25.91)	4.96 ± 0.12*** (28.73)	4.75 ± 0.27*** (28.89)
HEMa 500	2.29 ± 0.11	6.96 ± 0.16$^{###}$	5.23 ± 0.17*** (25,60)	5.06 ± 0.15*** (31,34)	4.83 ± 0.23*** (30.60)	4.66 ± 0, ±1.6*** (30.23)
Methotrexate 0.75	2.27 ± 0.16	6.38 ± 0.12$^{###}$	5.15 ± 0.21*** (26.74)	5.20 ± 0.22*** (29.44)	4.54 ± 0.19*** (34.77)	4.31 ± 0.26*** (35.47)

*### p< 0.001: significant difference compared to the mean leg thickness of the healthy control for each evaluation period. *** p< 0.001; n = 6: significant difference compared with the arthritic control for each evaluation period. HEMa: Hydroethanol extract of Malacantha alnifolia.*

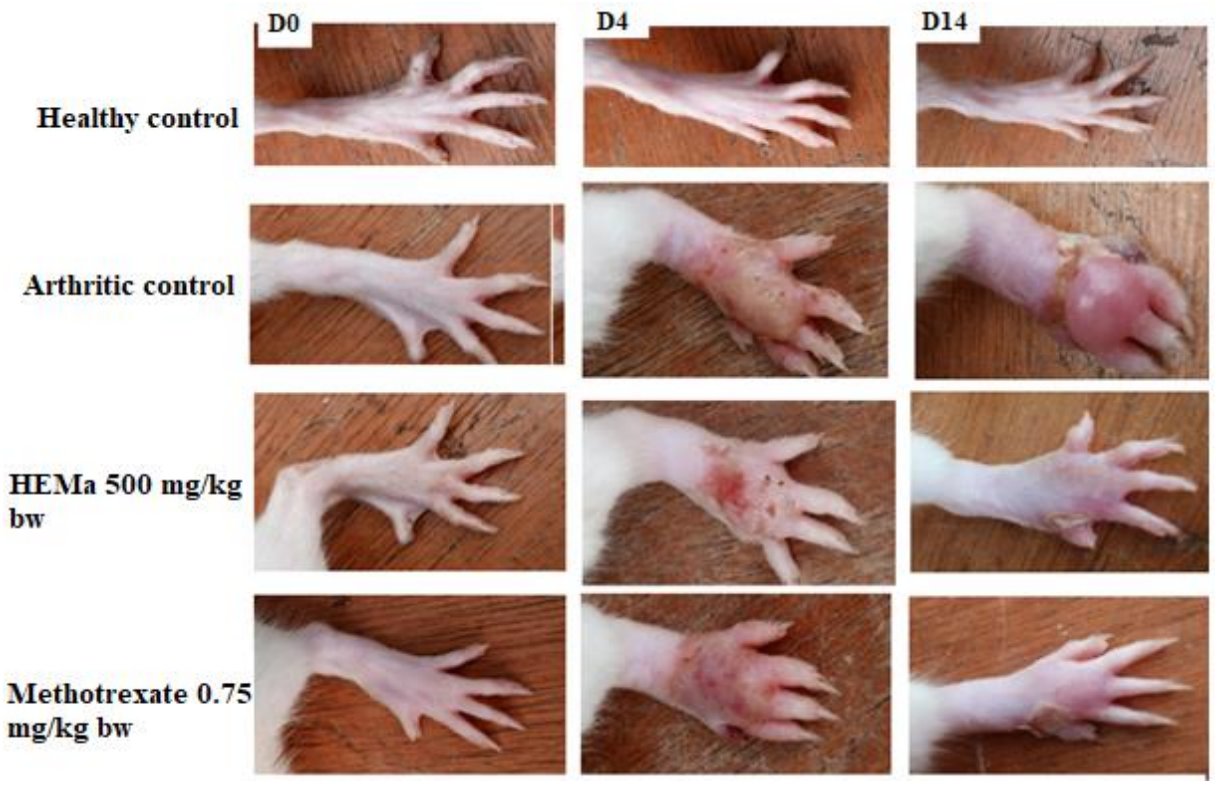

Fig. 1. Photographs of the right legs of rats before and after formaldehyde injection D0: day 0, D4: day 4 and D14: day 14

3.2 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Formalin-Induced Knee Oedema In Rats

The mean initial knee thickness of the rats ranged from 4.04 ± 0.22 to 4.19 ± 0.16 mm. Three days later of arthritis induction by formaldehyde, the knee of all rats (except healthy control rats) underwent a significant increase ($p< 0.001$) ranging from 6.64 ± 0.47 to 6.93 ± 0.23 mm compared with that of healthy controls (4.17 ± 0.19 mm). The mean knee thickness of rats treated with HEMa at 125 mg/kg bw was significantly reduced ($p<0.01$) from 11.77% to 16.74% compared with the mean knee thickness of arthritic control rats. Knees of arthritic rats treated with HEMa at 250 and 500 mg/kg bw had reductions in oedema ranging from 15.10% to 22.96% and from 18.03% to 31.11% respectively compared with the knees of arthritic control rats. Knees of rats treated with methotrexate underwent a significant ($p< 0.001$) slightly greater reduction than HEMa at these different doses. This reduction in knee thickness in rats pre-treated with methotrexate at 0.75 mg/kg bw corresponded to a maximum reduction of 35.34% (at day 7th post-treatment) compared with the arthritic control rat (Table 2).

3.3 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Scores During Formalin-Induced Arthritis In Rats

At day 0, absence of arthritic signs and inflammation in all rats corresponds to a score of 0. Three days later of arthritis induction by formaldehyde, arthritic and inflammatory signs appeared in all rats (except healthy control rats). These signs corresponded to mean scores ranging from 3.64 ± 0.38 to 3.98 ± 0.22. After treatment with HEMA at doses of 125 and 250 mg/kg bw, a reduction in arthritic and inflammatory signs corresponding respectively to a reduction in the arthritic index score ranging from 3.41 to 16.53% and from 5.14 to 38.13% compared with the score of arthritic control rats was recorded. The scores of rats treated with HEMa at 500 mg/kg and methotrexate at 0.75 mg/kg bw underwent significant ($p< 0.001$) and maximum decreases of 41.06% and 43.73% respectively compared with the scores of arthritic control rats (Table 3).

Table 2. Effect of hydroethanol extract of Malacantha alnifolia on knee thickness during formaldehyde-induced arthritis in rats

Lots (mg/kg bw)	Average knee thickness (mm) / Percentage reduction in oedema in brackets (%)					
	Day 0	Day 3 after induction	Days 1 after treatment	Days 3 after treatment	Days 7 after treatment	Days 10 after treatment
Healthy control (NaCl 9‰)	4.06 ± 0.15	4.17 ± 0.19	4.11 ± 0.31	4.16 ± 0.27	4.09 ± 0.18	4.18 ± 0.14
Arthritic control (NaCl 9‰)	4.10 ± 0.24	6.68 ± 0.19$^{###}$	6.82 ± 0.22$^{###}$	6.71 ± 0.47$^{###}$	6.62 ± 0.28$^{###}$	6.45 ± 0.14$^{###}$
HEMa 125	4.05 ± 0.23	6.67 ± 0.41$^{###}$	5.94 ± 0.18 (12.90)	5.92 ± 0.47* (11.77)	5.627 ± 0.18** (16.01)	5.37 ± 0.15*** (16.74)
HEMa 250	4.19 ± 0.16	6.93 ± 0.23$^{###}$	5.79 ± 0.25 (15.10)	5.61 ± 0.34*** (16.39)	5.10 ± 0.30*** (22.96)	5.14 ± 0.2*** (20.31)
HEMa 500	4.04 ± 0.22	6.65 ± 0.16$^{###}$	5.52 ± 0.22* (20.96)	5.50 ± 0.22*** (18,03)	4.56 ± 0.18*** (31.11)	4.61 ± 0.13*** (28,52)
Methotrexate 0.75	4.09 ± 0.13	6.64 ± 0.47$^{###}$	5.48 ± 0.48* (21.61)	5.05 ± 0.24*** (24.73)	4.28 ± 0.38*** (35.34)	4.46 ± 0.16*** (30.85)

*###p< 0.001: significant difference compared to the mean knee thickness of healthy control rats for each evaluation period. *p< 0.05; **p< 0.01; ***p< 0.001; n = 6: significant difference compared with the respective arthritic control for each evaluation period. HEMa: Hydroethanol extract of Malacantha alnifolia.*

Table 3. Effect of hydroethanol extract of Malacantha alnifolia on arthritis index scores

Lots (mg/kg bw)	Score/Percentage reduction in score in brackets (%)				
	Day 3 after induction	Days 1 after treatment	Days 3 after treatment	Days 7 after treatment	Days 10 after treatment
Healthy control (NaCl 9‰)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Arthritic control (NaCl 9‰)	3.91 ± 0.28	3.89 ± 0.37$^{###}$	3.81 ± 0.39$^{###}$	3.77 ± 0.36$^{###}$	3.75 ± 0.63$^{###}$
HEMa 125	3.95 ± 0.33	3.74 ± 0.27 (3.89)	3.68 ± 0.42 (3.41)	3.52 ± 0.62 (6.63)	3.13 ± 0.44** (16.53)
HEMa 250	3.97 ± 0.21	3.69 ± 0.41 (5.14)	3.59 ± 0.28 (5.77)	2.41 ± 0.36** (36.07)	2.32 ± 0.38*** (38.13)
HEMa 500	3.95 ± 0.32	3.65 ± 0.46 (6.16)	3.52 ± 0.47* (7.61)	2.39 ± 0.27*** (36.60)	2.21 ± 0.57*** (41.06)
Methotrexate 0.75	3.98 ± 0.22	3.64 ± 0.38 (6.42)	3.51 ± 0.51* (7.87)	2.29 ± 0.46*** (39.25)	2.11 ± 0.62*** (43.73)

*### p< 0.001: significant difference compared to the scores of the healthy control rats for each evaluation period. *p< 0.05, **p< 0.01; ***p< 0.001; n = 6: significant difference compared with the respective arthritic control for each evaluation period. HEMa: Hydroethanol extract of Malacantha alnifolia*

3.4 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Body Weight

On day 0, the average body weight of the rats varied non-significantly and ranged from 138.3 ± 2.65 to 146.5 ± 2.59. On day 4 later of arthritis induction, the mean body weight of the rats decreased significantly (p< 0.001) from 129.3 ± 3.30 to 134.5 ± 4.48 g compared with the mean weight of healthy control rats (141.9 ± 2.97 g). 14th day later of treatment, the mean body weights of rats treated with HEMa at doses of 250 and 500 mg/kg bw increased significantly by 3.5% and 5.89% respectively compared with the body weights of arthritic control rats. The mean body weight of methotrexate-treated rats increased by 6.30% compared with the body weight of arthritic control rats (Table 4).

3.5 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On White Blood Cells

At day 0, the mean white blood cell count varied non-significantly and ranged from 11.92 ± 2.54 to 12.98 ± 0.87.10^3/µL Three days later of arthritis induction, the mean white blood cell count increased significantly (p< 0.001) in all rats, from 17.4 ± 3.44 to 20.1 ±

3.07.10^3/µL compared with healthy control rats (12.03 ± 2.61). 14th day later, white blood cell counts of rats treated with HEMa at doses of 250 and 500 mg/kg bw were significantly ($p<0.05$) reduced by 19.01 % and 21.01 % respectively compared to the white blood cell counts of arthritic control rats (Table 5).

3.6 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Red Blood Cells

On day 0, the mean red blood cell count varied non-significantly from 6.79 ± 2.25 to 7.78 ± 3.14.10^6/µL. On day 4 later of arthritis induction, the mean red blood cell count showed no significant decrease ($p> 0.05$) with a maximum value of 13.02 % compared to healthy control rats. 14th day later of treatment, HEMa at the doses tested had no impact on the mean number of red blood cells compared with the number of red blood cells in the arthritic control rats. In rats treated with methotrexate, a non-significant variation in red blood cell count was also recorded compared with the red blood cell count of arthritic control rats (Table 6).

Table 4. Effect of hydroethanol extract of Malacantha alnifolia on body weight

Lots (mg/kg bw)	Average body weight of rats (g)/(percentage reduction or gain) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	141.2 ± 3.14	141.9 ± 2.97	144.62 ± 1.93
Arthritic control (NaCl 9‰)	144.0 ± 2.67	131.5 ± 3.57### (7.32)	127.62 ± 4.21###
HEMa 125	138.3 ± 2.65	129.3 ± 3.30### (8.87)	129.73 ± 3.51 (1.62)
HEMa 250	139.3 ± 4.21	130.0 ± 3.69### (8,38)	132.26 ± 2.71* (3.5)
HEMa 500	146.5 ± 2.598	133.0 ± 6.84### (6.27)	135.61 ± 3.52** (5.89)
Methotrexate 0.75	140.3 ± 3.67	134.5 ± 4.48### (4.58)	136.21 ± 3.19** (6.30)

*### p< 0.001: significant difference compared to the body weight of healthy control rats for each evaluation period. * p< 0.05, ** p< 0.01; n = 6: significant difference compared with the respective arthritic control for each evaluation period. HEMa: Hydroethanol extract of Malacantha alnifolia.*

Table 5. Effect of hydroethanol extract of Malacantha alnifolia on white blood cell count

Lots (mg/kg bw)	Mean white blood cell count (10^3/µL)/(percentage increase or reduction) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	12.74 ± 3.51	12.03 ± 2.61	12.61 ± 2.58
Arthritic control (NaCl 9‰)	12.48 ± 2.59	17.4 ± 3.44# (44,63)	16.51± 2.67#
HEMa 125	12.68 ± 1.28	17.6 ± 3.66# (46,3)	15.39 ± 2.44 (6.78)
HEMa 250	12.77 ± 1.45	20.1 ± 3.07### (67.08)	13.37 ± 2.11* (19.01)
HEMa 500	11.92 ± 2.54	18.8 ± 3.54## (56,27)	13.04 ± 2.63* (21.01)
Methotrexate 0.75	12.98 ± 0.87	19.7 ± 2.99### (63.75)	13.07 ± 2.17* (20.83)

*# p< 0.05; p< 0.01; p< 0.001: significant difference compared with the white blood cell count of healthy control rats for each evaluation period. * p< 0.05; n = 6: significant difference compared with the arthritic control. EHMa: hydroethanol extract of Malacantha alnifolia*

Table 6. Effect of hydroethanol extract of Malacantha alnifolia on red blood cell count

Lots (mg/kg bw)	Mean red blood cell count (10^6/µL)/(percentage increase or reduction) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	7.78 ± 3.14	7.22 ± 3.29	7.34 ± 2.62
Arthritic control (NaCl 9‰)	7.18 ± 2.40	6.58 ± 2.34 (8.86)	6.47 ± 2.91
HEMa 125	6.96 ± 2.42	6.66 ± 2.43 (7.75)	6.79 ± 2.72 (4.94)
HEMa 250	7.14 ± 3.33	6.28 ± 3.66 (13.02)	6.93 ± 2.04 (7.10)
HEMa 500	6.79 ± 2.25	6.31 ± 2.26 (12.60)	6.73 ± 2.61 (4.01)
Methotrexate 0.75	7.06 ± 2.37	6.75 ± 2.48 (6.50)	6.49± 2.55 (0.3)

*# p< 0.05: significant difference compared with the red blood cell count of healthy control rats for each evaluation period. * p< 0.05; n = 6: significant difference compared with the arthritic control. HEMa: Hydroethanol extract of Malacantha alnifolia.*

3.7 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Platelets

The mean initial platelet count varied non-significantly from 814 ± 56.6 and 971 ± 95.2.10^3/µL. On day 4 later of arthritis induction, the mean platelet count increased significantly from 4.36% to 15.38% compared with the platelet count of healthy control rats. 14th day later, the mean platelet count of rats treated with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw increased significantly (p< 0.01) compared with the platelet count of arthritic control rats. This increase corresponds to 10.52%, 11.84% and 15.70% respectively compared with the platelet count of arthritic control rats. The platelet count of methotrexate-treated rats also showed a significant increase of 14.56% compared with the platelet count of arthritic control rats (Table 7).

3.8 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Sedimentation Rate

At day 0, sedimentation rate varied non-significantly from 5.05 ± 2.83 to 6.83 ± 3.74 mm/h at hour 1 and from 13.5 ± 2.03 to 15.7 ± 1.17 mm/h at hour 2. On day 4 later of arthritis induction, a significant increase (p<0.001) in sedimentation rate from 105.28 to 139.29% at hour 1 and from 68.75 to 91.47% at hour 2. 14th day later of treatment with hydroethanol extract at doses of 125, 250 and 500 mg/kg bw, the sedimentation rate was significantly reduced by 7.76, 25.72 and 28.15% at hour 1 and by 27.79, 35.34 and 42.29% at hour 2, respectively, compared with the sedimentation rate of arthritic control rats. The sedimentation rate of methotrexate-treated rats was also significantly reduced by 29.12% at hour 1 and 47.12% at hour 2, compared with the sedimentation rate of arthritic control rats (Table 8).

Table 7. Effect of hydroethanol extract of Malacantha alnifolia on platelet count

Lots (mg/kg bw)	Mean platelet count (10^3/µL)/(percentage increase or decrease) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	950 ± 111	962 ± 98.2	977 ± 100.1
Arthritic control (NaCl 9‰)	815 ± 70.2	1109 ± 60.5$^{\#}$ (15.28)	1140 ± 89.1$^{\#}$
HEMa 125	960 ± 83.6	1110 ± 81.2$^{\#}$ (15.38)	1020 ± 81.2* (10.52)
HEMa 250	971 ± 95.2	1169 ± 104.6$^{\#\#}$ (21.51)	1005 ± 93.3* (11.84)
HEMa 500	814 ± 56,6	1061 ± 90.4 (10.29)	961 ± 86,6** (15.70)
Methotrexate 0.75	848 ± 61.5	1004 ± 94.1 (4.36)	974 ± 85.9** (14.56)

*#p< 0.05, ##p < 0.01: significant difference from platelet counts in healthy control rats for each evaluation period. *p < 0.05, **p< 0.01; n = 6: significant difference from arthritic control. HEMa: Hydroethanol extract of Malacantha alnifolia*

Table 8. Effect of hydroethanol extract of Malacantha alnifolia on sedimentation rate

Lots (mg/kg bw)	Sedimentation rate/(percentage increase or reduction) (%)					
	Day 0		Day 4		Day 14	
	1 h	2 h	1 h	2 h	1 h	2 h
Healthy control (NaCl 9‰)	6.83 ± 0.74	13.5 ± 2.03	7.94 ± 0.74	17.6 ± 4.75	6.44 ± 1.65	15.7 ± 5.35
Arthritic control (NaCl 9‰)	5.0 ± 1.83	14.8 ± 1.08	19.0 ± 1.83$^{###}$ (139.29)	30.7 ± 6.16$^{###}$ (74.43)	20.6± 4.61$^{###}$	33.1 ± 5.54$^{###}$
HEMa 125	6.0 ± 5.35	16.7 ± 1.17	17.0 ± 5.35$^{###}$ (114.10)	34.7 ± 8.49$^{###}$ (97.15)	19.0 ± 3.74 (7.76)	23.9 ± 6.73** (27.79)
HEMa 250	5.8 ± 3.89	15.2 ± 2.63	18.8 ± 3.89$^{###}$ (136.77)	29.7 ± 11.6$^{###}$ (68.75)	15.3 ± 5.53* (25.72)	21.4 ± 8.65** (35.34)
HEMa 500	5.3 ± 1.87	14.3 ± 1.87	16.3 ± 1.87$^{###}$ (105.28)	33.7 ± 0.84$^{###}$ (91.47)	14.8 ± 3.37* (28.15)	19.1 ± 0.76*** (42,29)
Methotrexate 0.75	7.0 ± 3.48	14.0 ± 2.24	17.0 ± 3.48$^{###}$ (114.10)	31.7 ± 3.66$^{###}$ (80.11)	14.6 ± 4.82* (29.12)	17.5 ± 9.52*** (47.12)

*###p< 0.001: significant difference from sedimentation rate of healthy control rats for each evaluation period. *p< 0.05, **p< 0.01; ***p< 0.001; n = 6: significant difference from the respective arthritic control for each evaluation period. HEMa: Hydroethanol extract of Malacantha alnifolia*

3.9 Effect Of Hydroethanol Extractof Malacantha Alnifolia On Total Protein Levels

Mean total protein count on day 0 varied non-significantly from 5.00 ± 0.21 to 6.34 ± 0.97 g/dL.

Three days later of arthritis induction, the mean number of total proteins suffered a significant decrease (p< 0.05) ranging from 29.60% to 42.19% compared to the mean number of total proteins of healthy control rats. 14th day later, the average number of total proteins of rats treated with hydroethanolic extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw underwent significant increase (p< 0.05) ranging from 11.92; 27.10 and 30.35% compared to the average number of total proteins of arthritic control rats. Mean total protein count of methotrexate-treated rats was significantly increased (p< 0.05) by 31.70% compared to the mean total protein count of arthritic control rats (Table 9).

3.10 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Albumin Levels

Mean albumin levels on day 0 ranged non-significantly from 3.11 ± 0.50 to 3.35 ± 0.57 g/dL. Three days later of arthritis induction, all rats showed a significant (p<0.001) decrease in albumin levels, ranging from 38.88% to 56.17% compared with healthy control rats. 14th day later, albumin levels in rats treated with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw increased significantly, by 26.25, 36.31 and 45.81% respectively, compared with albumin levels in arthritic control rats. Mean albumin content of methotrexate-treated rats increased significantly (p< 0.05) by 20.11% compared with the mean albumin content of arthritic control rats (Table 10).

Table 9. Effect of hydroethanol of Malacantha alnifolia extract on total proteins

Lots (mg/kg bw)	Average total protein (g/dL)/(percentage increase or decrease) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	5.39 ± 0.77	5.64 ± 0.53	5.51 ± 1.02
Arthritic control (NaCl 9‰)	5.64 ± 1.03	3.30 ± 0.56$^{#}$ (41.48)	3.69 ± 0.97$^{#}$
HEMa 125	6.34 ± 0.97	3.97 ± 0.38 (29.60)	4.13 ± 0.66 (11.92)
HEMa 250	5.76 ± 0.89	3.27 ± 0.56$^{#}$ (42.02)	4.69 ± 0.75 (27.10)
HEMa 500	5.47 ± 1.05	3.49 ± 0.60 (38.12)	4.81 ± 0.54* (30.35)
Mthotrexate 0.75	4.00 ± 0.21	3.26 ± 0.57$^{#}$ (42.19)	4.86 ± 0.82* (31.70)

 # p< 0.05: significant difference compared with total protein in healthy control rats for each evaluation period.

** p< 0.05; n = 6: significant difference from arthritic control. HEMa: Hydroethanol extract of Malacantha alnifolia.*

Table 10. Effect of hydroethanol extract of Malacantha alnifolia on albumin levels

Lots (mg/kg bw)	Mean albumin (g/dL)/(percentage increase or decrease) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	3.35 ± 0.57	3.24 ± 0.14	3.31± 0.44
Arthritic control (NaCl 9‰)	3.11 ± 0.50	1.42 ± 0.17### (56.17)	1.79 ± 0.37###
HEMa 125	2.99 ± 0.30	1.45 ± 0.32### (55.24)	2.26 ± 0.22* (26.25)
HEMa 250	3.27 ± 0.56	1.80 ± 0.38### (44.44)	2,44 ± 0,38* (36.31)
HEMa 500	3.13 ± 0.51	1.91 ± 0.40### (41.04)	2.61 ± 0.57* (45.81)
Methotrexate 0.75	3.24 ± 0.60	1.98 ± 0.40### (38.88)	2.15 ± 0.11* (20.11)

p< 0.001: significant difference from albumin levels in healthy control rats for each evaluation period.
** p< 0.05, ** p< 0.01; *** p< 0.001; n = 6: significant difference from arthritic control. HEMa: Hydroethanol extract of Malacantha alnifolia.*

3.11 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Alkaline Phosphatase Levels

Mean alkaline phosphatase levels varied non-significantly from 135 ± 14.9 to 155 ± 23.9 IU/L on day 0 of the experiment. Three days later of arthritis induction, the mean alkaline phosphatase count of all rats showed an increase ranging from 50.43 to 93.91% compared with the mean alkaline phosphatase count of healthy control rats. 14th day later, mean alkaline phosphatase levels in rats treated with hydroethanol extract of *Malacantha alnifolia* at doses of 250 and 500 mg/kg bw were significantly reduced (p< 0.05) by 10.92 and 14.20% respectively, compared with mean alkaline phosphatase levels in arthritic rats. Mean amount of alkaline phosphatase in methotrexate-treated rats increased significantly (p< 0.05) by 15.84% compared with the mean amount of albumin in arthritic control rats (Table 11).

3.12 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Aspartate Aminotransferase Levels

On day 0, mean aspartate aminotransferase (AST) levels ranged nonsignificantly from 111 ± 8.73 to 114 ± 4.11 IU/L. Three days later of arthritis induction, the mean AST levels of all rats increased significantly, from 88.99% to 94.49% compared with the mean AST levels of healthy control rats. 14th day later, the mean amount of AST at 500 mg/kg bw in rats treated with hydroethanol extract of *Malacantha alnifolia* was significantly reduced (p< 0.05) by 15.63% compared with the mean amount of AST in arthritic rats. Mean amount of AST in methotrexate-treated rats was significantly (p<0.05) reduced by 18.95% compared with the mean amount of AST in arthritic control rats (Table 12).

Table 11. Effect of hydroethanol extract of Malacantha alnifolia on alkaline phosphatase (ALP)

Lots (mg/kg bw)	Average amount of ALP (IU/L)/(percentage increase or decrease) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	148 ± 21.4	115 ± 14.4	123 ± 10.2
Arthritic control (NaCl 9‰)	165 ± 23.9	223 ± 18.2### (93.91)	183 ± 14.8###
HEMa 125	123 ± 16.4	213 ± 17.3### (85.21)	173 ± 18.5 (5.46)
HEMa 250	125 ± 14.9	173 ± 17.3### (50.43)	163 ± 15.7* (10.92)
HEMa 500	154 ± 14.4	220 ± 19.4### (91.30)	157 ± 15.6* (14.20)
Methotrexate 0.75	137 ± 19.8	210 ± 18.1### (82.60)	154 ± 16.5** (15.84)

*### p< 0.001: significant difference compared with alkaline phosphatase (ALP) levels in healthy control rats for each evaluation period. * p< 0.05, ** p< 0.01; *** p < 0.001; n = 6: significant difference from arthritic control. HEMa: hydroethanol extract of Malacantha alnifolia*

Table 12. Effect of hydroethanol extract of Malacantha alnifolia on aspartate aminotransferase (AST) levels

Lots (mg/kg bw)	Mean AST (UI/L)/(percentage increase or decrease) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	112 ± 6.25	109 ± 24.3	115 ± 38.3
Arthritic control (NaCl 9‰)	113 ± 8.16	208 ± 17.7### (90.82)	211 ± 21.1###
HEMa 125	118 ± 15.2	207 ± 19.7### (89.90)	202 ± 14.9 (4.26)
HEMa 250	111 ± 8.73	212 ± 10.6### (94.49)	192 ± 26.1 (9.00)
HEMa 500	122 ± 4.11	214 ± 28.4### (96,33)	178 ± 22.7* (15.63)
Methotrexate 0.75	114 ± 4.11	206 ± 28.7### (88.99)	171 ± 25.4* (18.95)

*### p< 0.001: significant difference compared with aspartate aminotransferase (AST) levels in healthy control rats for each evaluation period. * p< 0.05, ** p< 0.01; *** p< 0.001; n = 6: significant difference from arthritic control. HEMa: hydroethanol extract of Malacantha alnifolia.*

3.13 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Alanine Aminotransferase Levels

On day 0, mean alanine aminotransferase (ALT) levels ranged nonsignificantly from 60.3 ± 9.98 to 67.8 ± 4.43 IU/L. Three days later of arthritis induction, the mean ALT levels of all rats showed a significant increase ranging from 19.21 to 42.94% compared with the mean ALT levels of healthy control rats. 14th day later, mean ALT levels at doses of 250 and 500 mg/kg bw of rats treated with hydroethanol extract of *Malacantha alnifolia* were significantly reduced (p< 0.05) by 17.61 and 19.84% respectively, compared with the mean ALT levels of arthritic rats. Mean ALT levels in methotrexate-treated rats were non-significantly reduced by 12.82% compared with mean ALT levels in arthritic control rats (Table 13).

3.14 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Calcium Levels

On day 0, the mean amount of calcium varied non-significantly from 3.11 ± 0.72 to 4.06 ± 1.61. Three days later of arthritis induction, the mean calcium level of arthritic rats increased significantly, from 72.37% to 97.00%, compared with the mean level of healthy control rats. 14th day later, calcium levels in rats treated with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw were significantly reduced (p< 0.01) by 32.85 at 43.33% compared with the mean calcium levels in arthritic control rats. Mean calcium content of methotrexate-treated rats was also significantly reduced (p< 0.01) by 47.14% compared with the mean calcium content of arthritic control rats (Table 14).

Table 13. Effect of hydroethanol extract of Malacantha alnifolia on alanine aminotransferase (ALT) levels

Lots (mg/kg bw)	Mean ALT (IU/L)/(percentage increase or decrease) (%)		
	Day 0	Day 4	Day 14
Healthy control (NaCl 9‰)	65.5 ± 8.88	68.7 ± 9.36	60.3 ± 12.41
Arthritic control (NaCl 9‰)	60.3 ± 9.98	96.5 ± 10.39### (40.46)	89.7 ± 11.54##
HEMa 125	61.0 ± 5.29	89.6 ± 9.87## (30.42)	79.6 ± 9.87 (11.25)
HEMa 250	64.3 ± 11.4	93.9 ± 8.65### (36.68)	73.9 ± 8.65* (17.61)
HEMa 500	67.8 ± 4.43	81.9 ± 10.41# (19.21)	71.9 ± 10.41* (19.84)
Methotrexate 0.75	63.7 ± 5.42	98.2 ± 11.77###	78.2 ± 11.77

		(42.94)	(12.82)

*# p< 0.05; ## p< 0.01; ### p< 0.001: significant difference compared with alanine aminotransferase (ALT) levels in healthy control rats for each evaluation period. * p< 0.05, ** p < 0.01; *** p< 0.001; n = 6: significant difference from arthritic control. HEMa: hydroethanol extract of Malacantha alnifolia.*

Table 14. Effect of hydroethanol extract of Malacantha alnifolia on calcium levels

Lots (mg/kg bw)	**Average amount of calcium (IU/L)/(percentage increase or decrease) (%)**		
	Day 0	**Day 4**	**Day 14**
Healthy control (NaCl 9‰)	4.87 ± 0.95	4.67 ± 1.01	4.69 ± 0.83
Arthritic control (NaCl 9‰)	4.16 ± 1.61	9.2 ± 2.36$^{###}$ (97.00)	10.5 ± 2.14$^{###}$
HEMa 125	3.11 ± 0.72	8.55 ± 2.58$^{###}$ (83.08)	6.55 ± 2.46* (32.85)
HEMa 250	4.01 ± 1.53	9.06 ± 2.42$^{###}$ (94.00)	6.06 ± 2.67** (42.28)
HEMa 500	3.19 ± 1.77	8.05 ± 2.51$^{###}$ (72.37)	5.95 ± 2.65** (43.33)
Methotrexate 0.75	3.37 ± 1.61	8.95 ± 2.39$^{###}$ (91.64)	5.55 ± 2.54** (47.14)

*### p< 0.001: significant difference compared with calcium levels in healthy control rats for each evaluation period. * p< 0.05, ** p< 0.01; *** p< 0.001; n = 6: significant difference from arthritic control. HEMa: hydroethanol extract of Malacantha alnifolia.*

3.15 Effect Of Hydroethanol Extract Of Malacantha Alnifolia On Phosphorus Levels

At the start of the experiment, the average amount of phosphorus varied non-significantly from 5.06 ± 2.64 to 6.43 ± 2.84. On day 4 later of arthritis induction, the mean phosphorus content of all rats showed a significant increase ranging from 115.85 to 142.83% compared with the mean phosphorus content of healthy control rats. 14th day later, the mean phosphorus content of rats treated with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw was significantly reduced (p< 0.001) by 21.23 to 45.89% compared with the mean phosphorus content of arthritic control rats. Mean phosphorus content of methotrexate-treated rats was significantly reduced (p<0.001) by 53.49% compared with the mean phosphorus content of arthritic control rats (Table 15).

Table 15. Effect of hydroethanol extract of Malacantha alnifolia on phosphorus levels

Lots (mg/kg bw)	**Average amount of phosphorus (IU/L)/(percentage increase or reduction) (%)**		
	Day 0	**Day 4**	**Day 14**
Healthy control (NaCl 9‰)	5.93 ± 1.58	5.93 ± 1.58	6.07 ± 1.67
Arthritic control (NaCl 9‰)	6.18 ± 2.13	14.1 ± 2.13$^{###}$ (137.77)	14.6 ± 2.67$^{###}$
HEMa 125	5.96 ± 2.53	13.9 ± 2.53$^{###}$ (134.40)	11.5 ± 2.10* (21.23)
HEMa 250	6.43 ± 2.84	14.4 ± 2.84$^{###}$ (142.83)	10.1 ± 1.97** (30.82)
HEMa 500	5.71 ± 2.51	13.5 ± 2.51$^{###}$ (127.65)	7.90 ± 2.27*** (45.89)
Methotrexate 0.75	5.06 ± 2.64	12.8 ± 2.64$^{###}$ (115.85)	6.79 ± 2.23*** (53.49)

*### p< 0.001: significant difference compared with phosphorus levels in healthy control rats for each evaluation period. * p< 0.05, ** p< 0.01; *** p< 0.001; n = 6: significant difference from arthritic control. HEMa: hydroethanol extract of Malacantha alnifolia.*

4 Discussion

In this study, arthritis, a chronic inflammatory joint disease, was induced experimentally in rats using formaldehyde diluted to 2.5%. Injection of formaldehyde causes localized inflammation and pain in a 1st phase with the release of inflammatory mediators, followed

by another phase involving inflammatory systems [23]. This late phase produces a proliferative joint inflammation that is responsible for joint changes similar to those seen in rheumatoid arthritis [24]. In addition, chronic inflammation involves the release of numerous destructive mediators such as cytokines, interferons, histamine, prostaglandins, leukotrienes [25], platelet-activating factor, nitric oxide and tumor necrosis factor [26]. According to [27], these mediators are responsible for the presence of edema, persistent pain and destruction of cartilage and bone, and severe disability in animals.

Results reported in tables 1, 2 and 3 showed the presence of arthritic signs and inflammation with elevated scores in arthritic control lots compared to healthy control rats. Indeed, in this model, inflammation was established on days 1 and 3, leading to chronic inflammation. This chronic inflammation could lead to the presence of cells destroying the functional integrity of the animal's right hind leg [28]. However, after treatment with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw, reduction of signs of this pathology was observed. The hydroethanol extract of *Malacantha alnifolia* has anti-arthritic properties similar to methotrexate in the treatment of this pathology. These results are similar to those obtained by [17]. These authors showed that aqueous extract of *Pleurotus ostreatus* at doses of 100 and 300 mg/kg bw reduced the thickness of the paw rendered arthritic by formalin. Three days later of arthritis induction, body weight of arthritic rats decreased significantly. In fact, the injection of formaldehyde into the rat's leg resulted in a loss of body weight in arthritic rats, which would be due to a change in their metabolic activity, since, according to [29], the change in rat body weight also occurs during the arthritic experimental period. However, ten days later of treatment, the mean body weight of rats treated with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw increased significantly. These results are similar to those of [30], who showed that the loss of body weight obtained after three days of arthritis induction was restored by the aqueous extract of *Kirganelia reticulata*. In terms of white blood cells, three days later of arthritis induction, the number of white blood cells increased significantly ($p< 0.001$) compared with healthy control rats. In fact, injection of formaldehyde triggered inflammation resulting in the active release of leukocytes, which would maintain the defensive activity of the host defense system [31]. After treatment with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw, white blood cell counts were significantly reduced ($p< 0.05$) compared with arthritic control rats. These results are similar to those obtained by [32]. These authors showed that aqueous extract of *Enicostemma axillare* restored white blood cell counts.

Erythrocyte counts during arthritis induction with 2.5% formaldehyde decreased three day later induction. This average red blood cell count showed a maximum and significant ($p< 0.05$) decrease of 16.62% compared with the red blood cell count of healthy control rats. Indeed, according to [33], a cell's survival depends on the integrity of their membrane. Thus, exposure of the red cell membrane to a harmful substance such as formaldehyde could lead to reactions that would participate in red cell lysis. Ten days later of treatment with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw, the red blood cell count was virtually restored. These results are similar to those obtained by [32]. These authors showed that the aqueous extract of *Enicostemma axillare* restored red blood cell counts. Three day later arthritis induction, mean platelet count and sedimentation rate increased significantly ($p< 0.01$) compared with healthy control rats. Platelets are known to play a role in hemostasis and local vasoconstriction. In addition, formalin induced inflammation by activating factor XII, with proteolysis cascades leading to the production of fibrin from fibrinogen, triggering coagulation [34]. Thus, the increase in fibrinogen could be one of the causes of the increase, on the one hand, in the sedimentation rate, and on the other, in the platelet count. However, 14th day later of treatment with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw, the mean platelet count and sedimentation rate of treated rats were significantly reduced compared with arthritic control rats. Treatment of rats with hydroethanol extract of *Malacantha alnifolia* restored these parameters. These results are similar to those obtained [17], who showed that aqueous extract of *Pleurotus ostreatus* at doses of 100 and 300 mg/kg bw restored platelet count and sedimentation rate in their work.

4th day later of arthritis induction, mean numbers of total protein and albumin decreased significantly. In fact, formalin injection led to a decrease in total protein and albumin levels. This decrease in the level of these proteins could be due to denaturation and structural degradation of the proteins after formaldehyde injection [35,36] On the other hand, after 10 days' treatment with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw, total protein and albumin levels were restored. These results are similar to those obtained by [37]. These authors showed that the aqueous extract of *Murraya exotica* restored total protein and albumin levels.

Three days later of arthritis induction, arthritic rats showed significant increases in AST, ALT and ALP compared with healthy controls. The increase in these enzymes may be due to increased synthesis of inflammatory mediators by the liver. These enzymes present at very high concentrations in the liver can also elicit the presence of hepatocellular damage [38]. Indeed, formaldehyde injected into the rat's right hind leg triggered systemic inflammation following a complex enzymatic reaction process [39]. However, after treatment with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw, these parameters were restored. These results are similar to those obtained by [37]. These authors showed that aqueous extract of *Murraya exotica* restored AST, ALT and ALP levels. Calcium and phosphorus levels in arthritic rats were significantly higher than in healthy control rats. Mineralization is a process that requires calcium and phosphate ions, and involves small vesicles rich in osteoblasts, a reservoir of calcium and phosphate [40]. The destruction of bone tissue leads to the loss of certain bone tissues in the form of bone erosion by increasing plasma mineralization [41,42]. After treatment with hydroethanol extract of *Malacantha alnifolia* at doses of 125, 250 and 500 mg/kg bw, calcium and phosphorus levels were restored. These results are similar to those obtained by [43]. The latter showed that methanolic extract of *Barleria lupulina* at 300 and 600 mg/kg bw restored calcium and phosphorus levels.

5 CONCLUSION

Results showed that the hydroethanol extract of *Malacantha alnifolia* caused a reduction in signs of arthritis, decreases in white blood cell count, platelet count and sedimentation rate, as well as decreases in ALT, AST, ALP, calcium and phosphorus levels, and increases in rat body weight and red blood cell count previously increased or decreased in the presence of formaldehyde. The hydroethanol extract of *Malacantha alnifolia* has remarkable anti-arthritic properties similar to those of methotrexate.

REFERENCES

[1] Kumar V., Verma A., Ahmed V., Sachan N.K., Anwar F. and Mujeeb M. Fostered antiarthritic upshot of *Moringa oleifera* Lam. stem bark extract in diversely induced arthritis in *wistar* rats with plausible mechanism. *International Journal of Pharmaceutical science and Reasearch,* 4, 3894-901, 2013.

[2] Al-Nahain A., Jahan R. and Rahmatullah M. Review: Zingiber officinale: A potential plant against rheumatoid arthritis. *Hindawi Publishing Corporation,* 2104, 1-8, 2014.

[3] Diomandé M., Kaboré F., Soh E., Kouakou C.L., Koné M.G., Coulibaly A.K., Djaha K., Ouattara B., Eti E., Daboiko J.C., Kouakou M.N. Paraclinical, Therapeutic and Evolutionary Profile of Osteoarticular Tuberculosis: A Report about 550 Cases in Abidjan. *Open Journal of Rheumatology and Autoimmune Diseases,* 5, 131-136, 2015.

[4] McInnes I.B. and Schett G. Mechanisms of disease: The pathogenesis of rheumatoid arthritis. *New England Journal of Medecine,* 365, 2205-2219, 2011.

[5] Zhao H.S., Liu D., Huang Q., Xu, Shuto T. The protective effects of incadronate on inflammation and joint destruction in established rat adjuvant arthritis. *Rheumatology International,* 26, 732-740, 2006.

[6] Makinen H., Kautianinen H., Hannonen P., Mottonen M. and Leirisalo R.M. Sustained remission and reduced radiographic progression with combination disease modifying anti-rheumatic drugs in early rheumatoid arthritis. *Journal of Rheumatology,* 34, 316-321, 2007.

[7] Chiolero A., Würzner G. and Burnier M. Les inhibiteurs spécifiques de la cyclooxygénase de type 2: moins d'effets rénaux que les anti-inflammatoires non-stéroïdiens classiques ? Collection Serveur académique Lausannois, ID 2000: 10670/1.1ty33r, Site consulté 20/06/2022. https://isidore.science/document/10670/1.1ty33r.

[8] Ng S.C. Non-steroidal anti-inflammatory drugs-uses and complications. *Singapore Medical Journal,* 33, 510-513, 1992.

[9] Rubbert-Roth A. and Perniok A. Treatment of patients with rheumatoid arthritis with the interleukin-1 receptor antagonist anakinra (Kineret). Zeitschrift Fur Rheumatologie, https://www.semanticscholar.org/paper/Treatment-of-patients-with-rheumatoid-arthritis-the-Rubbert-Roth-Perniok/6c73703a3eb7fa7472abe9f25315af9422c33d0f. Site consulted on 06/20/2022.

[10] Lardry J.M. and Haberkorn V. L'aromathérapie et les huiles essentielles. *Kinestherapy Review,* 61, 14-17.2007.

[11] OMS (Organisation Mondiale de la Santé). Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005. WHO /EDM /TRM /2002.1.

[12] Aké Assi L. Rapport sur le colloque international de la médecine traditionnelle africaine à Abidjan-Côte d'Ivoire. *Bulletin de la médecine traditionnelle et pharmaceutique,* 4, 184-198, 1991.

[13] Tra Bi F.H., Irié G.M., N'Gaman K.C.C. and Mohou C.H.B. Études de quelques plantes thérapeutiques utilisées dans le traitement de l'hypertension artérielle et du diabète: deux maladies émergentes en Côte d'Ivoire. *Science et Nature,* 5, 39-48, 2008.

[14] Bouquet A. & Debray M. Plantes médicinales de Côte d'Ivoire. Edition de l'office de la recherche scientifique et technique outre-mer, 32, 10-12, 1974.

[15] EU (European Union). Commission implementing decision of 14 november 2012 establishing a common format for the submission of the information pursuant to Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (notified under document C (2012) 8064) text with EEA relevance. *Official Journal of European Union,* 320, 33-50, 2012.

[16] Zihiri G.N., Kra A.M., Bahi C. and Guédé G.F. Plantes médicinales immunostimulantes: critères de sélection, techniques rapides d'extraction des principes actifs et methods d'évaluation de l'activité immunogène. *Pharmacopée Africaine,* 17, 131-138, 2003.

[17] Singh V., Vyas D., Rajshree P. and Imtiyaz A.S. Pleurotus Ostreatus produces antioxidant and antiarthritic activity in Wistar Albino Rats. *World Journal of Pharmacy and Pharceutical Sciences,* 4, 1230-1246, 2015.

[18] Alamgeer, Uttra A.M. and Hasan U.H. Anti-arthritic activity of aqueous-methanolic extract and various fractions of *Berberis orthobotrys* Bien ex Aitch. *BMC Complematary and Alternative Medicine,* 17 (371), 1-16, 2017.

[19] Waynforth B.H. Injection Techniques-Experimental and Surgical Techniques in the Rat. Academic Press, London, 3-61, 1980.

[20] Tietz NW. Fundamentals of Clinical Chemistry, Ed. WB Saunders Co Philadelphia, 425-27, 1983.

[21] Murray W.D., Sowden L.C. and Colvin J.R. *Bacteroides cellulosolvens* sp. nov., a cellulolytic species from sewage sludge. *International Journal of Systematic Bacteriology,* 34 (2), 185-187, 1984.

[22] Tietz N.W. *Clinical Guide to Laboratory Tests (ELISA),* 3rd Edition, WB Saunders Co., Philadelphia, 22-23, 1995.

[23] Wheeler-Aceto H. and Cowan A. Neurogenic and tissue-mediated components of formalin induced edema: Evidence for supraspinal regulation. Agents Actions, 34, 264-269, 1991.

[24] Okoli C.O., Akah P.A. and Nwafor S.V. Anti-inflammatory activity of plants. *Journal of Natural Remedies,* 3 (1), 1-30, 2003.
[25] Ammon H.P., Safayhi H., Mack T. and Sabieraj J. Mechanism of antiinflammatory actions of *curcumine* and *bowellic* acids. *Journal of Ethnopharmacology,* 38 (2-3), 113-119, 1993.
[26] McIntosh J.C., Mervin-Blake S., Conner E. and Wrigth J.R. Surfactant protein A protects growing cells and reduces TN F-alpha activity from LPS-stimulated macrophages. *American Journal of Physiology,* 271 (2), 310-319, 1996.
[27] Eric G.B. and Lawrence J.L. Rheumatoid arthritis and its therapy, The Textbook of therapeutic drugs and disease management. 6th editions Baltimore Williams and Wilkins Company 1, 579-595, 1996.
[28] Carl M.P. Experimental joint disease: Observations on adjuvant-induced arthritis. *Journal of Chronic Diseases,* 16 (8), 863-864, 1963.
[29] Walz D.T., DiMartino M.J. and Misher A. Adjuvant induced arthritis in rats. II. Drug effects on physiologic, biochemical and immunologic parameters. *Journal of Pharmacology and Experimental Therapeutics,* 178 (1), 223-231, 1971.
[30] Shruthi S.D., Sujan G.P.S., Padmalatha R.S. and Ramachandra Y.L. *In vitro, in vivo* and *in silico* antiarthritic studies of polyprenol from *Kirganelia reticulata* Baill. *Jounal of Biochemical Technology,* 3 (5), 221-226, 2012.
[31] Mahgoub O., Kadim I.T., Tageldin M.H., Al-Marzooqui W.S., Khalaf S.Q. and Ambu Ali A. Clinical profile of sheep fed non-conventional feeds containing phenols and condensed tannins. *Small Ruminant Research,* 78 (1-3), 115-122, 2008.
[32] Shafeeq K.M., Senthamarai R. and Sundari S.K.K. *In vivo* antiarthritic activity of aqueous extract of *Enicostemma axillare* against formaldehyde induced arthritis. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 4 (3), 110-115, 2013.
[33] Rahman H., Eswaraiah M.C. and Dutta M. *In-vitro* anti-inflammatory and anti-arthritic activity of *Oryza sativa Var. Joha Rice* (An Aromatic Indigenous Rice of Assam). *American-Eurasian Journal of Agricultural and Environmental Sciences,* 15 (1), 115-121, 2015.
[34] Ingebrigt T. and Jan S.G. Factors interfering with the cell morphology in smears. *Scandinavian Journal Haematology,* 23 (3), 197-203, 1979.
[35] Weissmann G. The role of lysosomes in inflammation and disease. *Annual Review of Medicine,* 18: 97-112, 1976.
[36] Thompson D.J. and Stout D.G. Mixtures of Persian clover with Italian ryegrass or barley-Italian ryegrass for annual forage. *Canadian Journal of Plant Science,* 77 (4), 579-585, 1997.
[37] Aishwarya M.N.L., Mounika P., Mounika J., Shankar M., Mallikarjuna G., Niranjan Babu M. and Nagaraju N. Antiarthritic Activity of *Murraya exotica* Linn against Formaldehyde Induced Arthritis in *Wistar* Rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 9 (3), 205-17, 2018.
[38] Gad F.A., Abd Allah O.A., Farid A.S. and Fararh K.M. Biochemical, histopatological and immunohistochemical changes in paracetamol, fish oil and thioacetamide treated rat. *Benha Veterinary Medical Journal,* 25 (1), 18-28, 2013.
[39] Das S., Hopkins P.M., Khambadakone D. and Durica D.S. RNAi mediated disruption of ecdysteroid signaling during limb regeneration in the fiddler crab, Uca pugilator. *Integrative and Comparative Biology,* 50, e 221, 2010.
[40] Anderson H.C. Molecular biology of matrix vesicles. *Clinical Orthopaedics and Related Research,* 314, 266-280, 1995.
[41] Pulla Reddy A.C.H. and Lokesh B.R. Effect of curcumin and eugenol on iron-induced hepatic toxicity in rats. *Toxicology,* 107 (1), 39-45, 1996.
[42] Arafa H.M.M. Curcumin attenuates diet-induced hypercholesterolemia in rats. Medical Science Monitor: Inter. *Medical Journal of Experimental and Clinical Research,* 11 (7), 228-234, 2005.
[43] Mazumder P.M., Mondal A., Sasmal D., Arulmozhi S. and Rathinavelusamy P. Evaluation of antiarthritic and immunomodulatory activity of *Barleria lupulina*. *Asian Pacific Journal of Tropical Biomedicine,* 2 (3), 1400-1406, 2012.