Original ResearchArticle

The Effect of the Methanolic Extract of *Siphonochilusaethiopicus* on Biochemical, Hematological Parameters, and Oxidative Stress in the Experimentally Induced Arthritis Model in Mice.

ABSTRACT

Background *: Siphonochilusaethiopicus*belongs to the gingerfamily, Zingiberaceae. In Chinesemedicine, Zingiberaceae are acknowledged for their anti-inflammatory and antioxidantproperties, whichcontribute to immune strengthening. Theypromote digestion, alleviatenausea and motion sickness, and also help in combatingflu and colds. The currentstudywasconductedtoinvestigate the antioxidant and anti-inflammatoryproperties of the methanolicextract of *Siphonochilusaethiopicus* (MESA).

Methodology : The *in vivo*studyexamined the anti-inflammatory impact of the extract at doses of 75, 150, and 300 mg/kg using a formaldehyde-induced inflammation model in mice. The anti-arthriticeffect of the methanolicextract of Siphonochilusaethiopicus (MESA) wasdetermined by pawdiameter, body weight, biochemicalparameters (C-reactiveprotein and rheumatoid factor), oxidative stress (malondialdehyde, superoxide dismutase, catalase, and nitric oxide), and hematologicalparameters. Additionally, a histopathologicalexaminationwasconducted to evaluate the outcomes.

Results *:*The resultsshowedthat the methanolicextract of *Siphonochilusaethiopicus* (MESA) significantlydecreasedpaw inflammation ($P< 0.05$ to $P< 0.001$) and enhancedhematological and biochemicalparameters. Additionally, MESA significantlyamelioratedoxidative stress parameters ($P< 0.05$ to $P < 0.001$).

Conclusion*:*The results of the studysuggestthat*Siphonochilusaethiopicus*possessesstrongantioxidant and anti-arthriticactivity, supportingitsconventional application as a remedy for rheumatoidarthritis."

Key words: Antioxidant activities, Anti-arthritic, Anti-inflammatory, *Siphonochilusaethiopicus*

1. INTRODUCTION

Inflammation is a response of the immune system to variousaggressions and isimplicated in a wide range of human pathologies such as diabetes, cardiovasculardiseases, includingarthritis[1][2]. Rheumatoidarthritisis the mostcommoninflammatoryrheumatismthat affects the synovial membrane [3]. Formalhistopathological changes observed in patients withrheumatoidarthritisinclude persistent inflammation and cellproliferation in the synovial membrane of the joint, infiltration of variousinflammatorycells, pannus formation, cartilage and bone tissue destruction, leading to eventual joint deformation and loss of function[3]. The pathologyiscurrentlyexperiencing a global resurgence, affecting 2% to 4% of the general population [4]. The main eventsthatoccurduring the inflammatoryreactionlikelyinvolve the imbalance of nitric oxide, lipidperoxidation, cytokine release, and formation of reactiveoxygenspeciesderivedfromneutrophils[5]. Free radical instabilityisfundamentally the result of electronlossleading to increasedreactivity and electron stripping fromothermolecules, initiating a dangerouschainreactionknown as "free radical damage" [6][5]. The main targets of these free radicals are proteins, lipids, and DNA/RNA, and all these modifications in different and multiple molecules can increase the chances of mutagenesis. In fact, overproduction of ROS over an extendedperiod can cause severe damage to cellular structure and function[7]. Hence, rapidremovalismandatory. Free radicals are important mediatorsthatinitiateinflammatoryprocesses, and therefore, theirneutralization by antioxidants and radical scavengers can attenuate inflammation. To minimize damage caused by free radicals, the body employsseveral enzymes such as superoxide dismutase and catalase and cofactorssuch as glutathione[6]. However, the endogenousphysiologicalresponseestablished by antioxidant enzymes may not besufficient to limit ROS production [6][8].

In fact, the primarytreatmentinvolves the use of a combination of non-steroidal anti-inflammatorydrugs, glucocorticosteroids, disease-modifyingantirheumaticdrugs, and biologicaltherapiessuch as rituximab and infliximab [9]. This therapeuticapproachislengthy and focuses on inhibiting the main mediators of the chronic inflammation process to regulate or reducetheirdegenerative impact on joint tissues [10]. Althoughthesemedications are effective, their long-term use in treatingchronic inflammation isassociatedwithundesirablesideeffects. These adverse effectsinclude gastrointestinal lesions, skin rashes, allergicreactions, hepatitis, renaltoxicity and evencardiac complications [11].

Phytotherapyisbecomingincreasinglypopular in the modern world to addressdisease progression and extra-articular manifestations of rheumatoidarthritis due to

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cardiovascularcomplications, mooddisorders, gastrointestinal and respiratorydisorders, immunodeficiency, and blooddisordersassociatedwithvariousavailablerheumatoidarthritistherapies[12]. Among the plants used in the treatment of inflammatorydiseases in Cameroon, *Siphonochilusaethiopicus*stands out.

*Siphonochilusaethiopicus*istraditionallyused in the treatment of cough, cold, asthma, headache, pain, inflammation, and malaria [13]. Itsantibacterial[14], antimalarial[15], and anti-candida [16]properties have been studied in rhizome extracts. A few bioactive compounds wereisolatedfrom the rhizome of *S. aethiopicus*, includingtwofuranoterpenoids, whichaccounted for 20% of the oil composition of the extract[17].

The currentstudyaims to investigate the antioxidant and anti-inflammatoryproperties of methanolicextract of *Siphonochilusaethiopicus*in miceusing the formaldehyde-induced inflammation model.

2. MATERIALS AND METHODS

2.1.Plant Material and Extract Preparation

The roots of *G. kaussiana* were collected in Tokombere subdivision (Far-North region of Cameroon: longitude E 14°08'35"; latitude N 10°52'18"; altitude 746 m) after the approval of the research project by the University of Maroua (Faculty of Science).The fresh material was firstly transported in polystyrene bags to the Laboratory of Biochemistry and Biological Chemistry of the Faculty of Science of the University of Maroua and identified by Professor Tchopsala, botanist in the same University. A voucher specimen was deposited at the Cameroon National Herbarium and compared to specimen N° 45836/HEFG. Then, the roots were washed three times with tap water and dried at room temperature (35 ± 3 °C). The dried materials were grinded. The powder was sieved (0.5 mm) and kept until extraction.
The extraction was achieved by macerating of 300 g of powder in 3000 mL of methanol (80%) for 72 hours under stirring at room temperature [18]. The mixture was filtered using Wathman 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

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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, Animal Allocation, and Treatment

The animalsweredividedinto 6 groups of 5 animalseach and treateddaily for tendays as follows:

- Two control groups (normal and negative) treatedwithdistilled water (10 mL/kg, *p.o*) each;
- One positive control group treatedwith the referencedrug:diclofenac 5 mg/kg, *p.o*;
- Three groups treatedwith MESA at doses of 75, 150, and 300 mg/kg, *p.o*respectively.

One hourafterthesevarioustreatments, a volume of 0.04 mL of a 1.4% formaldehyde solution wasinjectedunder the plantaraponeurosis of the lefthindpaw of the animals in all groups except the normal control group, whichreceived 0.04 mL of distilled water by the same route. This formaldehyde injection wasrepeated on the thirdday of the experiment[19].

2.4.Evaluation of PawEdema and Animal Weight

The evolution of edemawasmonitored by measuring the diameter of the edematouspaw (mm) of each animal everydaythroughout the experimentperiodusing a vernier caliper. The evolution of edema in the different groups wasdeterminedusing the followingformula:

$$\Delta E = Edx - Ed0$$

ΔE = difference in edemabetweenday-x and day-0; Ed0= initial thickness (mm) of the left leg (beforeformaldehyde injection); Edx = thickness of the left leg (mm) on day "d" afterformaldehyde injection.

The percentage of inhibition "% Inh" wascalculated for each group of treatedmicecomparedwith the negative group.

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

ΔEt = difference in edemabetween day-0 and day-x of the leftpaw of the treatedmouse; ΔEC = represents the difference in edemabetweenday-0 and day-x of the leftpaw of the negative control group.

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2.5.Blood and Organ Sampling

On the 10th day, after the final measurement of edema and weight, the animalsweresacrificedafteranaesthetizing by intraperitoneal injection of thiopental at the dose of 50 mg/kg. Blood wasimmediatelycollectedintoheparin tubes and used to evaluatehematologicaland biochimicalparameters. Liver and spleen sampleswerecollected, homogenized in Tris-HCl buffer (50 mM; pH 7.4), centrifuged at 3000 rpm for 15 minutes, stored in the freezer and subsequentlyused for the assay of malondialdehyde, superoxide dismutase, catalase and nitric oxide.

2.6.Assessment of Hematological and BiochemicalParameters

Hematologicalparameterswereconductedusing a MINDRAY brand multiparametricautomatedanalyzer (BC-2800). The parameters of interest in ourstudyincludedredbloodcell count, white bloodcell count, hemoglobinlevel, hematocritlevel and platelet count.

CRP levelsweremeasuredusing an immunoturbidimetricassay in the medicallaboratoryfollowingthe protocoloutlined by Pepys [20].

Qualitative testing for rheumatoid factor wasperformedaccording to the methoddescribed by Young [21].

2.7.Superoxide Dismutase (SOD) Assay

Superoxide dismutase (SOD) activitywasevaluatedusing the methoddescried by Misra and Fridovish[22]. In a test tube, 134 µL of the homogenatewas mixed with 1666 µL of carbonate buffer (0.05 M, pH = 10.2). The reactionwasinitiated by adding 0.2 mL of freshlypreparedadrenaline (0.3 mM). This mixture washomogenized 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 wasrecordedbetween 20 and 80 seconds at 480 nm. The specificactivity of SOD wasevaluated in SOD units per milligram of protein.

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

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

2.8.Catalase (CAT) Assay

Catalase activitywasevaluatedaccording to the methoddescribed by Sinha [23]. 50 µL of sample and 50 µL of distilled water wereintroducedinto the test tubes and the blank tube, respectively. Subsequently, 750 µL of a phosphate buffer solution (0.1 mM; pH 7.5) and 200 µL of hydrogenperoxide (50 mM) wereadded to all tubes at room temperature for one minute, and the reactionwasstopped by adding 2 mL of potassium dichromate (5%) preparedin 1% aceticacid. The tubes werethenincubated for 10 minutes in a boiling water bath and cooledwithtap water. Optical densitieswereread at 570 nm. The specificactivity of catalase wasdeterminedfrom a previouslyestablished standard curveaccording to the equation:

$y = ax + b.$

The specificactivity of catalase isexpressed in µM of H_2O_2/min/mg of tissu.

2.9.Measurement of Malondialdehyde (MDA) Content:

Briefly, 125 µL of 20% trichloroaceticacid and 250 µL of 0.67% thiobarbituricacidwereadded to the test tubes containing 250 µL of the sample, and to the blank tube containing 250 µL of Tris-HCl buffer (50 mM; pH 7.4). All tubes weresealedwith glass beads, heated at 90°C in a water bath for 10 minutes, thencooled and centrifuged at 3000 rpm at room temperature for 15 minutes. The opticaldensities of the supernatantsfrom the test tubes wereread at 532 nm against the blank[24].

The concentration of MDA wasdeterminedusing the formula below. The MDA levelwasexpressed in mmol/g of tissu.

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

ΔAbs : Change in absorbance; ε : Molar extinction coefficient (15600 mol^{-1}) ; L : Path length(1cm) et q : gramme of orgn in the sample.

2.10. Determination of Nitric Oxide (NO) Concentration:

In test tubes, 100 µL of eachsample (homogenate) wasdilutedin 400 µL of distilled water. 5 mL of distilled water wereadded to the test tubes and to the blank tube, respectively. Then,

500 µL of Griessreagentwasadded to each tube. The mixture washomogenized and incubated at room temperature, protectedfrom light, for 10 minutes, and the absorbance wasreadagainst the blank at 546 nm [25]. The concentration of nitric oxide wasexpressed in µmol/g of tissue. The NO concentration wascalculatedusing the followingformula:

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

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

2.11. HistologicalStudy

At the end of the experiment, the hindpaws of the micewereremoved and fixedin 10% formalin. Decalcificationwasperformedusing a 3% nitricacid solution at room temperature, and the decalcification solution wasreplacedevery 48 hours for a total of 12 times. Subsequently, the samplesweredehydrated in alcohol (50, 75, 85, 95, and 100% ethanol), embedded in paraffin, sectioned (thickness, 5 µm), and stainedwithhematoxylin (8 min) and eosin (1 min) at room temperature for morphological observation under an optical microscope. Images werecapturedusing an Olympus CKX3 optical microscope (magnification, x 100; six fields; Olympus Corporation).

2.12. Statistical Analysis

Resultswereexpressed as mean ± standard deviation of five animals per group. Data wereanalyzedusing one-wayanalysis of variance (ANOVA) performed by GraphPadPrism 8.01. Multiple comparisonswereachievedusingDunnett's tests and significancewasconsidered at $P< 0.05$.

3. ESULTSAND DISCUSSION

3.1.Results

3.1.1. Effect of MEGK on micepawedema

Figure 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. This edema reached its maximum on the 6th day of the experiment after the second formaldehyde injection. In the various groups treated with MESA at doses of 150 and 300 mg/kg, paw edema variation decreased significantly (*P*<

0.05) from the first day of the experiment. From the 5th to the 10th day, MESA at doses of 150 and 300 mg reduced (P< 0.001) paw edema variation compared to the negative control.

Compared to the negative control group, diclofenac also reduced paw edema variation from the first day, but more significantly (P< 0.01 and P< 0.001) from the 5th day of the experiment.

The percentages of inhibition calculated to assess the inhibitory effect of MESA (75, 150, and 300 mg/kg) and diclofenac (5 mg/kg) on mouse paw edema are presented in Table I."

MESA at doses of 150 and 300 mg/kg exhibited significant inhibition of mouse 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 (Table I).

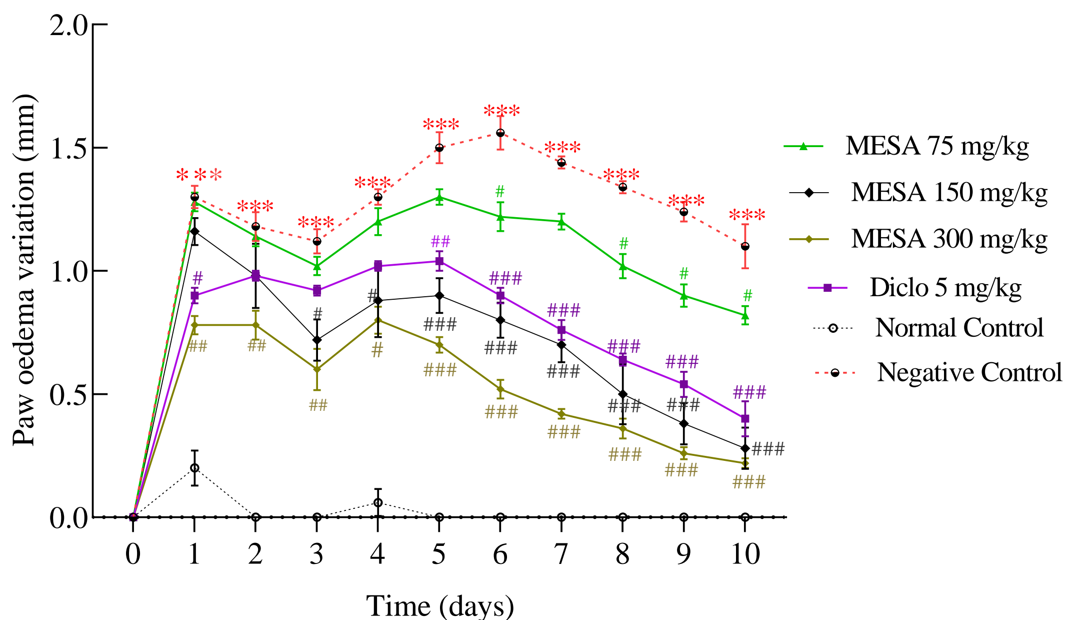

Figure 1:Effect of MESA on the Evolution of EdemaduringFormaldehyde-Induced Inflammation

Values are presented as means ± SEM, (n = 5). Significantdifferencescompared 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:MethanolicExtract of *S. aethioicus*; Dilco: Diclofenac.

Table 1:Effect of MESA and diclofenac on the percentage inhibition of pawedemainduced 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 : MethanolicExtract of *S. aethioicus*;

3.1.2. Evolution of mice body weightduringformaldehyde-induced inflammation

Table 2illustrates the changes in body weightobserved in treatedmice over the course of the experimentalperiod (10 days). Startingfrom the 6thday, a significantdecrease (*P*< 0.01) in body weightwasnotedamongmice in the negative control group compared to the normal control. This decline in body weightpersisteduntil the 10thday in this group. Conversely, in groups treatedwithdiclofenac and MESA, therewas an improvement in weightlossduring the later stages of the experiment.

Table 2: Changes in The Body Weight in FormaldehydeInducedArthritis 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##

The values are presented as means ± SEM, (n = 5). Significantdifferencescompared 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:MethanolicExtract of *S. aethioicus*.

3.1.3. Effect of MESA on HematologicalParametersduringFormaldehyde-Induced Inflammation

Table 3 shows the effect of differenttreatments on hematologicalparameters 10 daysafter inflammation induction by formaldehyde. It demonstrates a significantincrease in white bloodcell count (*P*< 0.001) and platelets (*P*< 0.05) in the negative control group compared to the normal group. Diclofenac (5 mg/kg) significantlydecreased the white bloodcell count (*P*<

0.01). MESA (75, 150, and 300 mg/kg) significantlyreduced the white bloodcell count (*P*< 0.05 and *P*< 0.01) compared to the negative control group. Treatmentwithdiclofenacand MESA,maintainedplatelet count close to normal levels.

It isalsonotedthat in arthriticmice (negative control), therewere significantlyreducedlevels of redbloodcells (*P*< 0.001), hemoglobin (*P*< 0.01), and hematocrit (*P*< 0.05) compared to animals in the normal control group. Treatmentwithdiclofenac, MESAsignificantlyincreased (*P*< 0.001 –*P*< 0.05) the levels of redbloodcells and hemoglobincompared to animals in the negative control group. This treatmentmaintainedhematocritlevels close to normal.

Table 3 : Effect of MESA on hematologicalparametersduringformaldehyde-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	$16.08 \pm 1.03^{***}$	$3.64 \pm 0.28^{***}$	$87.35 \pm 4.25^{***}$	$33.42 \pm 2.44^{**}$	$577.42 \pm 19.12^{*}$
Diclofenac5 mg/kg	$12.14 \pm 0.81^{\#\#}$	4.27 ± 0.51	$107.32 \pm 4.94^{\#}$	37.67 ± 2.27	483.36 ± 14.13
MESA75 mg/kg	$14.6 \pm 1.04^{\#}$	3.96 ± 0.28	97.87 ± 4.68	36.64 ± 1.92	505.61 ± 15.03
MESA150 mg/kg	$13.24 \pm 0.86^{\#}$	$4.87 \pm 0.35^{\#}$	100.25 ± 5.38	38.64 ± 2.08	449.54 ± 13.58
MESA300 mg/kg	$10.73 \pm 0.65^{\#\#}$	$5.68 \pm 0.34^{\#\#}$	$125.46 \pm 6.05^{\#\#\#}$	44.98 ± 4.73	497.21 ± 18.38

The values are presented as means ± SEM, (n = 5). Significantdifferencescompared 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:methanolicextract of *S. aethioicus*; WBC: white bloodcells; PLT: platelets; RBC: redbloodcells; HCT: hematocrit; HB: hemoglobin.

3.1.4. Effect of MESA on biochemical markers (FR and CRP) duringformaldehyde-induced inflammation

The data presented in Table 4showthat the concentration of CRP in the negative control group significantlyincreasedcompared to the normal control group. Treatment of animalswithMESA (75, 150, and 300 mg/kg) and diclofenac at 5 mg/kg significantlyreduced (*P*< 0.01) CRP levelscompared to the negative control group. Furthermore, the qualitative test for rheumatoid factor (RF) wascorrelatedwith plasma CRP levels. This test was positive in the negative control group, where CRP levelswereelevated.

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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	Négatif
Negative Control	24.06 ± 2.14 ***	Positif
Diclofenac 5 mg/kg	10.87 ± 2.4 ###	Négatif
MESA 75 mg/kg	16.86 ± 2.93 ##	Positif
MESA 150 mg/kg	12.18 ± 1.86 ###	Négatif
MESA 300 mg/kg	7.23 ± 1.45 ###	Négatif

CRP values are presented as means ± SEM, (n = 5). The significantdifferencecompared to control groups ispresented as follows: *** $P< 0.001$ compared to the normal control; ## $P < 0.01$ and ### $P < 0.001$ compared to the negative control. MESA:methanolicextract of . *S. aethiopicus*;; CRP: C-reactiveprotein; RF: rheumatoid factor.

3.1.5. **Effect of MESA on nitric oxide (NO)and malondialdehyde (MDA) levelsduringformaldehyde-induced inflammation**

Regarding the effect of MESA on NO and MDAlevelsduringformaldehyde-induced inflammation, as shown in Figure 2, a significantincrease ($P< 0.001$) in NO and MDAconcentration wasobserved in the liver (AC) and spleen (BD) homogenates of negativecontrolscompared to those of the normal control. MESA at doses of 150 and 300 mg/kg, significantlyreduced ($P< 0.001$- $P< 0.01$) this NO and MDAlevels in the liver and spleen homogenatescompared to the negative control.

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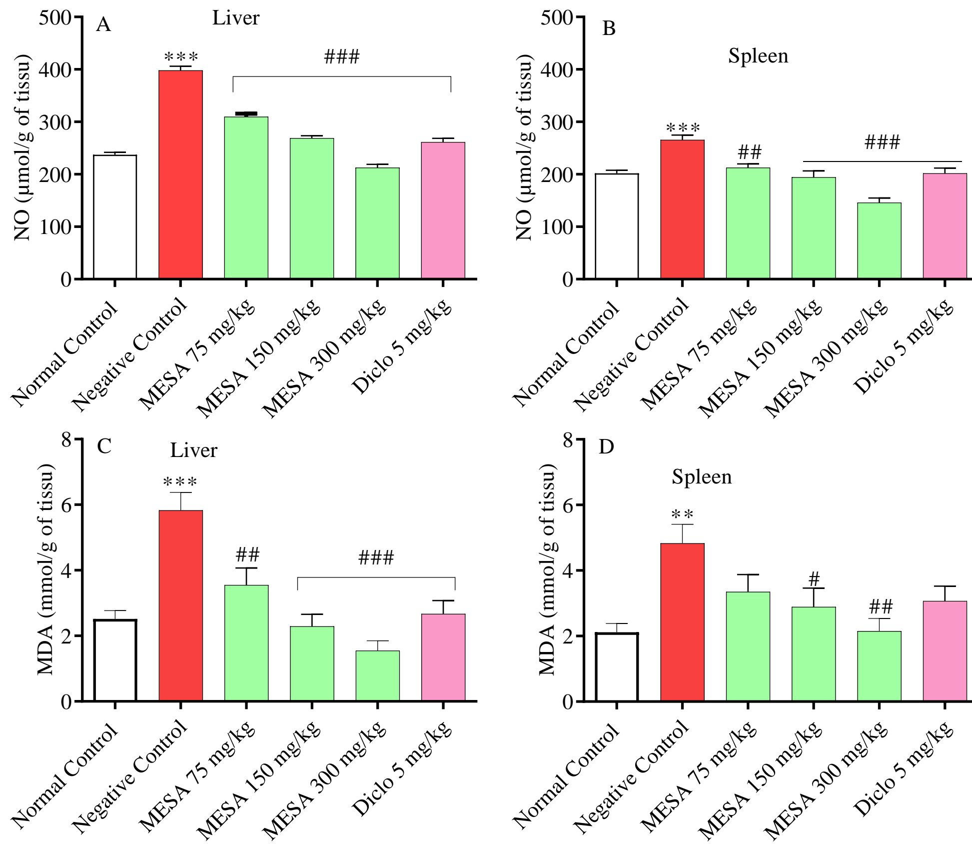

Figure 2:Effect of MESA on hepatic (AC) and splenic (BD) nitric oxide and malondialdehydelevels in mice

Values are presented as means ± SEM, (n = 5). The significantdifferencecompared to the control groups isshown as follows: *** *P*< 0.001 compared to the Normal Control; ### *P* < 0.001 compared to the Negative Control. EMSA:Methanolicextract of S. aethioicus; MESA: Methanolicextract of *S.aethiopicus*; Dilco: Diclofenac; NO: Nitric Oxide ; MDA :malondialdehyde.

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

Figure 3illustrates the effect of MESA on the activity of Superoxide Dismutase and Catalase in the liver(AC) and spleen (BD)homogenates. It isobservedthatcompared to the animals in the normal control group, the SOD and CAT activity in the negative control group wassignificantlyreduced (*P*< 0.05) in bothliver and spleen homogenates. However, in the groups treatedwith MESA at doses of 150 and 300 mg/kg, SOD and CAT activitysignificantlyincreased (*P*< 0.001 –*P* < 0.05) in the homogenates of theseorgans.

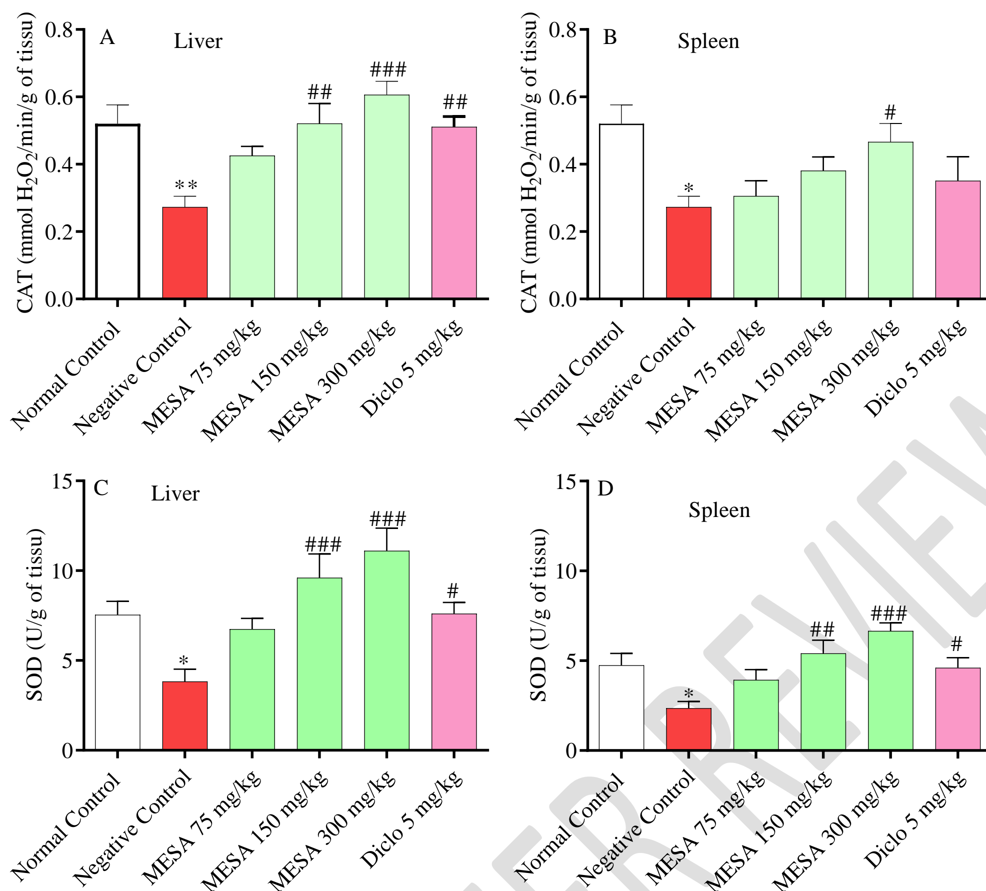

Figure 3:Effect of EMGK on Hepatic (AC) and Splenic (BD) Superoxide Dismutase (SOD) and Catalase (CAT) Activity in Mice

Values are represented as means ± SEM, (n = 5). Significantdifferencecompared to the negative control group ispresented 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:Methanolicextract of *S.aethiopicus*

3.1.7. HistologicalExamination of Mouse PawBones

The histology of cross-sectional views of mouse pawbonestreatedwithdifferent substances isdepicted in Figure 4. Pawsamplesfrom the untreatedarthritic group exhibited a severeinflammatoryresponsecharacterized by articular cartilage destruction and pronouncedboneresorption. It isalsoevidentthat the histology of pawsfromanimalstreatedwith MESA (75, 150, and 300 mg/kg) showedmildarticular cartilage destruction and minimal boneresorption."

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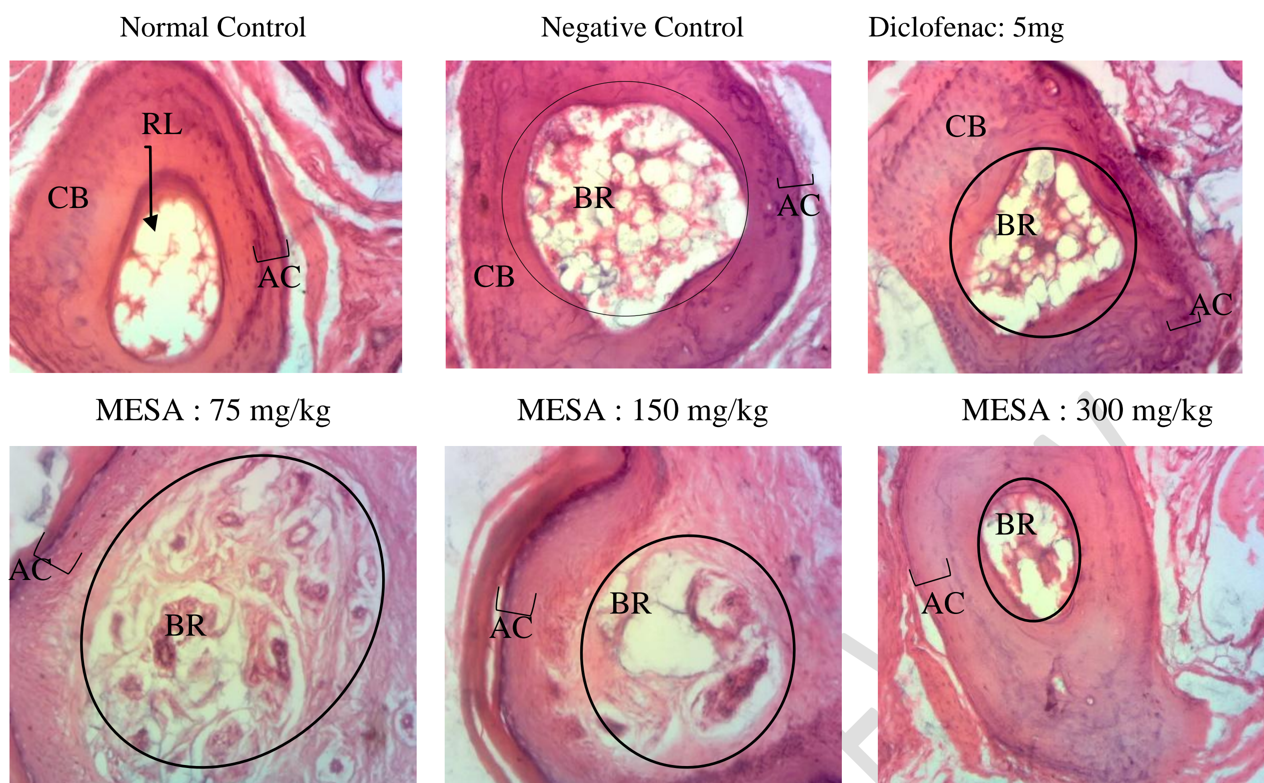

Figure 4:Microphotographs (HE × 100) of the cross-sectional view of mouse pawbonestreatedwithdifferent substances for 10 days

BR = Bone resorption; RL = Resorptionlacuna; AC = Articular cartilage; CB = Cortical bone. MESA:Methanolicextract of *S.aethiopicus*.

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4. DISCUSSION

The presentstudyaimed to evaluate the anti-inflammatory and antioxidanteffects of methanolicextracts of *S. aethiopicus*. The anti-inflammatoryactivitywasassessed in vivo using the formaldehyde-inducedpaw inflammation model. To achievethis objective, variousbiochemical, hematological, histological, and oxidative stress parameterswereexplored.

Articular cartilage iscomposed of proteins[26]. Denaturation of theseproteins leads to the development of inflammation [26]. Articular cartilage can bedestroyed by several agents, including substances like formaldehyde[26][27]. The arthriticeffectproduced by formaldehyde injection occurs in twodifferentstages: in the first stage, neurogenic histamine, bradykinins, and serotonin are secreted, followed by initiation of a tissue-mediated phase, duringwhich release of prostaglandin-like substances occurs.

It has been demonstratedthatdrugs acting on the central nervous system are also effective in both phases, but the effect of drugs on the late phase of the diseaseisunclearwhenthesedrugsact on the peripheralnervous system. Effective anti-rheumaticdrugsshouldimprovethese phases [28]. Afterformaldehyde 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 rheumatoidarthritis[29].

Pawedemaisassociatedwith cellular inflammation in the affected area, increasedfluid flow and vascularpermeability[30]. Pawmeasurementis the simplestway to determine the level of disease, providing a generalparameter for assessing the effectiveness of anti-arthriticdrugs[31]. A decrease in the level of inflammatoryintermediatesdirectlyreducespawdiameter and indicates the effectiveness of therapy[32].

In the presentstudy, MESA at doses of 150 and 300 mg/kg significantlyreducedpawedemainduced by formaldehyde on days 2, 4, 6, 8, and 10. Thus, MESAdemonstrated anti-inflammatoryactivity comparable to that of diclofenac, a well-knownreference anti-inflammatory. Theseresults are consistent withseveralstudiesshowingthat the anti-inflammatoryactivity of plant extracts can bepartlyexplained by the presence of bioactive moleculesresponsible for thiseffect.

Animals in the negative control group developedchronicpawedemawithinflammatorycell infiltration, articular cartilage erosion, and bone destruction and remodelingsimilar to humanrheumatoidarthritis[33]. In thisstudy, werecorded a significantreduction in all parametersused to studyarthriticactivity. Indeed, MESA has protective effectsagainstformaldehyde-induced damage. This protective effectcouldbe due to itspotential inhibition of proteindenaturationwithsubsequent inhibition of the release of inflammatorymediators (histamine, serotonin, prostaglandin, etc.) responsible for inflammation [34][35].

The resultsobtained in this test alsosuggestthat the anti-inflammatoryactivity of MESAislargelyrelated to the extract'seffect on inflammatorycell infiltration and theirmediatorsreleased at the inflammatory site. Therefore, cell migration and the production of some key inflammatory markers are verified in thisstudy.

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Complete blood count (CBC) wasperformed in thisstudy to quantify the leukocyte count, mainly monocytes, neutrophils, and lymphocytes, in the blood of experimentalanimals. Indeed, duringformaldehyde-induced inflammation, monocytes and neutrophils are recruitedfrom the blood to the site of disorder to fulfilltheirphagocyticfunctions[36]. Hematological analyses indicated a high level of white bloodcells in untreatededematousanimalscompared to treated and normal control groups. The administeredMESAwould have reduced the leukocyte count (WBC) throughpolyphenols, flavonoids, and tannins contained in theseextracts, known as potentinhibitors of leukocyte migration [37].

The increase in platelet count in the body istermedthrombocytosis. This condition isparticularlyassociatedwithsevere cases of rheumatoidarthritis. The elevation of CRP levels in animals in the diseased group leads to platelet activation, ultimatelyexacerbatingrheumatoidarthritis[38][39].

Similarly, the analysis of hematologicalparametersindicatedthat groups treatedwithMESAhadplateletlevelsvery close to those of the normal control group. This decreasecouldbeattributed to the reduction in the production of inflammatory markers such as CRP and RF, as demonstrated in thisstudy. Indeed, in addition to theirprimaryrole in plateletaggregationduringbloodclotting, plateletsalso have the ability to recognizeforeign agents and initiate or modulateinflammatoryresponses[40][41]. Duringinflammatoryprocesses, releasedinflammatorymediators lead to increasedplatelet production [42]. Thus, MESAmayact on thesemediators by inhibitingtheirsynthesis, explaining the significantdifference in plateletlevelsbetweentreated and untreatededematousanimals.

The body weight of miceconstitutesanotherclinicalparametermonitored in thisstudy. In thiswork, a significant ($P< 0.001$) loss of body weightwasobserved in untreatedarthritic rats compared to rats in the normal control group. This islikely due to reducedmobility of the rats due to paw inflammation, limitingtheiraccess to food. On the other hand, the inflammatoryresponseprovoked by ACF (adjuvant complet de Freund) injection stimulates the production of leptin (satiety hormone), a cytokine-like hormone, leading to reducedfoodintake and consequentlyweightloss[43]. Similarresults have been reported by someauthorsshowingthatweightlossoftenaccompaniesprolongedarthritis due to the systemic or local action of inflammatory cytokines such as TNF-α and IL-1β, primarilyproduced by monocytes and macrophages, whichstimulate muscle degeneration[44][45]. Furthermore, a

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significant (P< 0.01) weight gain wasobserved in rats treatedwithdiclofenac (3 mg/kg) or EMGK (25, 50, and 100 mg/kg). This islikelylinked to their anti-inflammatoryproperties, thusreversing the effects of arthritisthrough bioactive molecules, namelypolyphenols.

Meanwhile, it can beanticipatedthat a reduction in oxidative stress by MESAcouldbe part of the main mechanisms for the observed anti-inflammatoryactivity in thisstudy (in arthriticanimals). Phytochemicalanalysis has shownthat the extractscontainsignificantlevels of total phenolic compounds, tannins, and flavonoids .These compounds are known to possessantioxidant and anti-inflammatorycapacities[46]. Plant polyphenols and flavonoids, due to their high hydroxyl group content, scavengereactiveoxygenspecies (ROS) to reduceoxidative stress[47]. In formaldehyde-inducedRA in mice, the level of oxidative stress wassignificantlyreduced in polyarthritic rats treatedwith MESA. Conversely, increasedlipidperoxidationrepresented by elevated MDA levelswasobserved in negative control mice, correlatedwithlowactivity of antioxidant enzymes such as SOD and CAT.

A previousstudy[48]reportedthatinflammatoryreactions lead to overproduction of reactiveoxygenspecies (ROS) such as superoxide anions (O_2^-), hydrogenperoxide (H_2O_2), and hydroxylradicals (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 convertsitinto H_2O_2, whichisthenconvertedinto H_2O and O_2 by catalase [50].

Analysis of oxidative stress parameters shows a decrease in lipidperoxidation concomitant with an increase in enzymaticactivity of SOD, catalase, and glutathione in tissue homogenates, thusreflecting the antioxidanteffect of these plants. The anti-inflammatoryactivity of these plants isbelieved to bepartially due to modulation of oxidative stress. Diclofenac (5 mg/kg) and MESArestored (P< 0.001) the levels of SOD, CAT, NO, and MDA in treatedarthriticanimalscompared to negativecontrols.

During the inflammatory process, nitric oxide (NO) isproducedfromL-arginine via induciblenitric oxide synthase (iNOS) in monocytes and macrophages [51]. Excessive concentrations of NO combine withsuperoxide anions to formperoxynitrite, whichinduces nitration of nucleicacids, proteins, and lipids, therebydistortingtheir structure and impairingtheirfunction[5]. Thesefindingssuggestthat MESAcouldprevent damage to certain biomoleculescaused by chronicdegenerativediseases. Such damage has been histologicallydemonstrated by tissue lesions in rheumatoidarthritis (RA).

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Histologicalexamination of the paws in arthriticanimalsrevealedleukocyte infiltration. Treatmentwith MEGK protectedagainstarthritis damage induced by formaldehyde.

Furthermore, the increase in SOD activitycouldindicate an increase in O_2^- production. TreatmentwithMESAsignificantlydecreased MDA concentrations in the liver and spleen homogenates. The observedincrease in MDA levels in untreatedarthriticanimalscontributes to increased free radical generation and decreasedantioxidantdefensecapacity of the immune system, leading to the observeddecrease in SOD and CAT [52].

A previousstudyconfirmedourfindingsthatantioxidantssuch as CAT and SOD weresignificantlyreduced in arthriticanimalscompared to control animals. Theselevels of antioxidant enzymes wererestored to near-normal levels in animalsreceiving*S. anacardiumnut*extract[53]. It shouldbenotedthatoxidative stress negatively affects gene transcription[54], and somestudies report thatduring inflammation, inflammatory cytokines and reactiveoxygenspeciesproduced prompt immune cells to release enzymes and mediators to exacerbate RA [35]. Thus, itcouldbepostulatedthatattenuation of oxidative stress by MESAmaybe one of the primarymechanisms for suppressinggene expression of cytokines involved in inflammation in RA.

Therefore, the inhibition of free radical scavenging, proteindenaturation inhibition, reduction of pawedema, improvement in hematological and biochemical variables, and histopathologicalexamination support the antiarthriticpotential of MESA, whichmaybeattributed to itspolyphenolicconstituents.

CONCLUSION

The main objective of thisstudywas to evaluate the antioxidant and anti-inflammatoryproperties of the methanolicextract of *S. aethiopicus* in the formaldehyde-induced inflammation model. It emergesfromthisworkthattreatmentwithMESAreducesedema, stabilizes and improveshematological and biochemicalparameters, prevents pannus formation, and cartilage and bonelesionsduringthisstudy. Moreover, itenhances the body'santioxidantcapacities and inhibitslipidperoxidation. Thus, the presentstudysuggeststhat the methanolicextract of *S. aethiopicus*couldbeused for the treatment of rheumatoidarthritis;however, furtherstudieswillbenecessary to proveitseffectiveness and precisemechanism of action in arthritis.

ETHICAL APPROVAL

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All the experimentswerecarriedfollowing the guidelines of the CameroonianBioethicsCommittee (reg N° FWAIRB00001954) and in accordance with NIH-Care and Use of Laboratory Animals (8th edition).

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