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Antioxidant and anti-inflammatory properties of the methanolic extract of *Siphonochilus aethiopicus* rhizomes

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ABSTRACT

Introduction: *Siphonochilus aethiopicus* is a medicinal plant widely used in the treatment of many inflammatory conditions such as arthritis. The objective of this study was therefore to evaluate the antioxidant and anti-inflammatory properties of methanolic extract of *S. aethiopicus* rhizomes.

Methods: The total phenolic compounds, flavonoid, and tannin content, as well as the *in vitro* antioxidant activity of the extract, were estimated. The *in vivo* anti-inflammatory activity was then evaluated in male mice aged 3 to 4 months using the arthritic mouse model induced by carrageenan (0.05 ml; 1%) and monosodium urate (MSU) crystals (26.6 mg/mL). Mice were treated with the methanolic extract of *S. aethiopicus* (75, 150, 300 mg/kg) and the reference drugs: indomethacin (3 mg/kg) and colchicine (1 mg/kg). The serum, splenic, and hepatic lysosomal enzymes were determined, and oxidative stress biomarkers were estimated. Paws were sectioned for histological analysis.

Results: Results showed that *S. aethiopicus* extract had non-negligible concentrations of polyphenols, flavonoids, and tannins, which could confer it an antioxidant effect. Further, the methanolic extract of *S. aethiopicus* at different doses significantly ($P<0.05$) reduced paw swelling, attenuated joint inflammation, limited the release of lysosomal enzymes, and improved antioxidant enzymes.

Conclusion: The methanolic extract of *S. aethiopicus* has anti-inflammatory and antioxidant properties, and can be used to treat acute forms of gouty arthritis.

Implication for health policy/practice/research/medical education:
There is progressive increase in failure of conventional drugs in the management of health challenges including arthritis. This paper valorises *Siphonochilus aethiopicus,* an alternative phytomedicine endowed with anti inflammatory and antioxidant potentials.

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Introduction

Inflammation is an indispensable defence mechanism for the integrity of the body. It is implicated in a very large number of human pathologies such as arthritis (1). Rheumatoid arthritis is the most common inflammatory rheumatism that affects the synovial membrane, leads to degeneration of joint tissue, and later causes bone erosion. It affects 2% to 4% of individuals in the general population (2). As the disease progresses, patients present mobility disorders; finally, 15% to 20% of patients will require surgery within five years, giving a high impact on the quality of patients' lives (3). Rheumatoid arthritis is characterized by increased levels of immune cells, such as macrophages and lymphocytes in the synovial space, and a high concentration of free radicals, mainly reactive oxygen species, which could irreversibly affect

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joint tissue through the oxidation of its biomolecules, thus contributing to the progression of the disease (4).

Several drugs have been used as anti-arthritic. A combination of non-steroidal anti-inflammatory drugs, glucocorticosteroids, disease-modifying anti-rheumatic drugs, and biological therapies such as rituximab and infliximab (5) are mainly prescribed. However, this therapeutic approach is lengthy and focuses on inhibiting the main mediators of the chronic inflammation process in order to regulate them or reduce their degenerative impact on joint tissue (6). Although these drugs are effective, their use is associated with undesirable side effects, especially long-term administration in the treatment of chronic inflammation. These adverse effects include digestive tract damage (peptic ulcer, stenosis, perforation), fever, skin rashes, allergic reactions, hepatitis, renal toxicity, nephropathy and even cardiac complications (7). This, therefore, imposes the quest for a therapeutic alternative with little or no side effect. One of the most reliable sources of new compounds is medicinal plants. Approximately 80% of the African population uses medicinal plants in health care (8). Plants are often used as an alternative therapy to modern medicine due to the welter of properties they possess, including antiseptic, analgesic, and anti-inflammatory properties.

In Cameroon, *Siphonochilus aethiopicus* is traditionally used in the treatment of cough, cold, asthma, headache, pain, inflammation, and malaria (9-11). Its antibacterial (12), antimalarial (13), and anti-candida (10) properties have been studied in rhizome extracts. A few bioactive compounds were isolated from the rhizome of *S. aethiopicus,* including two furanoterpenoids, which accounted for 20% of the oil composition of the extract (14). To our knowledge, there is no scientific report on the anti-arthritic activity of *S. aethiopicus* rhizome. So, the present work was achieved to evaluate the effect of the hydromethanolic extract of *S. aethiopicus* on carrageenan and MSU-induced arthritis in mice.

Materials and Methods

Plant material

The rhizomes of *S. aethiopicus* were collected in Tokombéré subdivision (Far-North region of Cameroon: longitude E 14°08'35"; latitude N 10°52'18"; altitude 746 m). The fresh material was put in polystyrene bags before sending to the Laboratory of Biochemistry and Biological Chemistry of the Faculty of Science of the University of Maroua and identified by Professor Tchobsala (botanist in the same university). A voucher specimen was deposited at the Herbarium of the Garoua Wild Life School (Cameroon) to compare with a specimen recorded under number 45836/HEFG. Then, the rhizomes were washed three times with tap water and dried at room temperature (35 ± 3°C). The dried materials were ground. Powder was sieved (0.5 mm) and stored until extraction.

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° FWA-IRB00001954) and in accordance with *NIH-Care and Use of Laboratory Animals* (8th edition).

Extract preparation

Extraction was achieved by macerating 250 g of powder in 2500 mL of methanol (80%) for 72 hours under stirring at room temperature. The mixture was filtered using Whatman filter paper (N°1) and the resulting filtrate was collected. The solvent was evaporated from the filtrate at 64°C using rotavapor. The extract was stored at 4°C in a sealed sterile vial until use.

Acute toxicity test

The toxicity test was conducted according to the Organisation for Economic Co-operation and Development (OECD) guideline 423 (15). Three groups (n=3) of female Wistar rats weighing 150-200 g were used. Distilled water (10 mL/kg) was given to one control group; the other two groups respectively received high doses (2000 and 4000 mg/kg) of methanolic extract of *S. aethiopicus*. Behaviour and lethality of the rats were observed during the first four hours after administration of the extract, then on a daily basis for 14 days.

Phytochemical analysis

The total phenolic content of the methanolic extract was evaluated using Folin-Ciocalteu as described by Singleton et al (16). In a test tube containing 0.2 mL of the sample previously diluted in 80% methanol, 1 mL of Folin-Ciocalteu (10 %) was introduced. The mixture was stirred for 5 minutes. Then, 0.8 mL of sodium bicarbonate solution (7.5%) was added in the tube before incubating at room temperature for 30 minutes. The absorbance of the mixture was measured at 745 nm using UV-VIS spectrophotometer (PRIM Light & Advanced, Germany). Quantification of polyphenols was achieved using the standard curve of gallic acid (0-250 µg/mL) and the result was expressed as milligrams of gallic acid equivalent per 100 g of dry weight (mg GAE/100 g).

The method described by Mimica-Dukić et al (17) was used to determine the flavonoid content of the methanolic extract of *S. aethiopicus*. One milliliter of the extract and 1 mL of $AlCl_3$ reagent (130 mg of $AlCl_3$ and 400 mg sodium acetate in 100 mL of methanol) were mixed in a

tube and stirred for 5 minutes before adding two drops of concentrated acetic acid. The absorbance of the mixture was measured at 430 nm. The standard curve of optical density against quercetin concentration (0-100 µg/mL) was used to express the result as milligrams of quercetin equivalent per 100 grams of dry weight (mg QE/100 g).

Tannin content was evaluated as described by Bainbridge et al (18). 0.2 mL of the methanolic extract and 2 mL of vanillin (1 g vanillin/100 mL of HCl) were introduced, respectively, into a tube and incubated for 30 minutes in the dark place. The absorbance of the mixture was read at 500 nm. The calibration curve was established with catechin (0-50 µg/mL) and results were expressed as milligrams of catechin equivalent per 100 grams of dry weight (mgCaE/100 g).

Evaluation of the *in vitro* antioxidant activity of the methanolic extract

The ferric reducing antioxidant potential (FRAP) was determined as described by Benzie et al (19). Briefly, 1 mL of sample or standard (Trolox) solution was mixed with 3 mL of FRAP reagent (Fe (III)-2,4,6-Tri(2-pyridyl)-s-triazine). The mixture was shaken for 60 s and incubated for 40 minutes at room temperature. The absorbance was measured at 593 nm using UV-VIS spectrophotometer. The calibration curve was established with ascorbic acid (0-25 µg/mL), and the results were expressed as ascorbic acid milligram equivalent per 100 grams dry weight (mg AAE/100 g).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out according to the method described by Sun et al (20) with some modifications. One milliliter of sample or standard (Trolox) was mixed with 3 mL of 1mM DPPH. The mixture was stirred for 2 minutes and incubated for 40 minutes in a dark place at room temperature. Absorbance was measured at 517 nm using UV-VIS spectrophotometer. The calibration curve was established with Trolox (0-200 µM), and the results were expressed in Trolox millimole equivalent per 100 grams of dry weight (mmol TE/100 g).

Evaluation of the anti-inflammatory activity induced by Carrageenan

Six groups of five animals each were constituted with male mice weighing between 25 and 30 g. Three groups were treated with the methanolic extract of *S. aethiopicus* (MESA) (75, 150, and 300 mg/kg, p.o.). The positive control group received diclofenac sodium solution (10 mg/kg, p.o.) while the normal and negative control groups received 10 mL/kg of distilled water. After 1 hour of the various treatments, 0.05 mL of a freshly prepared 1% carrageenan suspension in saline (0.9 %) solution was injected into the sub plantar tissue of the left hind leg of all mice except the normal control group. The thickness of paws was measured every hour for 6 hours using a caliper (21).

Evaluation of the anti-inflammatory activity induced by MSU

Preparation of MSU

Monosodium urate (MSU) crystals were prepared according to Schorn et al (22) method with slight modifications. Briefly, 3 g of uric acid salt were dissolved in 600 mL of 0.03M NaOH. The mixture was incubated in a water bath for 2 hours, allowed to cool and the pH was adjusted at 7.5. It was then added 6 mL of 5M NaCl to the mixture and all was stirred for 24 hours at room temperature. The mixture was then centrifuged and the urate crystal were collected, washed and sterilized in a Pasteur furnace at 180°C for 2 hours.

Induction of inflammation process

Mice were randomly divided into seven groups of five animals each and treated daily for 3 days as follows: 2 control groups (normal and negative control) treated with physiological water (NaCl: 9 ‰); 2 positive control groups treated with indomethacin (3 mg/kg) and colchicine (1 mg/kg); 3 other groups were treated with methanolic extract of rhizomes from *S. aethiopicus* (75, 150, and 300 mg/kg). After 1 hour, an aliquot (0.15 mL) of MSU (26.6 mg/mL) was injected in the intra-articular region of the left paw for all the groups except the normal control, which received just 0.15 mL of normal saline (23). The diameter of paw was measured at 0, 4, 24, 48, and 72 hours after oedema induction. The evolution of the rectal temperature (°C) was also registered using a thermometer (CHICCO) and the paw thickness was measured using an electronic calliper. At the end of the experiment, the mice were sacrificed after anaesthetizing by intraperitoneal injection of thiopental at the dose of 50 mg/kg. The blood was recovered in a heparin tube, centrifuged at 3000 g for 15 minutes, and the serum was collected in microtubes for lysosomal enzymes analysis. The liver and spleen were isolated and homogenized in Tris buffer (50mM; pH 7.4) and the slurry was centrifuged. The supernatant was used for the evaluation of the lysosomal enzyme activities and antioxidant parameters (malondialdehyde [MDA], superoxide dismutase [SOD]), catalase [CAT], and glutathione [GSH]). Histological studies were done on the posterior paws of the animals.

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 $P < 0.05$.

Results

Phytochemical screening of the extract

The total phenolic, flavonoid, and tannin contents of the methanolic extract of *S. aethiopicus* rhizome are reported in Table 1. The scavenging capacity estimated by the

Table 1. Extraction yield, total polyphenols, flavonoids, and tannins contents; FRAP, ABTS, DPPH scavenging activities of methanolic extract of *S. aethiopicus*

Yield (%)	Polyphenols (mg/100 g)	Flavonoids (mg/100 g)	Tannins (mg/100 g)	FRAP (mg/100 g)	DPPH (mM/100 g)	ABTS (mM/100 g)
16.23 ± 0.54	271.48 ± 9.56	50.93 ± 2.46	37.63 ± 8.52	89.43 ± 7.31	122.25 ± 10.89	113.17 ± 9.124

Values are given in mg or mM for 100 g of dry weight (mean ± standard deviation of three repetitions).

ABTS method was 113.17 mg/100 g, while the value found by the method using the DPPH method was 122.25 mM.Eq.T/100 g DW).

Acute toxicity of methanolic extract of *Siphonochilus aethiopicus*

Oral administration of a single dose of 2000 mg/kg of methanolic extract of *S. aethiopicus* did not result in any lethality or signs of characteristic toxicity such as decreased sensitivity to pain, noise, and locomotion. No significant changes in body weight were observed in animals during the 14 days of observation (Figure 1).

Four hours after the administration of *S. aethiopicus* extract, all rats treated with 4 g/kg showed signs of moderate toxicity, including drowsiness, isolation, hair straightening and hypo-activity. On the second day, a doughy stool was observed with an insignificant change in weight. On the third day and 1 week after extract administration, rats recovered normal behaviour comparable to that of the control group (Figure 1). In addition, no signs of toxicity were observed in the groups treated with the extract at 2 and 4 g/kg. The LD_{50} of the extract is, therefore, higher than 4000 mg/kg.

Effect of methanolic extract of *Siphonochilus aethiopicus* on carrageenan-induced paw oedema

The effect of the methanolic extract *S. aethiopicus* on carrageenan-induced paw oedema in mice is presented in Figure 2. In untreated animals (negative control) paw oedema increased progressively over time and reached its peak 4 hours after carrageenan injection. Treatment of mice with methanolic extract *S. aethiopicus* significantly ($P<0.01$) reduced inflammation compared to negative control mice.

The maximum inhibition percentages (6th hour) of oedema caused by MESA were 16.67%, 35.76%, 44.44% corresponding to the doses of 75 mg/kg, 150 mg/kg, and 300 mg/kg, respectively (Table 2). On the other hand, the percentage of inhibition of oedema caused by diclofenac (10 mg/kg) was 29.25%. From the 4th hour onwards, the percentage inhibition of the methanolic extract *S. aethiopicus* (150 and 300 mg/kg) was higher than that of diclofenac (10 mg), which represents the reference molecule.

MSU-induced variation of paw oedema

Paw oedema measurements were recorded before (0 hours) and at 4, 24, 48, and 72 hours after every treatment (Figure 3). In negative control mice, a significant ($P<0.001$) increase in leg oedema was observed 4 and 24 hours after the injection of the MSU. On the last 2 days (at 48 and 72 hours), a successive decrease in oedema was noted but remained significantly ($P<0.001$) higher than the neutral control.

Furthermore, a significant ($P<0.001$) decrease in oedema was noted in the *S. aethiopicus* methanolic extract-treated groups (75, 150, 300 mg/kg) as well as those treated with the reference drugs (indomethacin and colchicine). This effect became visible 24 hours after treatment and interestingly on the last two days of the experiment (48 and 72 hours).

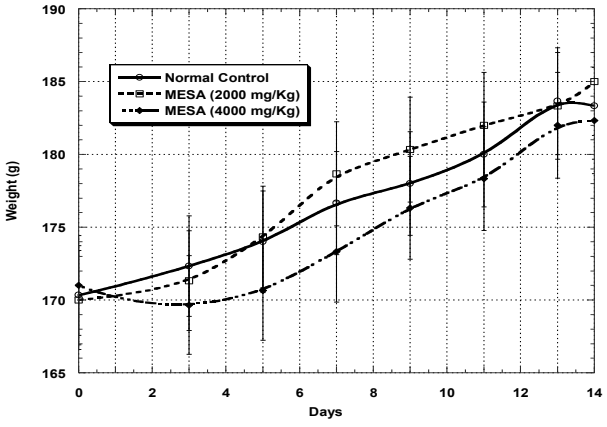

Figure 1. Changes in rats' weight during toxicity evaluation of MESA (methanolic extract of *Siphonochilus aethiopicus*).

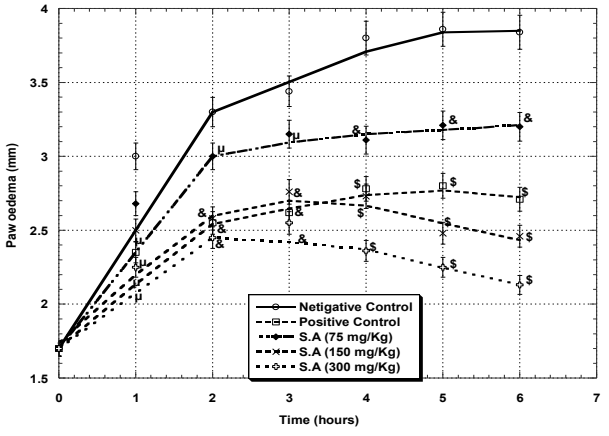

Figure 2. Effect of the methanolic extract of *Siphonochilus aethiopicus* (S.A) on carrageenan-induced paw oedema in mice, 0 to 6 hours after carrageenan injection (mean ± SEM, n = 5; $^{\mu}$ $P < 0.05$, $^{\&}$ $P < 0.01$, and $^{\$}$ $P < 0.001$ compared to the negative control group at the same time).

Table 2. Effect of methanolic extract of *Siphonochilus aethiopicus* on percentage inhibition of paw oedema induced in mice by carrageenan

Time (h)	Positive control	*Siphonochilus aethiopicus* (75 mg/kg)	*Siphonochilus aethiopicus* (150 mg/kg)	*Siphonochilus aethiopicus* (300 mg/kg)
0	0	0	0	0
1	21.67*	10.55*	16.67*	25**
2	22.72**	9.09*	21 .71**	25.75**
3	23.93**	8.43*	19.57**	25.87**
4	26.75***	17.9 8**	28 .07***	37.72***
5	27.46***	16.67 **	35.66***	41 .72***
6	29.25***	16.67**	35.76***	44. 44***

Mean ± SEM, n = 5; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared to the negative control group at the same time.

Evolution of body temperature

The treatment of the mice with the methanolic extract of *S. aethiopicus* affected their body temperature with a significant decrease ($P<0.05$) as early as 4 hours and interestingly within 48 hours (Figure 4).

Lysosomal enzymes activities

Results of lysosomal enzymes (phosphatase acid and β-galactosidase) activity in serum, spleen, and liver of mice are shown in Figure 5. All three doses of methanolic extract of *S. aethiopicus* (75, 150, and 300 mg/kg) significantly ($P < 0.001$) reduced lysosomal enzymes activities compared to the negative control group.

Tissue-level of oxidative stress

Results of the oxidative stress markers obtained in the liver and spleen homogenates are presented in Table 3 and Table 4. The assay of these markers showed a significant increase in the MDA level ($P<0.001$), while a decrease in CAT, SOD and GSH activities was observed in the treated groups, compared to the normal control group.

The treatment of rats with the methanolic extract of *S. aethiopicus* (75, 150, and 300 mg/kg/d) for 3 days showed that MESA induced a dose-dependent regulation of the disturbance of tissue's antioxidant enzyme activity and MDA level. MESA was able to restore and maintain them at values close to those of the normal control group.

Histological study of the legs

Figure 6 shows the results of the histopathological analysis of the skin and bones of mice paws. The skin and bones of each mouse were stained using the classical H&E technique. Normal morphology was observed in normal mice (Figure 6).

In the negative control animals, hemorrhages were noted in the subcutaneous tissues while the joint junction space was reduced. The deformed joints observed in the negative control animals revealed the arthritic effect of

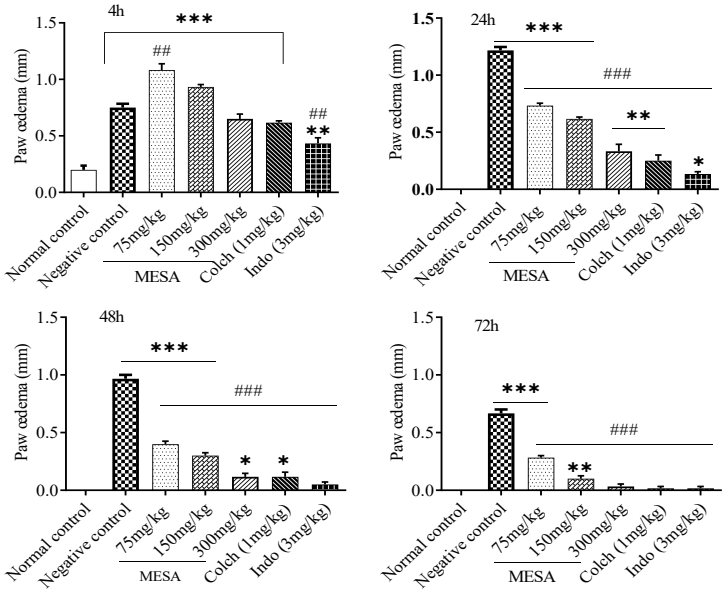

Figure 3. Anti-oedema effect of methanolic extract of *Siphonochilus aethiopicus* (MESA), colchicine (Colch) and indometacin (Indo) on monosodium urate-induced inflammation. Values were recorded 4, 24, 48, and 72 hours after treatment (Mean± SEM, n = 5, *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$, compared to the normal control. #$P < 0.05$, ##$P < 0.01$; ###$P < 0.001$ compared to the negative control).

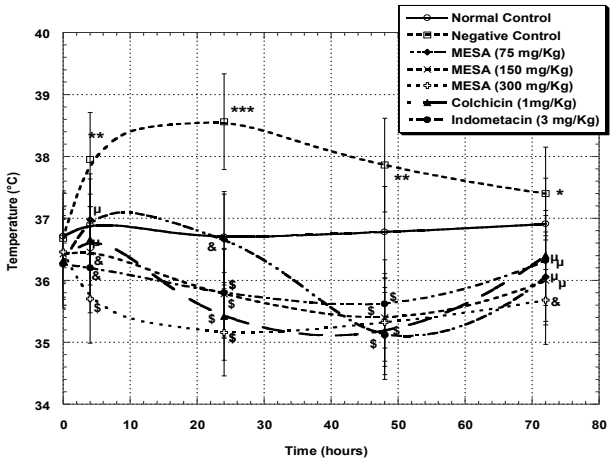

Figure 4. Effects of methanolic extract of *Siphonochilus aethiopicus* (MESA), colchicine and indometacin on body temperature of the mice in parallel MSU-induced inflammation (Mean ± SEM, n = 5, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to normal control, $^{\mu}P < 0.05$, $^{\&}P < 0.01$, $^{\$}P < 0.001$ compared to negative control).

MSU crystals. In synovial inflammation with pannus formation and severe leukocyte infiltration, indomethacin and colchicine resulted in an overlay of normal leg skin morphology. The junction spaces were almost normal and the articular cartilage also appeared normal, indicating an anti-inflammatory effect (Figure 6).

Like the reference compounds used, the methanolic extract of *S. aethiopicus* resulted in a dose-dependent recovery of the mouse paw skin and a normal inter articular junction space. Histological sections of the mice paws showed moderate infiltration of inflammatory cells. Methanolic extract of *S. aethiopicus* decreased the inflammatory response and synovial hyperplasia following intra-articular injection of MSU in the paw of mice compared to negative control mice (Figure 6). In addition, the methanolic extract of *S. aethiopicus* prevented the deformation of articular cartilage.

Discussion

The present study aimed at characterizing and evaluating the anti-inflammatory and antioxidant effects of the methanolic extract of *S. aethiopicus* rhizomes. The anti-inflammatory activity was evaluated using the carrageenan and MSU-induced paw oedema models.

Several aggressors, including substances like carrageenan and crystals, can destroy articular cartilage. Carrageenan induces local oedema, which peaks after 4 hours. The molecular mechanism of this inflammation is characterized by the release of several mediators responsible for the inflammatory process (24). The activation of mast cells releases serotonin and histamine, stimulating a cascade of events that produce several chemotactic factors such as C5a and chemokines. These mediators lead to intense leukocyte migration to the inflamed site (25). In the carrageenan-induced model of inflammation in mice, there is a progression of oedema after subcutaneous injection of carrageenan into the paw of the mice in two phases. The first phase, characterized by the release of histamine, bradykinin, and serotonin, which promote vasodilatation, plasma transudation, and oedema, occurs immediately and lasts 2 hours 30

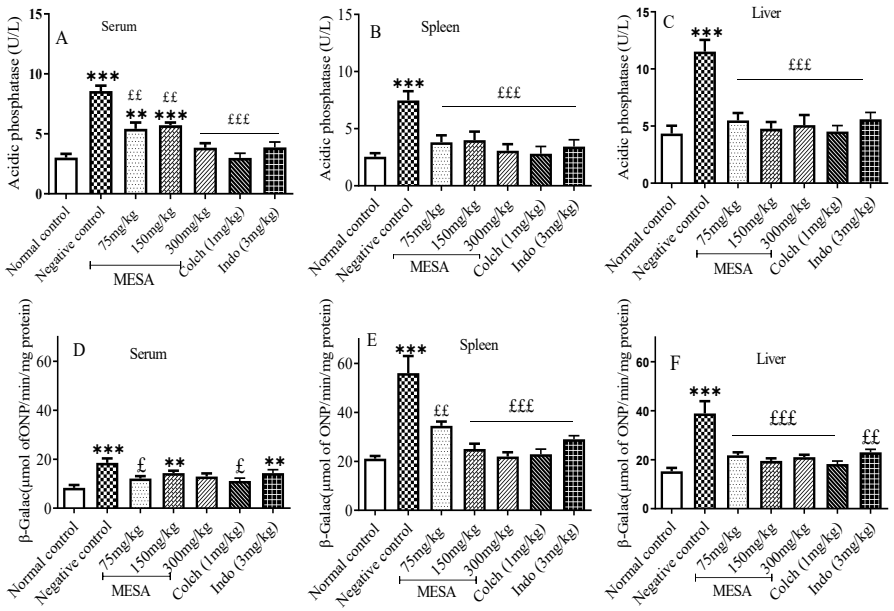

Figure 5. Effect of methanolic extract of *S. aethiopicus* (MESA), colchicine and indomethacin on the activity of lysosomal enzymes (acid phosphatase and β-galactosidase) of plasma, liver, and spleen of MSU-induced arthritis (Mean ± SEM, n = 5, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to normal groups. $^{£}P < 0.05$, $^{££}P < 0.01$; $^{£££}P < 0.001$, significant difference compared to negative control groups.

Table 3. Effect of methanolic extract of *Siphonochilus aethiopicus*, colchicine and indomethacin on oxidative parameters (malondialdehyde, superoxyde dismutase) of liver and spleen

Parameters	Malondialdehyde		Superoxide dismutase	
	Liver	Spleen	Liver	Spleen
Normal (N)	20.07 ± 2.72	12.25 ± 1.64	232.88 ± 40.08	226.43 ± 26.13
Arthritis (NC)	53.76 ± 10.19**	36.24 ± 6.47***	144.11 ± 22.24*	100.38 ± 9.73$^{***£}$
MESA				
75 mg/kg	28.84 ± 3.94$^{£££}$	25.34 ± 3.01$^{***££}$	220.93 ± 45.10$^{£££}$	182.46 ± 30.28$^{£££}$
150 mg/kg	22.30 ± 3.01$^{£££}$	15.50 ± 3.66$^{£££}$	296.61 ± 25.87$^{£££}$	214.87 ± 29.97$^{£££}$
300 mg/kg	20.09 ± 1.19$^{£££}$	15.17 ± 2.32$^{£££}$	334.63 ± 40.6$^{**£££}$	278.63 ± 40.84$^{£££}$
Colchicine (1 mg/kg)	17.81 ± 1.75$^{£££}$	10.99 ± 1.24$^{£££}$	346.53±40.36$^{***£££}$	245.98 ± 48.86$^{£££}$
Indo (3 mg/kg)	24.03 ± 2.44$^{£££}$	14.15 ± 1.52$^{£££}$	306.83 ± 39.56$^{£££}$	209.43 ± 25.67$^{££}$

MESA, methanolic extract of *S. aethiopicus.*
The values are expressed in mean ± SEM (n = 5, $^{*}P < 0.05$, $^{**}P < 0.01$, *** $P < 0.001$ significant compared to normal control $^{£}P < 0.05$, $^{££}P < 0.01$; $^{£££}P < 0.001$ significant difference compared to negative control).

Table 4. Effect of methanolic extract of *Siphonochilus* aethiopicus, colchicine and indomethacin on oxidative parameters (catalase and glutathione) of liver and spleen

Parameters	Catalase		Glutathione	
	Liver	Spleen	Liver	Spleen
Normal (N)	117.58 ± 19.41	83.58 ± 24.23	52.43 ± 10.09	36.43 ± 6.96
Arthritis (NC)	43.12 ± 11.02***	32.52 ± 10.83***	27.46 ± 7.61**	21.46 ± 3.59**
MESA				
75 mg/kg	73.30 ± 7.03$^{*£}$	39.30 ± 16.95**	41.58 ± 8.31	24.78 ± 5.36
150 mg/kg	84.01 ± 10.49$^{££}$	51.41 ± 10.07*	39.20 ± 7.66	31.40 ± 6.09
300 mg/kg	113.10 ± 16.53$^{£££}$	73.70 ± 11.95$^{££}$	44.23 ± 14.07	31.63 ± 5.81
Colchicine (1 mg/kg)	103.90 ± 11.81$^{£££}$	77.30 ± 15.36$^{££}$	49.98 ± 10.69$^{£}$	30.38 ± 8.10
Indo (3 mg/kg)	80.90 ± 13.09$^{*£}$	40.70 ± 13.12**	34.43 ± 9.21	24.43 ± 5.53

MESA, methanolic extract of *S. aethiopicus.*
The values are expressed in mean ± SEM (n = 5, $^{*}P < 0.05$, $^{**}P < 0.01$, *** $P < 0.001$ significant compared to normal control $^{£}P < 0.05$, $^{££}P < 0.01$; $^{£££}P < 0.001$ significant difference compared to negative control).

minutes after carrageenan injection. The second phase is characterized by the release of prostaglandin, protease, lysosomes, and migration of leukocytes to the inflamed site and lasts up to 6 hours after carrageenan injection (25,26). In the present study, the predominant anti-inflammatory action of the extract was observed at the last phase where prostaglandins are the main substances that maintain the inflammatory reaction. This indicates that the methanolic extract of *S. aethiopicus* could act via the inhibition of prostaglandin activity.

It is known that the formation of MSU in the articular synovium triggers an inflammatory response. By mimicking this physiopathology of gout, MSU crystal was injected into the hind paw of mice. Then, oedema and body temperature, lysosomal enzyme level, oxidative stress, and histological variations at the articulation were measured. The changes in oedema and body temperature were measured within a time interval of 4, 24, 48, and 72 hours. A significant increase ($P < 0.001$) in paw oedema was noted 4 hours after injection of MSU crystals in the negative control mice, reaching a maximum at 24 hours. From the 48th hour onwards, we noticed a slight progressive decrease of oedema, which by the 72nd hour was not comparable to initial paw volume. This proves that MSU induced an inflammatory reaction similar to those gotten by previous authors (27). In fact, the injection of MSU induced the release of several chemical mediators that amplified inflammation. In *S. aethiopicus* methanolic extract treated groups (75, 150, and 300 mg/kg), a significant decrease ($P < 0.001$) in paw oedema was observed from 24th hour post-treatment. At the 4th hour, a significant reduction ($P < 0.05$) of oedema was visible at 75 and 150 mg/kg extract doses, which remained significantly ($P < 0.001$) high compared to the neutral control. This could be due to delayed metabolism of extract whose anti-inflammatory constituents' bioavailability at the inflammation site (target) was effective only 24 hours after administration as reported in another finding (28). However, it may also be indirectly related to the route of administration, as the oral route used in this study would not have allowed the active ingredients of this extract to be rapidly released into the bloodstream (29).

Between 48th and 72nd hours, there was a significant ($P < 0.001$) reduction in oedema in the extract-treated

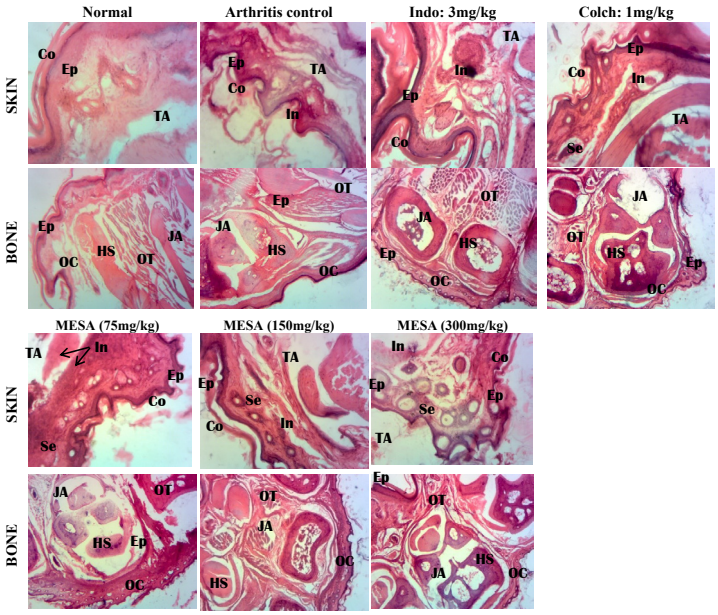

Figure 6. Microphotographies (HE × 100) of paw transverse section of normal, arthritic, and treated mice with colchicine, indomethacin, and methanolic extract of *S. aethiopicus*. Normal = Animals receiving distilled water; Arthritis control = Arthrosis induced animals receiving distilled water; Indo: 3 mg = Arthrosis induced animal receiving indomethacin 3 mg/kg; Col: 1 mg = Arthrosis induced animals receiving colchicine 1 mg/kg, MESA = Arthrosis induced animals receiving methanolic extract of *S. aethiopicus* 75, 150, and 300 mg/kg doses. JA = Intra-articular joint; OC = Cortical bone; TA =Adipose tissue; Se = Eosinophilic secretion, OT = Trabecular bone; HS = Synovial hyperplasia, In = Inflammatory cells; Co = Cornea layer; Ep = Stratified and keratinized malpighium epithelium.

mice, especially at the dose of 150 and 300 mg/kg, comparable to the reference drugs (colchicine and indomethacin). These drugs used act by almost different mechanisms. Colchicine, for example, acts by inhibiting inflammasome NLRP3, case-1, IL-1β production, and recruitment of leukocytes at the inflammation site (30). In addition, it reduces the level of histamine released by mast cells (31), leading to the total disappearance of the oedema. Indomethacin-induced oedema reduction is linked to several mechanisms, the main one being COX2 inhibition (32). Indomethacin reduces the physiological effect of prostaglandins (33) and prevents infiltration of lymphocytes, monocytes, and macrophages into the synovial cavity (34). It is, therefore, likely that *S. aethiopicus* extract acted by the same mechanisms of action as these drugs to exert its anti-inflammatory effect.

MSU crystals have been reported to induce inflammation by activating specific receptors via mediators and cytokines released after activation of different cells (35). These cytokines, such as IL-1, can stimulate the thermoregulatory center and increase the temperature of subjects. In this work, the significant temperature increase in negative control mice was linked to the massive release of these cytokines. This parameter was reversed by *S. aethiopicus* extract, which showed a significant ($P < 0.001$) decrease 24-48 hours post-treatment. This extract would have inhibited the inflammatory response by reducing the high level of inflammatory mediators in MSU-injected mice.

The interaction between MSU and macrophages causes lysosomal membrane rupture. This results in the release of lysosomal enzymes (β-glucuronidase, β-galactosidase, N-acetyl glucosaminidase, and acid phosphatase) and increase of these enzymes in the blood, spleen, and liver. These enzymes are also responsible for inflammation, degradation of proteins, glycosaminoglycans, and lipids. Therefore, lowering the level of extracellular lysosomal enzymes may be beneficial in the control of arthritic inflammation (36). In the present study, the methanolic extract of *S. aethiopicus* significantly reduced the activities of lysosomal enzymes in MSU crystals-treated mice. Methanolic extract of *S. aethiopicus* restored lysosomal enzyme levels close to normal values probably by stabilizing the lysosomal membrane.

Increased oxidative stress in inflamed joints is considered as a mediator of inflammation, which appears after arthritis induction. During inflammation, different cell types, such as macrophages and neutrophils, play a major role in the production of reactive oxygen species, leading to lipid peroxidation and imbalance in antioxidant status (37). The role of the joint in the production of reactive oxygen species is also important in the development of arthritis. Oxidative stress is one of the main causes of acute and chronic disorders (38). After injection of MSU crystals, levels of various tissue antioxidant enzymes such as SOD, glutathione peroxidase, and CAT decreased significantly in mice. This decrease in antioxidant enzyme levels was due to increased lipid peroxidation, which also plays a major role in severe arthritic disease. Certain natural flavonoids have strong antioxidant and anti-inflammatory

activities (39). In this study, treatment with *S. aethiopicus* methanolic extract reduced oxidative stress by inhibiting lipid peroxidation, which decreases the activity of the SOD, CAT, and GSH.

In addition, during the progression of polyarthritis, pro-inflammatory cytokines (in particular TNF-α and IL-1ß) may activate the nicotinamide adenine dinucleotide phosphate oxidase, which through the respiratory chain could cause the release of superoxide and then induce oxidative stress. Excessive production of free radicals and pro-inflammatory cytokines accelerates the degradation of articular cartilage, bone destruction, and stimulation of osteoclasts (40). Thus, the antioxidant potential of the extract was tested *in vitro* by DPPH, ABTS, and FRAP tests. Results obtained revealed that *S. aethiopicus* methanolic extract had a free radical scavenging capacity confirmed by DPPH and ABTS tests. In addition, the extract showed a strong ability to reduce ferric ions. This finding is in agreement with Mokgehle et al (41) results, who studied the detail antioxidant effect of *S. aethiopicus*. Previous results have shown that the antioxidant activities of plant extracts are related to their polyphenolic content (42), which exert their antioxidant potential by acting as reducing agents, hydrogen or electron donors, and metal chelators (43). In this work, phytochemical analysis showed that the extract was quantitatively rich in polyphenols, flavonoids and tannins. The values obtained for the polyphenol and flavonoid contents were much higher than those previously observed by Mokgehle et al. (44). This difference might be attributed to geographical, climatic and environmental factors, genotype, harvesting seasons, storage time, plant maturity, cultivation practices, temperature, and solvents used for extraction (45). Polyphenols, flavonoids, and tannins have great antioxidant potential (46). Inflammation caused the joint space to shrink, leading to cartilage degradation. A treatment that can reduce inflammation by blocking cytokines may help overcome the problem of joint space narrowing and bone degradation (47). In the present work, *S. aethiopicus* extract limited inflammation in mice and prevented joint space narrowing, infiltration of inflammatory cells, and cartilage degradation.

Conclusion

The present work aimed to evaluate the antioxidant and anti-inflammatory effects of the methanolic extract of *S. aethiopicus* rhizomes. Phytochemical analysis revealed that this extract was rich in polyphenolic compounds. *In vitro*, the methanolic extract of *S. aethiopicus* showed an important anti-arthritic activity by inhibiting of protein denaturation, an antioxidant capacity with respect to the DPPH and ABTS radicals, and a significant chelating effect with respect to ferrous iron. The *In vivo* study also showed the extract could reduce joint inflammation, inhibit the release of lysosomal enzymes, and restore oxidative stress markers balance. In addition, no evidence of acute oral toxicity was observed after administration of methanolic extract of *S. aethiopicus* at oral doses of 2000 and 4000 mg/kg body weight in female rats. These results may justify the use of *S. aethiopicus* rhizome extract to treat inflammation in folk medicine.

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Authors' contributions

BBK and DN conceived and designed the experiment, ED performed the experiments and wrote the paper, HHNA revised the manuscript, SZ interpreted data. All authors confirmed the final version for publication.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The study was approved by the Ethic Committee of the Faculty of Sciences of the University of Maroua (Ref. N°14/0261/ Uma/D/FS/VD-RC), according to the guidelines of Cameroonian bioethics committee (reg N°. FWA-IRB00001954).

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