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Effect of *Siphonochilus aethiopicus* Methanolic Extract on Oxidative Stress Markers, Biochemical and Hematological Parameters in Experimentally Induced Arthritis Model in Mice

Elizé Dadaya [a*], Jeweldai Vedekoi [b], Tchoubou Zacharie [c], Hervé Ngatanko Abaissou [c] and Ndjonka Dieudonné [d]

[a] Department of Life Sciences, Higher Teachers Training College, University of Bertoua, P.O Box 652, Bertoua, Cameroon.
[b] Department of Biotechnology and Pharmacognosy, Faculty of Science, University of Ebolowa, P.O. Box 118, Ebolowa, Cameroon.
[c] Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon.
[d] Department of Biological Sciences, Faculty of Science, University of Garoua, P.O. Box 346, Garoua, Cameroon.

Authors' contributions

This work is a product of all authors' collaboration. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: elizedadaya@yahoo.fr;*

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ABSTRACT

Background: *Siphonochilus aethiopicus* belongs to the *Zingiberaceae* family. In Chinese medicine, *Zingiberaceae* are acknowledged for their anti-inflammatory and antioxidant properties, which contribute to immune system reinforcement. They are known to promote digestion, alleviate nausea, motion sickness and are involved incombating flu and cold. The current study was conducted to investigate the antioxidant and anti-inflammatory properties of the methanolic extract of *Siphonochilus aethiopicus* (MESA).
Methodology: The *in vivo* study examined the anti-inflammatory impact of the extract (75, 150, and 300 mg/kg) using a formaldehyde-induced inflammation mouse model. The anti-arthritic effect of the MESA was determined by measuring paw diameter, body weight, biochemical parameters (C-reactive protein and rheumatoid factor) including oxidative stress markers (malondialdehyde, superoxide dismutase, catalase, and nitric oxide), and hematological parameters. Additionally, a histopathological examination was conducted to evaluate the outcome of treatment on tissue integrity.
Results: Results obtained show that the MESA significantly decreased paw inflammation ($P < 0.05$ to $P < 0.001$) and enhanced hematological and biochemical parameters. Additionally, MESA significantly ameliorated oxidative stress parameters ($P < 0.05$ to $P < 0.001$).
Conclusion: This finding suggests that *Siphonochilus aethiopicus* possesses strong antioxidant and anti-arthritic activity, supporting its traditional application as a remedy for rheumatoid arthritis.

Keywords: Antioxidant activities; anti-arthritic; anti-inflammatory; Siphonochilus aethiopicus.

1. INTRODUCTION

Inflammation is a response of the immune system to various aggressions and is implicated in a wide range of human pathologies such as diabetes, cardiovascular diseases, including arthritis [1,2] Rheumatoid arthritis is the most common inflammatory rheumatism that affects the synovial membrane [3]. Formal histopathological changes observed in patients with rheumatoid arthritis include persistent inflammation and cell proliferation in the synovial membrane of the joint, infiltration of various inflammatory cells, pannus formation, cartilage and bone tissue destruction, leading to eventual joint deformation and loss of function [3]. The pathology is currently experiencing a global resurgence, affecting 2%-4% of the general population" [4]. The main events that occur during the inflammatory reaction likely involves imbalance of nitric oxide, lipid peroxidation, cytokine release, and formation of reactive oxygen species derived from neutrophils [5]. Free radical instability is fundamentally the result of electron loss leading to increased reactivity and electron stripping from other molecules, initiating a dangerous chain reaction known as free radical damage [5,6]. "Main targets of these free radicals are proteins, lipids, and DNA/RNA. All these modifications in different and multiple molecules can increase the chances of mutagenesis. In fact, overproduction of ROS over an extended period can cause severe damage to cellular structure and function [7]; hence, rapid removal is mandatory. Free radicals are important mediators that initiate inflammatory processes, and therefore, their neutralization by antioxidants and radical scavengers can attenuate inflammation. To minimize damages caused by free radicals, the body employs several enzymes (such as superoxide dismutase and catalase) and cofactors (such as glutathione) [6]. However, the endogenous physiological response established by antioxidant enzymes may not be sufficient to limit ROS production [6,8].

In fact, the primary treatment involves the use of a combination of non-steroidal anti-inflammatory drugs, glucocorticosteroids, disease-modifying antirheumatic drugs, and biological therapies such as rituximab and infliximab [9]. This therapeutic approach is lengthy and focuses on inhibiting main mediators of the chronic inflammation process to regulate or reduce their degenerative impact on joint tissues [10]. Although these medications are effective, their long-term use in treating chronic inflammation is associated with undesirable side effects. These adverse effects include gastrointestinal lesions, skin rashes, allergic reactions, hepatitis, renal toxicity and even cardiac complications [11].

Phytotherapy is becoming increasingly popular in the modern world to address disease progression and extra-articular manifestations of rheumatoid arthritis due to cardiovascular complications, mood disorders, gastrointestinal

and respiratory disorders, immunodeficiency, and blood disorders associated with various available rheumatoid arthritis therapies [12]. Among plants traditinally used in the treatment of inflammatory diseases in Cameroon, *Siphonochilus aethiopicus* is very much prized.

Siphonochilus aethiopicus isused traditionally in the treatment of cough, cold, asthma, headache, pain, inflammation, and malaria [13]. Its antibacterial [14], antimalarial [15], and anti-candida [16] properties have been achieved in rhizome extracts. A few bioactive compounds were isolated from *S. aethiopicus* rhizomes, including two furanoterpenoids, which accounted for 20% of the oil composition of the extract [17].

The current study aims to investigate the antioxidant and anti-inflammatory potential of the methanolic extract of *Siphonochilus aethiopicus* in mice using the formaldehyde-induced inflammation mouse model.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

S. aethiopicus rhizomes were collected in the Tokombere subdivision (Far-North region of Cameroon: longitude E 14°08'35"; latitude N 10°52'18"; altitude 746 m).The fresh material was first transported in polystyrene bags to the Laboratory of Biochemistry and Biological Chemistry of the Faculty of Science of the University of Maroua. Professor Tchopsala, botanist in the same University, did identification of the plant. A voucher specimen was deposited at the Cameroon National Herbarium by comparing to specimen N° 45836/HEFG. Rhizomes were washed three times with tap water and dried at room temperature (35 ± 3 °C). Dry material obtained was reduced into a powder, sieved (0.5 mm partical size) and kept until extraction.

The extraction was achieved by macerating 300 g of powder in 3000 mL of methanol (80%) for 72 hours under stirring at room temperature [18]. The mixture was filtered using Wattman filter paper (N°1) and the filtrate was collected. The solvent of the filtrate obtained was concentrated with a rotary evaporator set at 70˚C. The residue was kept at - 4˚C.

2.2 Experimental Animals

The study was conducted on male *albino Swiss* mice weighing between 25 and 30 g, purchased from LANAVET (Laboratoire National Vétérinaire, Garoua). All animals aged 4 months were bred in the animal house of the Department of Biological Sciences (University of Maroua) at room temperature, natural light/dark cycle, and given access to water and food *ad libitum*. Animals were housed in polyacrylic cages (5 mice/cage) and were acclimatized for 14 days. Mice were treated following the guidelines of the Cameroonian Bioethics Committee (reg N° FWAIRB00001954) and in accordance with *NIH-Care and Use of Laboratory Animals (8th edition)*.

2.3 Induction of Arthritis, Mice Grouping, and Treatment

Mice were divided into 6 groups (n= 5) and treated daily for ten days as follows:

- Two control groups (normal and negative) treated with distilled water (10 mL/kg, *p.o*) each;
- One positive control group treated with the reference drug: diclofenac 5 mg/kg, *p.o*;
- Three test groups treated with MESA (75, 150, and 300 mg/kg) *p.o* respectively.

One hour after these various treatments, a volume of 0.04 mL of a 1.4% formaldehyde solution was injected under the plantar aponeurosis of the left hind paw of the animals in all groups except the normal control group, which received 0.04 mL of distilled water by the same route. This formaldehyde injection was repeated on the third day of the experiment [19].

2.4 Evaluation of Paw Edema and Animal Weight

The evolution of edema was monitored by measuring the diameter of the edematous paw (mm) of each animal every day throughout the experimental period using a vernier caliper. The evolution of edema in different groups was determined using the following formula:

$$\Delta E = Edx - Ed0$$

ΔE = difference in edema between day-x and day-0; Ed0= initial thickness (mm) of the left leg (before formaldehyde injection); Edx = thickness of the left leg (mm) on day "d" after formaldehyde injection.

The percentage of inhibition (% Inh) was calculated for each group of treated mice compared with the negative control group.

$$\% \text{ Inh} = 100\left[1-\frac{\Delta Et}{\Delta EC}\right]$$

∆Et = difference in edema between day-0 and day-x of the left paw of the treated mice; ∆EC = represents the difference in edema between day-0 and day-x of the left paw of the negative control group.

2.5 Blood and Organ Sampling

On the 10th day, after the final measurement of edema and weight, animals were euthanized after anaesthetizing by an intraperitoneal injection of thiopental (50 mg/kg). Blood was immediately collected into heparin tubes and used to evaluate hematological and biochimical parameters. Liver and spleen samples were collected, homogenized in Tris-HCl buffer (50 mM; pH 7.4), centrifuged at 3000 rpm for 15 minutes, stored in the freezer. These samples were subsequently used for the assay of malondialdehyde, superoxide dismutase, catalase and nitric oxide.

2.6 Assessment of Hematological and Biochemical Parameters

Hematological parameters were conducted using a MINDRAY brand multiparametric automated analyzer (BC-2800). Parameters of interest in included red blood cell count, white blood cell count, hemoglobin level, hematocrit level and platelet count.

CRP level was measured using an immunoturbidimetric assay following the protocol outlined by Pepys [20].

Qualitative testing for rheumatoid factor was performed according to the method described by Young [21].

2.7 Superoxide Dismutase (SOD) Assay

Superoxide dismutase (SOD) activity was evaluated using the method described by Misra and Fridovish [22]. In a test tube, 134 µL of the homogenate was mixed with 1666 µL of carbonate buffer (0.05 M, pH = 10.2). The reaction was initiated by adding 0.2 mL of freshly prepared adrenaline (0.3 mM). This mixture was homogenized by rapid inversion of the test tube. The blank tube consisted of 134 µL of distilled water, 1660 µL of carbonate buffer, and 0.2 mL of adrenaline solution. The increase in absorbance was recorded between 20 and 80 seconds at 480 nm. The specific activity of SOD was evaluated in SOD units per gram of organ.

$$\%\,\mathrm{Inh} = 100 - \frac{(\mathrm{Abs20s}-\mathrm{Abs80s})\mathrm{Sample}}{(\mathrm{Abs20s}-\mathrm{Abs80s})\mathrm{Blank}}\ X\ 100$$

Abs20s (absorbance measured at 20 seconds) and Abs80s (absorbance measured at 80 seconds). The specific activity of SOD (SOD units/g of tissue) = (Number of SOD units/mL)/g of tissue x dilution factor.

2.8 Catalase (CAT) Assay

Catalase activity was evaluated according to the method described by Sinha [23]. Equivolumes (50 µL) of sample and distilled water were introduced into test tubes and a blank tube was also prepared. Subsequently, 750 µL of a phosphate buffer solution (0.1 mM; pH 7.5) and 200 µL of hydrogen peroxide (50 mM) were added to all tubes at room temperature for one minute, and the reaction was stopped by adding 2 mL of potassium dichromate (5%) prepared in 1% acetic acid. Tubes were then incubated for 10 minutes in a boiling water bath and cooled with tap water. Optical densities were read at 570 nm. Specific catalase activity was determined from a previously established standard curve according to the equation:

$$y = ax + b.$$

Catalase specific activity was expressed in mM of H_2O_2/min/g of tissue.

2.9 Measurement of Malondialdehyde (MDA) Content

Briefly, 125 µL of 20% trichloroacetic acid and 250 µL of 0.67% thiobarbituric acid were added to test tubes containing 250 µL of the sample, and to a blank tube containing 250 µL of Tris-HCl buffer (50 mM; pH 7.4). All tubes were sealed with glass beads, heated at 90°C in a water bath for 10 minutes, cooled and centrifuged at 3000 rpm at room temperature for 15 minutes. Optical densities of supernatants from test tubes were read at 532 nm against the blank [24].

The concentration of MDA was determined using the formula below. MDA level was expressed in mmol/g of tissue.

$$[MDA] = \frac{(\Delta Abs\ Sample)}{(\varepsilon * L * q)}$$

∆Abs: Change in absorbance; ε: Molar extinction coefficient (15600 mol^{-1}); L: Path length (1cm) and q: gram of organ in the sample.

2.10 Determination of Nitric Oxide (NO) Concentration

In test tubes, 100 µL of each sample (homogenate) was diluted in 400 µL of distilled water. 5 mL of distilled water were added to the test tubes and to the blank tube, respectively. Then, 500 µL of Griess reagent was added to each tube. The mixture was homogenized and incubated at room temperature, protected from light, for 10 minutes, and the absorbance was read against the blank at 546 nm [25]. The concentration of nitric oxide was expressed in µmol/g of tissue. The NO concentration was calculated using the following formula:

$$[NO] = \frac{(Abs\ Sample - AbsBlank)}{(\alpha * p)}$$

[NO]: Nitric oxide concentration, α: Slope of the standard curve and p: Weight of the organ

2.11 Histological Study

At the end of the experiment, hind paws of mice were sectioned and fixed in 10% formalin. Decalcification was performed using a 3% nitric acid solution at room temperature, and the decalcification solution was replaced every 48 hours for a total of 12 times. Subsequently, samples were dehydrated in alcohol (50, 75, 85, 95, and 100% ethanol), embedded in paraffin, sectioned (thickness, 5 µm), and stained with hematoxylin (8 min) and eosin (1 min) at room temperature for morphological observation under an optical microscope. Images were captured using an Olympus CKX3 optical microscope (magnification x 100; six fields; Olympus Corporation).

2.12 Statistical Analysis

Results were expressed as mean ± standard deviation of five animals per group. Data were analyzed using one-way analysis of variance (ANOVA) performed by GraphPad Prism 8.01. Multiple comparisons were achieved using Dunnett's tests and significance was considered at $P < 0.05$.

3. RESULTS

3.1 Effect of MESA on Mice Paw Edema

Fig. 1 depicts the evolution of paw edema variation (ΔE) during the experimental period. A significant increase ($P < 0.001$) in paw edema variation was observed in the negative control group compared to the normal control. edemas reached their maximum sizes on the 6th day of the experiment after the second formaldehyde injection. In MESA (150 and 300 mg/kg) treated groups however, paw edema variation decreased significantly ($P < 0.05$) from the first day of the experiment. From the 5th to the 10th day, MESA (150 and 300 mg/kg) reduced ($P < 0.001$) paw edema variation compared to the negative control.

Diclofenac also reduced paw edema variation from the first day with a more significantly ($P < 0.01$ and $P < 0.001$) effect from the 5th day of the experiment compared to the negative control group.

Inhibition percentages calculated to evaluate the inhibitory effect of MESA (75, 150, and 300 mg/kg) and diclofenac (5 mg/kg) on mice paw edema are presented in Table 1."

MESA (150 and 300 mg/kg) exhibited significant inhibition of mice paw edema, ranging from 16.94% to 80.36%. The highest inhibition (80.36%) of paw edema variation in animals at the end of the experiment was observed in the group treated with 300 mg/kg extract dose (Table 1).

3.2 Evolution of Mice Body Weight during Formaldehyde-Induced Inflammation

Table 2 illustrates changes in body weight observed in treated mice throughout the experimental period (10 days). Starting from the 6th day, a significant decrease ($P < 0.01$) in body weight was noted among mice in the negative control group compared to the normal control. This decline in body weight persisted until the 10th day in this group. Conversely, groups treated with diclofenac and MESA showed improvement in weight loss during the latest stages of the experiment.

3.3 Effect of MESA on Hematological Parameters during Formaldehyde-Induced Inflammation

Table 3 shows the effect of different treatments on hematological parameters 10 days after inflammation induction by formaldehyde. A significant increase in white blood cell count ($P <$

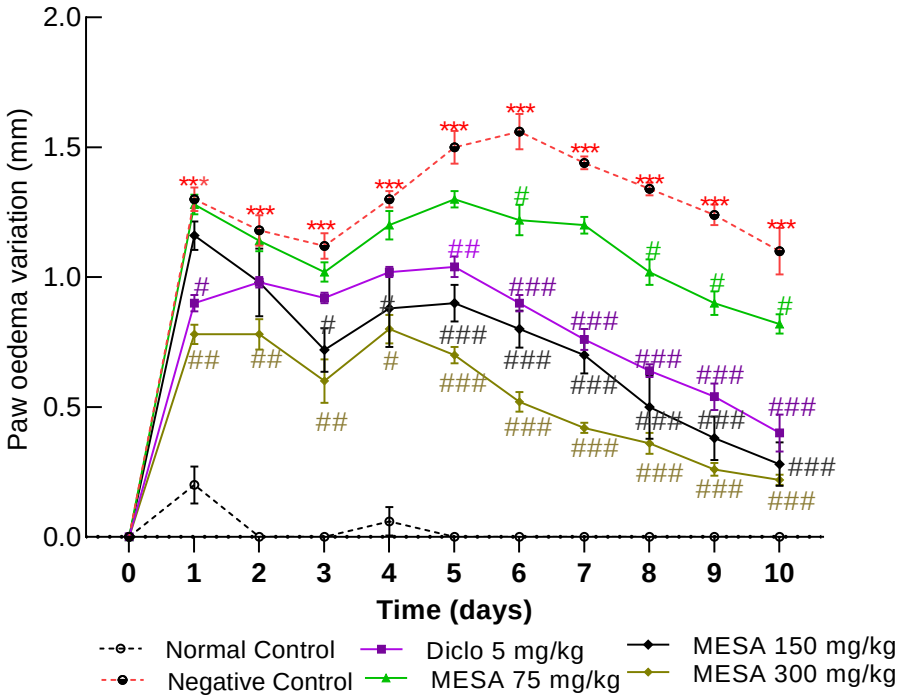

Fig. 1. Effect of MESA on the evolution of edema during formaldehyde-induced inflammation

*Values are presented as means ± SEM, (n = 5). Significant differences compared to control groups are indicated as follows: *** P < 0.001 compared to the normal control; # P < 0.05, ## P < 0.01, and ### P < 0.001 compared to the negative control. MESA: methanolic extract of S. aethiopicus; Diclo: diclofenac*

Table 1. Effect of MESA and diclofenac on the percentage inhibition of paw edema induced by formaldehyde in mice over 10 days

	Time (Days)				
Groups	**2**	**4**	**6**	**8**	**10**
Diclofenac 5 mg/kg	16.94%	21.53%	42.30%	52.30%	54.54%
MESA 75 mg/kg	6.77%	10.76%	30.76%	47.69%	49.81%
MESA 150 mg/kg	16.94%	32.30%	37.17%	63.15%	70.45%
MESA 300 mg/kg	33.89%	39.46%	62.82%	70.76%	80.36%

MESA: methanolic extract of S. aethioicus

0.001) and platelets ($P < 0.05$) in the negative control group compared to the normal group. Diclofenac (5 mg/kg) significantly decreased the white blood cell count ($P < 0.01$). MESA (75, 150, and 300 mg/kg) significantly reduced the white blood cell count ($P < 0.05$ and $P < 0.01$) compared to the negative control group. Treatment with diclofenac and MESA, maintained platelet count close to normal levels.

It is also noted that in arthritic mice (negative control), there were significantly reduced levels of red blood cells ($P < 0.001$), hemoglobin ($P < 0.01$), and hematocrit ($P < 0.05$) compared to animals in the normal control group. Treatment with diclofenac, MESA significantly increased ($P < 0.001$ – $P < 0.05$) the number of red blood cells and hemoglobin compared to the negative control group. This treatment maintained hematocrit levels close to normal.

3.4 Effect of MESA on Biochemical Markers (RF and CRP) during Formaldehyde-Induced Inflammation

Data presented in Table 4 shows that the concentration of CRP in the negative control group significantly increased compared to the

Table 2. Variation in body weight in formaldehyde induced arthritis in mice

	Period (Days)					
Groups	**0**	**2**	**4**	**6**	**8**	**10**
Normal Control	25.62 ± 2.20	25.9 ±2.23	26.52 ± 2.12	27.68 ± 2.03	28.22 ±1.78	28.62 ±1.89
Negative Control	25.96 ± 1.86	25.5 ± 1.65	24.84 ± 0.6	24.18 ± 0.57**	24.02 ± 0.87**	23.4 ± 0.78**
Diclofenac 5 mg/kg	26.04 ± 1.54	25.13 ± 1.65	24.76 ± 1.94	24.3 ± 1.72	24.74 ± 1.84	24.64 ± 1.68
MESA 75 mg/kg	26.7 ± 1.79	26.44 ± 1.76	25.84 ± 1.91	25.5 ± 2.13	24.98 ± 2.06	25.06 ± 1.96
MESA 150 mg/kg	26.02 ± 2.11	25.62 ± 1.73	25.34 ± 2.02	25.72 ± 1.92	26.37 ± 1.65$^{\#}$	26.84 ± 1.25$^{\#}$
MESA 300 mg/kg	26.5 ± 2.13	26.54 ± 2.15	26.56 ± 2.31	26.74 ± 1.75$^{\#}$	27.46 ± 1.77$^{\#\#}$	27.5 ± 1.72$^{\#\#}$

*Values are presented as means ± SEM, (n = 5). Significant differences compared to the control groups are presented as follows: ** P < 0.01 compared to the normal control; # P < 0.05, ## P < 0.01, and ### P < 0.001 compared to the negative control. MESA: methanolic extract of S. aethiopicus*

Table 3. Effect of MESA on hematological parameters during formaldehyde-induced inflammation

	Hematological parameters				
Groups	**WBC (x10^9/L)**	**RBC (x10^9/L)**	**HB (g/L)**	**HCT (%)**	**PLT (x10^9/L)**
Normal Control	7.32 ± 0.47	6.19 ± 0.42	130.54 ± 6.54	44.76 ± 4.33	413.75 ± 2.14
Negative Control	16.08 ± 1.03***	3.64 ± 0.28 ***	87.35 ± 4.25***	33.42 ± 2.44**	577.42 ± 19.12*
Diclofenac 5 mg/kg	12.14 ± 0.81$^{\#\#}$	4.27 ± 0.51	107.32 ± 4.94$^{\#}$	37.67 ± 2.27	483.36 ± 14.13
MESA 75 mg/kg	14.6 ± 1.04$^{\#}$	3.96 ± 0.28	97.87 ± 4.68	36.64 ± 1.92	505.61 ± 15.03
MESA 150 mg/kg	13.24 ± 0.86$^{\#}$	4.87 ± 0.35$^{\#}$	100.25 ± 5.38	38.64 ± 2.08	449.54 ± 13.58
MESA 300 mg/kg	10.73 ± 0.65$^{\#\#}$	5.68 ± 0.34$^{\#\#}$	125.46 ± 6.05$^{\#\#\#}$	44.98 ± 4.73	497.21 ± 18.38

*Values are presented as means ± SEM, (n = 5). Significant differences compared to the control groups are indicated as follows: * P < 0.05, ** P < 0.01, and *** P < 0.001 compared to the normal control; # P < 0.05, ## P < 0.01 and ### P < 0.001 compared to the negative control. MESA: methanolic extract of S. aethiopicus; WBC: white blood cells; PLT: platelets; RBC: red blood cells; HCT: hematocrit; HB: hemoglobin*

Table 4. Effect of MESA on CRP and RF during by formaldehyde-induced inflammation

	Biochimical parameters	
Groups	**CRP (U/L)**	**RF**
Normal Control	5.98 ± 0.78	Negative
Negative Control	24.06 ± 2.14 ***	Positive
Diclofenac 5 mg/kg	10.87 ± 2.4 ###	Negative
MESA 75 mg/kg	16.86 ± 2.93 ##	Positive
MESA 150 mg/kg	12.18 ± 1.86 ###	Negative
MESA 300 mg/kg	7.23 ± 1.45 ###	Negative

*CRP values are presented as means ± SEM, (n = 5). The significant difference compared to control groups is presented as follows: *** P < 0.001 compared to the normal control; ## P < 0.01 and ### P < 0.001 compared to the negative control. MESA: methanolic extract of S. aethiopicus, CRP: C-reactive protein; RF: rheumatoid factor*

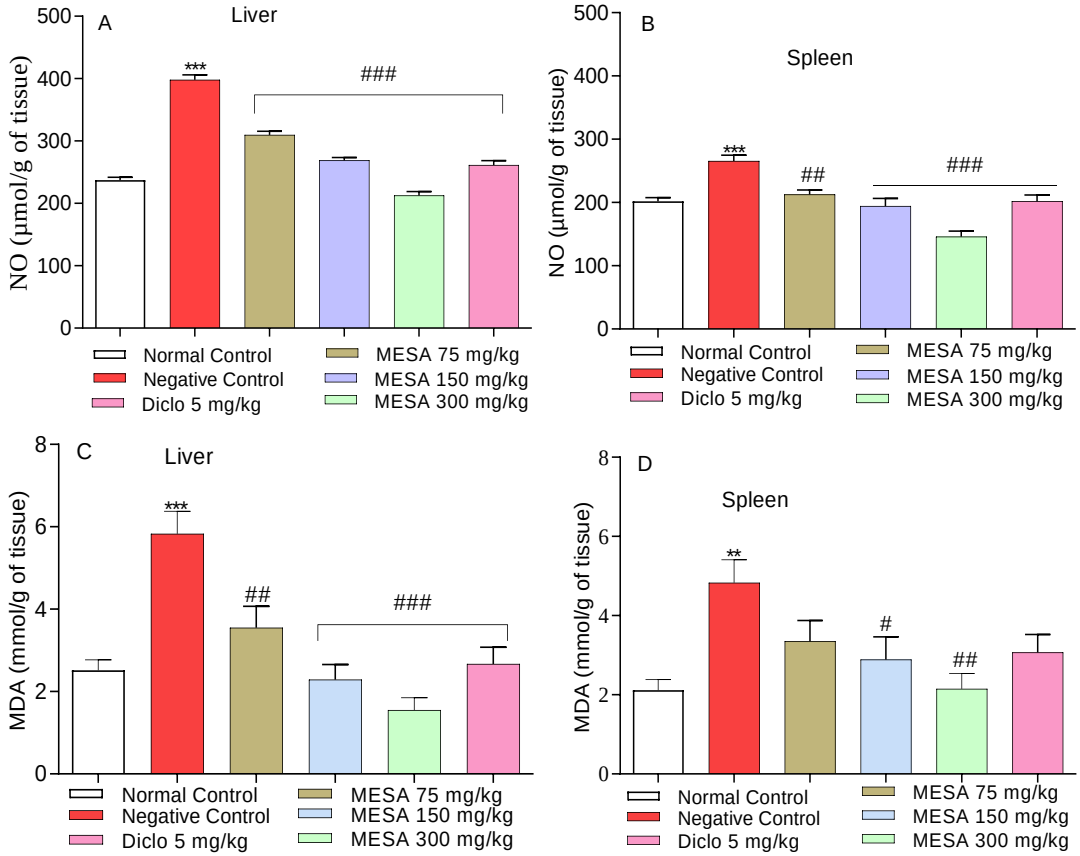

Fig. 2. Effect of MESA on hepatic (AC) and splenic (BD) nitric oxide and malondialdehyde levels in mice

*Values are presented as means ± SEM, (n = 5). The significant difference compared to the control groups is shown as follows: *** P < 0.001 compared to the Normal Control; ### P < 0.001 compared to the Negative Control. MESA: methanolic extract of S. aethiopicus; Diclo: diclofenac; NO: Nitric Oxide; MDA: malondialdehyde*

normal control group. Treatment of animals with MESA (75, 150, and 300 mg/kg) and diclofenac (5 mg/kg) significantly reduced (P < 0.01) CRP levels compared to the negative control group. Furthermore, the qualitative test for rheumatoid factor (RF) was correlated with plasma CRP levels. This test was positive in the negative control group, where CRP levels were elevated.

3.5 Effect of MESA on Nitric Oxide (NO) and Malondialdehyde (MDA) Levels during the Formaldehyde- Induced Inflammation

The effect of MESA on NO and MDA levels during formaldehyde-induced inflammation is shown in Fig. 2. A significant increase ($P < 0.001$) in NO and MDA concentration was observed in the liver (AC) and spleen (BD) homogenates of negative controls compared to the normal control. MESA (150 and 300 mg/kg), significantly reduced ($P < 0.001$; $P < 0.01$ respectively) NO and MDA levels in the liver and spleen homogenates compared to the negative control.

3.6 Effect of MESA on Superoxide Dismutase (SOD) and Catalase (CAT) Activity

Fig. 3 illustrates the effect of MESA on Superoxide Dismutase and Catalase activity in the liver (AC) and spleen (BD) homogenates. The SOD and CAT activity in the negative control group was significantly reduced ($P < 0.05$) in both liver and spleen homogenates compared to the normal control group. However, in groups treated with MESA (150 and 300 mg/kg), SOD and CAT activity significantly increased ($P < 0.001$ – $P < 0.05$) in homogenates of these organs.

3.7 Histological Examination of Mice Paw Bones

The cross-section histology of paw bones treated with different substances is depicted in Fig. 4. Paw samples from the untreated arthritic group exhibited a severe inflammatory response characterized by articular cartilage destruction and pronounced bone resorption. It is also evident that the histology of paws of MESA (75, 150, and 300 mg/kg) treated animals showed mild articular cartilage destruction and minimal bone resorption.

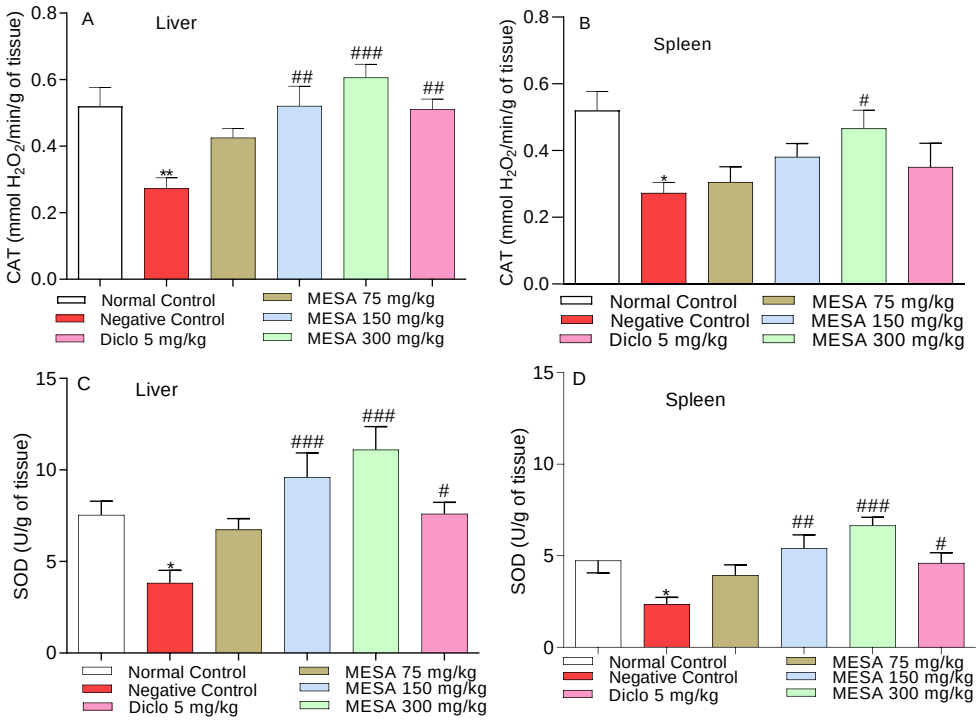

Fig. 3. Effect of MESA on hepatic (AC) and splenic (BD) superoxide dismutase (SOD) and catalase (CAT) activity in mice

*Values are represented as means ± SEM, (n = 5). Significant difference compared to the negative control group is presented as follows: *P < 0.05 compared to the Normal Control. # P < 0.05, ## P < 0.01, and ### P < 0.001 compared to the Negative Control. MESA: methanolic extract of S. aethiopicus*

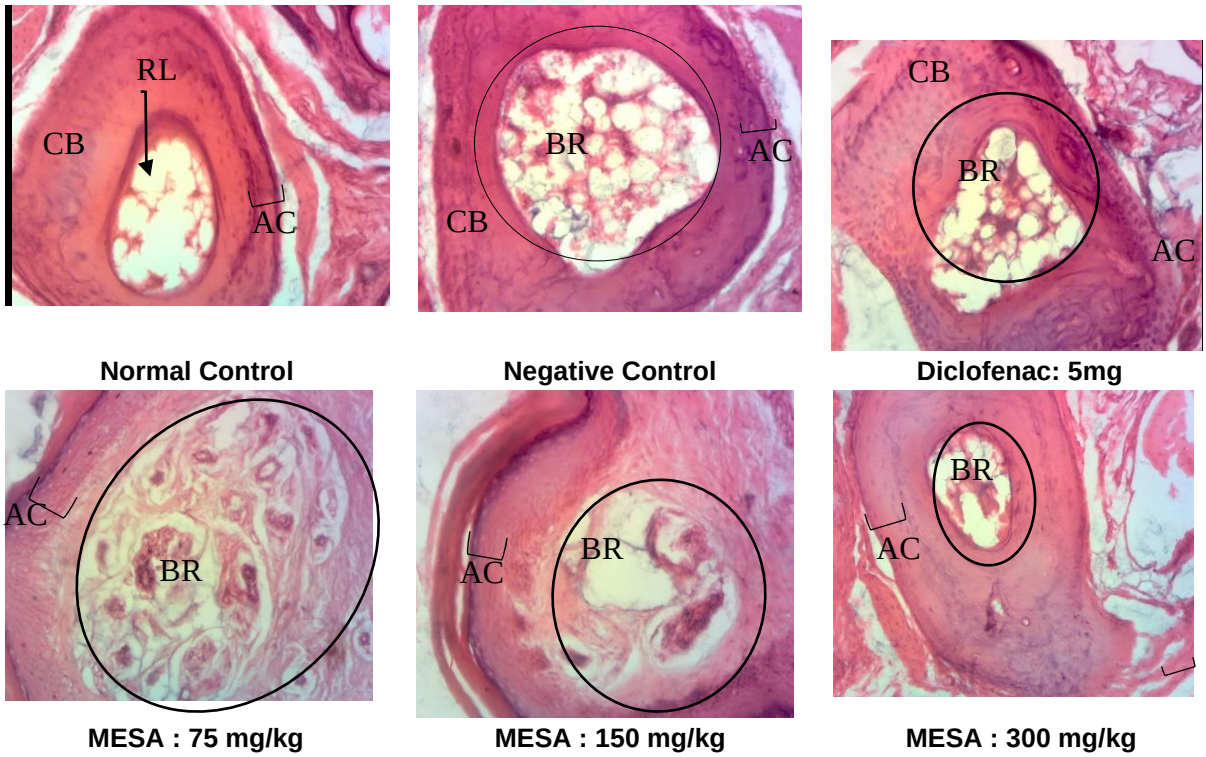

Fig. 4. Microphotographs (HE × 100) of the cross-sectional view of mouse paw bones treated with different substances for 10 days

BR = Bone resorption; RL = Resorption lacuna; AC = Articular cartilage; CB = Cortical bone. MESA: methanolic extract of S. aethiopicus

4. DISCUSSION

The present study aimed to evaluate the anti-inflammatory and antioxidant effects of the methanolic extracts of *S. aethiopicus*. The anti-inflammatory activity was assessed *in vivo* using the formaldehyde-induced paw inflammation mouse model. Subsequently, various biochemical, hematological, histological, and oxidative stress parameters were assessed.

Articular cartilage is composed of proteins [26]. Denaturation of these proteins leads to the development of inflammation [26]. Several agents, including substance like formaldehyde can destroy articular cartilage [26,27]. The arthritic effect produced by formaldehyde injection occurs in two different stages. In the first stage, neurogenic histamine, bradykinins, and serotonin are secreted, followed by initiation of a tissue-mediated phase, during which release of prostaglandin-like substances occurs.

It has been demonstrated that drugs acting on the central nervous system are also effective in both phases, but the effect of drugs on the late phase of the disease is unclear when these drugs act on the peripheral nervous system. Effective anti-rheumatic drugs should improve these phases [28]. After formaldehyde injection, prostaglandins are generated in the early phase, causing inflammation. Autoantibodies are generated in the later phase. Release of pro-inflammatory cytokines plays an important role in various complications of rheumatoid arthritis [29].

"Paw edema is associated with cellular inflammation in the affected area, increased fluid flow and vascular permeability" [30]. "Paw measurement is the simplest way to determine the level of disease, providing a general parameter for assessing the effectiveness of anti-arthritic drugs" [31]. "A decrease in the level of inflammatory intermediates directly reduces paw diameter and indicates the effectiveness of therapy" [32].

In the present study, MESA (150 and 300 mg/kg) significantly reduced paw edema induced by formaldehyde on days 2, 4, 6, 8, and 10. MESA thus demonstrated anti-inflammatory activity comparable to that of diclofenac, a well-known reference anti-inflammatory. These results are consistent with several studies showing that the

anti-inflammatory activity of plant extracts can be partly explained by the presence of bioactive molecules responsible for this effect.

Animals in the negative control group developed chronic paw edema with inflammatory cell infiltration, articular cartilage erosion, and bone destruction and remodeling similar to human rheumatoid arthritis [33]. In this study, we recorded a significant reduction in all parameters used to study arthritic activity. This reveals MESA protective effect against formaldehyde-induced damage. This protective effect could be due to its potential inhibition of protein denaturation with subsequent inhibition on the release of inflammatory mediators (histamine, serotonin, prostaglandin, etc.) responsible for inflammation [34] [35].

The result obtained in this test also suggests that the anti-inflammatory activity of MESA is largely related to the extract's effect on inflammatory cell infiltration and their mediators released at the inflammatory site. Therefore, cell migration and the production of some key inflammatory markers are verified in this study.

Complete blood count (CBC) was performed in this study to quantify the leukocyte count, mainly monocytes, neutrophils, and lymphocytes, in the blood of experimental animals. During formaldehyde-induced inflammation, monocytes and neutrophils are recruited from the blood to the site of disorder to fulfill their phagocytic functions [36]. Hematological analyses indicated a high level of white blood cells in untreated edematous animals compared to treated and normal control groups. The administered MESA could have reduced the leukocyte count (WBC) through polyphenols, flavonoids, and tannins contained in these extracts, known as potent inhibitors of leukocyte migration [37].

The increase in platelet count in the body is termed thrombocytosis. This condition is particularly associated with severe cases of rheumatoid arthritis. The elevation of CRP values in the diseased group animals leads to platelet activation, ultimately exacerbating rheumatoid arthritis [38,39].

Similarly, the analysis of hematological parameters indicated platelet levels in groups treated with MESA very close to those of the normal control group. This decrease could be attributed to the reduction in the production of inflammatory markers such as CRP and RF, as demonstrated in this study. In addition to their primary role in platelet aggregation during blood clotting, platelets also have the ability to recognize foreign agents and initiate or modulate inflammatory responses [40,41]. During inflammatory processes, released inflammatory mediators lead to increased platelet production [42]. Thus, MESA may act on these mediators by inhibiting their synthesis, explaining the significant difference in platelet levels between treated and untreated edematous animals.

The body weight of mice constitutes another clinical parameter monitored in this study. A significant ($P < 0.001$) loss of body weight was observed in untreated arthritic mice compared normal control group mice. This is likely due to reduced mobility of mice resulting from paw inflammation, limiting their locomotor ability to access food. On the other hand, the inflammatory response provoked by ACF (adjuvant complet de Freund) injection stimulates the production of leptin (satiety hormone), a cytokine-like hormone, leading to reduced food intake and consequently weight loss [43]. Similar results have been reported by some authors showing that weight loss often accompanies prolonged arthritis due to the systemic or local action of inflammatory cytokines such as TNF-α and IL-1β, primarily produced by monocytes and macrophages, which stimulate muscle degeneration [44,45]. Furthermore, a significant ($P < 0.01$) weight gain was observed in mice treated with diclofenac (3 mg/kg) or MESA (75, 150, and 300 mg/kg). This is likely linked to their anti-inflammatory properties, thus reversing the effects of arthritis through bioactive molecules, namely polyphenols.

The reduction in oxidative stress by MESA could be part of the main mechanisms for the observed anti-inflammatory activity in this study (in arthritic animals). Phytochemical analysis has shown that the extract contains high concentration of total phenolic compounds, tannins, and flavonoids. These compounds are known to possess antioxidant and anti-inflammatory capacities [46]. Plant polyphenols and flavonoids, due to their high hydroxyl group content, scavenge reactive oxygen species (ROS) to reduce oxidative stress [47]. In formaldehyde-induced RA in mice, the level of oxidative stress was significantly reduced in polyarthritic mice treated with MESA. Conversely, increased lipid peroxidation represented by elevated MDA levels was observed in negative control mice, correlated with low activity of antioxidant enzymes such as SOD and CAT.

A previous study [48] reported that inflammatory reactions lead to overproduction of reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), resulting in oxidative stress [49], which can damage DNA, proteins, and lipids [49]. In the body, superoxide dismutase (SOD) prevents the accumulation of O_2^- and converts it into H_2O_2, which is then converted into H_2O and O_2 by catalase [50].

Analysis of oxidative stress parameters shows a decrease in lipid peroxidation concomitant with an increase in enzymatic activity of SOD, catalase, and glutathione in tissue homogenates, thus reflecting the antioxidant effect of this plant. The anti-inflammatory activity of this plant is believed to be partially due to modulation of oxidative stress. Diclofenac (5 mg/kg) and MESA restored ($P < 0.001$) the levels of SOD, CAT, NO, and MDA in treated arthritic animals compared to negative controls.

Inducible nitric oxide synthase (iNOS) in monocytes and macrophages converts L-arginine into nitric oxide (NO) during the inflammatory process [51]. Overabundance of NO reacts with superoxide anions to generate peroxynitrite, which converts lipids, proteins, and nucleic acids to nitrate, changing their structure and function [5]. These findings suggest that MESA could prevent damage to certain biomolecules caused by chronic degenerative diseases. Such damage have been histologically demonstrated by tissue lesions in rheumatoid arthritis (RA). Histological examination of paws in arthritic animals revealed leukocyte infiltration. Treatment with MESA protected against arthritis damage induced by formaldehyde.

Furthermore, the increase in SOD activity could indicate an increase in O_2^- production. Treatment with MESA significantly decreased MDA concentrations in the liver and spleen homogenates. The observed increase in MDA levels in untreated arthritic animals contributes to increased free radical generation and decreased antioxidant defense capacity of the immune system, leading to the observed decrease in SOD and CAT [52].

A previous study confirmed this finding that antioxidants such as CAT and SOD were significantly reduced in arthritic animals compared to control animals. These levels of antioxidant enzymes were restored to values closer to their normal levels in animals receiving *S. anacardium* nut extract" [53]. It should be noted that oxidative stress negatively affects gene transcription [54], and some studies report that during inflammation, inflammatory cytokines and reactive oxygen species produced prompt immune cells to release enzymes and mediators that exacerbate RA [35]. Thus, it could be postulated that attenuation of oxidative stress by MESA may be one of the primary mechanisms for suppressing gene expression of cytokines involved in inflammation in RA.

Therefore, the inhibition of free radical scavenging, protein denaturation inhibition, reduction of paw edema, improvement in hematological and biochemical variables, and histopathological examination sustain the antiarthritic potential of MESA, which may be attributed to its polyphenolic constituents [52].

5. CONCLUSION

The main objective of this study was to evaluate the antioxidant and anti-inflammatory properties of the methanolic extract of *S. aethiopicus* in the formaldehyde-induced inflammation model. From this work it appears that treatment with MESA reduces edema, stabilizes and improves hematological and biochemical parameters, prevents pannus formation, and cartilage and bone lesions during this study. Moreover, it enhances the body's antioxidant capacities and inhibits lipid peroxidation. Thus, the present study suggests that the methanolic extract of *S. aethiopicus* could be used for the treatment of rheumatoid arthritis; however, further studies will be necessary to prove its effectiveness and precise its anti-arthritic mechanism of action.

ETHICAL APPROVAL

All experiments were carried following the guidelines of the Cameroonian Bioethics Committee (reg N° FWAIRB00001954) and in accordance with NIH-Care and Use of Laboratory Animals (8th edition).

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COMPETING INTERESTS

Authors declare that no competing interests exist.

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