

Assessment of phytochemical composition and anti-inflammatory activity of methanolic roots extract of *Gnidia kraussiana* (Thymeleaceae)

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Inflammation plays a significant role in many chronic health conditions, posing a major threat to public health, especially in developing countries. Various medicinal plants and formulations have been proven to exhibit anti-inflammatory properties. *Gnidia kraussiana* is a plant used in traditional African medicine to treat inflammatory diseases such as gout, rheumatism, and jaundice. The present study was undertaken to explore the anti-inflammatory potential of the methanolic root extract of *Gnidia kraussiana* (MREGK). Material and Methods: Phytochemical characterization studies and *in vitro* antioxidant activity through DPPH, ABTS, and FRAP tests were achieved on MREGK. The safety of MREGK was evaluated by an acute oral toxicity test in conformity with OECD 423 guidelines. The *in vitro* anti-inflammatory activity of MREGK was evaluated utilizing the protein denaturation inhibition assay. The anti-inflammatory effect of MREGK (25, 50, and 100 mg/kg) was explored *in vivo* in a carrageenan-induced acute inflammation mouse model. The anti-inflammatory activity was assessed by measuring the percentage inhibition of edema. Phytochemical screening revealed varying levels of total phenolic compounds, flavonoids, and tannins. *In vitro* antioxidant tests showed antiradical capacity against DPPH free radical (426.36 mmol T.Eq/100 g) and ABTS (349.14 mmol T.Eq/100 g) as well as a strong iron reducing power (75.05 mg vitC.Eq/100 g). The acute oral toxicity evaluation of MREGK revealed a LD₅₀ greater than 2000 mg/kg. The MREGK significantly inhibited protein denaturation comparable to the standard drug diclofenac. A significant inhibition of paw oedema was noted *in vitro* with MREGK comparable to diclofenac. Results suggest that *Gnidia kraussiana* exhibits significant antioxidant and anti-inflammatory activity. Consequently, its chemical constituents may hold promise for use as drugs with antioxidant and anti-inflammatory properties.

Keywords: *Gnidia kraussiana*, carrageenan, antioxidant, anti-inflammatory, protein denaturation.

Introduction

Inflammation is a complex set of interactions among different types of cells forming a communication network involving numerous pro- or anti-inflammatory chemical mediators that can perpetuate, or modify a response¹. Its primary function is to eliminate, or isolate the aggressor agent from the rest of the organism, and to facilitate tissue repair as quickly as possible². However, when the

body fails to regulate it, this inflammation persists and becomes chronic, leading to severe tissue damage accompanied by the development of certain pathophysiological processes and diseases such as cancer, atherosclerosis, osteoarthritis, Alzheimer's disease, rheumatoid arthritis, chronic asthma, sclerosis, and Crohn's disease. These diseases are prevalent worldwide and constitute one of the greatest challenges in current conventional medicine³. Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are commonly used to treat inflammatory conditions. These medications play a crucial role in reducing inflammation and

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disease progression⁴. However, they have several disadvantages such as short duration of action and long-term adverse effects including nephrotoxicity, gastrointestinal tract irritation, hematological disorders, increased risk of cardiovascular diseases, muscle weakness, cataracts, etc.⁵. All these adverse effects have shifted research concerns regarding inflammation towards safer and less toxic indigenous medicines.

Medicinal plants have long been recognized as valuable sources of therapeutic agents because of their rich diversity of specialized secondary metabolites.^{6,7} Among these bioactive constituents are phenolic compounds, phenolic acids, flavonoids, tannins, terpenoids, and other phytochemicals that exhibit a broad range of biological activities.⁸ Increasing evidence suggests that these phytochemicals constitute an important natural reservoir of antioxidants, capable of scavenging reactive oxygen species, enhancing the body's antioxidant defense system, and protecting against oxidative stress-related disorders.^{8,9} These antioxidant properties are believed to contribute significantly to their therapeutic potential and their protective effects against numerous chronic diseases.¹⁰

Gnidia kraussiana, is a plant employed in Central African folk medicine in the treatment of various ailments including edema, fever, bronchitis, hepatitis, snakebites, earworms, leprosy, and acute gout^{11,12}. The phytochemical analysis of this plant has revealed substantial levels of flavonoids, coumarins, and terpenes¹³. Other studies have shown that phenolic compounds present in this plant extract promote immunoglobulin synthesis in case of immune-deficient infections, pediatric pulmonary infections, chronic bronchitis in adults, HIV/AIDS, leukemia and cancer^{12,14}. The current work aims to study the ability of the MREGK roots to inhibit protein denaturation and its effect on carrageenan-induced inflammation in mice.

Materials and Methods

Preparation of plant material and extraction

The roots of *Gnidia kraussiana* were sourced from the '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 freshly collected material was conveyed in polystyrene bags to the Laboratory of

Biochemistry and Biological Chemistry within the Faculty of Science at the University of Maroua, where it was identified and authenticated by Professor Tchopsala, botanist at the same University. A voucher specimen was submitted to the Cameroon National Herbarium for comparison with specimen number 322643/HNC. The roots were rinsed three times with tap water and air-dried at room temperature. The dried materials were ground into a powder, sieved (0.5 mm), and stored prior to extraction.

Extraction involved soaking 300 grams of the powder in 3000 mL of 80% methanol for 72 hours with continuous stirring at room temperature¹⁵. The mixture was passed through Whatman filter paper (N^o. 1) to obtain the filtrate, which was then concentrated using a rotary evaporator operating at 70°C. The resulting residue was stored at -4°C.

Experimental animals

The research involved male albino Swiss mice weighing between 25 and 30 g, obtained from LANAVET ('Laboratoire National Vétérinaire, Garoua'). All animals aged 4 months were reared in the animal breeding facility within the Department of Biological Sciences (University of Maroua). During 14 days of acclimatization, conditions of room temperature, natural light/dark cycle, free access to water and food were met for animals housed in polyacrylic cages (n=5). The animals were treated in compliance with the guidelines of the Cameroonian Bioethics Committee (reg N^o FWAIRB00001954) and adhered to the *NIH Guide for the Care and Use of Laboratory Animals* (8th edition).

Phytochemical analysis

Total polyphenol content

The total polyphenol content of the MREGK was determined using the Folin-Ciocalteu colorimetric method described by Singleton *et al.*¹⁶. Briefly, a mixture of 0.2 mL of the sample diluted in 80% methanol and 1 mL of 10% Folin-Ciocalteu reagent was prepared in a test tube. Following 5 minutes of stirring, 0.8 mL of a 7.5% sodium bicarbonate solution was introduced. After a 30-minute incubation period at room temperature, absorbance was recorded at 745 nm using a UV-vis spectrophotometer (PRIM Light & Advanced, Germany). Polyphenol quantification was based on a gallic acid standard curve (0-250 µg/mL; R² = 0.9921), with results reported as mg GAE/100 g (milligrams of gallic acid equivalent per 100 grams of dry weight).

$$TPC \left(\frac{\text{mgGAE}}{100 \text{ g}} \right)_{DE} = \frac{OD * df * V}{TP * \alpha}$$

TPC= total polyphenol content; mg GAE= milligrams of gallic acid equivalent; DE= dry extract; OD= optical density; df= dilution factor; V= total extraction volume (10 mL); TP= test portion; α = slope of the calibration.

Flavonoids content

Flavonoid content in the MREGK was assessed following the method outlined by Mimica-Dukic *et al.*¹⁷. 1 mL of the extract was mixed with 1 mL of AlCl_3 reagent (prepared by dissolving 130 mg of AlCl_3 and 400 mg of sodium acetate in 100 mL of methanol) in a tube. After stirring for 5 minutes, two drops of acetic acid were introduced into the mixture. The absorbance of the resulting solution was recorded at 430 nm. The outcome was reported as milligrams of quercetin equivalent per 100 grams of dry weight (mg QE/100 g), utilizing a standard curve correlating optical density with quercetin concentration (0-100 $\mu\text{g/mL}$; $R^2 = 0.9924$).

$$FLAC \left(\frac{\text{mgQE}}{100 \text{ g}} \right)_{DE} = \frac{OD * df * V}{TP * \alpha}$$

Tannins content

The tannin content was assessed following the method described by Bainbridge *et al.*¹⁸. In the experimental procedure, 200 μL of the MREGK and 2 mL of vanillin solution (1 g vanillin/ 100 mL of HCl conc.) were added to a test tube. The mixture was then incubated in darkness for 30 minutes. After incubation, the absorbance of the resulting solution was measured at 500 nm. A calibration curve was constructed using catechin (0-50 $\mu\text{g/mL}$; $R^2 = 0.9879$), and the results were expressed as milligrams of catechin equivalent per 100 grams of dry weight (mg CE/100 g).

$$TANC \left(\frac{\text{mgCE}}{100 \text{ g}} \right)_{DE} = \frac{OD * df * V}{TP * \alpha}$$

Evaluation of the in-vitro antioxidant activities of the MREGK

The FRAP assay, as outlined by Benzie and Strain¹⁹, involved combining 1 mL of either the sample or Trolox standard solution with 3 mL of FRAP reagent (Fe (III)-2,4,6-Tri(2-pyridyl)-s-triazine). After shaking for 60 seconds, the mixture was allowed to incubate for 40 minutes at room temperature. Subsequently, the solution's absorbance was measured at 593 nm using a UV-vis

spectrophotometer. A calibration curve was established using ascorbic acid (0-25 $\mu\text{g/mL}$; $R^2 = 0.9846$), and the results were reported as milligrams of ascorbic acid (Vitamin C) equivalent per 100 grams of dry weight (mg vitCE/100g).

$$FRAP \left(\frac{\text{mg vitCE}}{100 \text{ g}} \right)_{DE} = \frac{OD * df * V}{TP * \alpha}$$

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed according to the procedure described by Sun *et al.*²⁰ with slight modifications. 1 mL of either the sample or standard solution (Trolox) was combined with 3 mL of 1 mM DPPH. The mixture was agitated for 2 minutes and subsequently incubated for 40 minutes in darkness at room temperature. Absorbance at 517 nm was determined using a UV-vis spectrophotometer. We constructed a calibration curve using Trolox (0-200 μM ; $R^2 = 0.9844$), and the results were expressed in millimoles of Trolox equivalents per 100 grams of dry weight (mmol TE/100g).

$$DPPH \left(\frac{\text{mmol TE}}{100 \text{ g}} \right)_{DE} = \frac{OD * df * V}{TP * \alpha}$$

ABTS radical scavenging activity was conducted following the methodology outlined by Re *et al.*²¹. In brief, 1 mL of each sample or standard (Trolox) was combined with 3 mL of 7 mM ABTS+ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)). Following mixing, the solution was agitated for 2 minutes and subsequently allowed to incubate in darkness at room temperature for 40 minutes before measuring absorbance at 734 nm. We created a calibration curve using Trolox (0-200 μM ; $R^2 = 0.9883$), and the outcomes were reported as Trolox millimoles equivalent per 100 grams of dry weight (mmol TE/100 g).

$$ABTS \left(\frac{\text{mmol TE}}{100 \text{ g}} \right)_{DE} = \frac{OD * df * V}{TP * \alpha}$$

Assessment of the in vitro anti-inflammatory potential of MREGK

We assessed the *in vitro* anti-inflammatory activity of MREGK using the protein denaturation inhibition assay²². The procedure begins with the preparation of three solutions:

The experimental solution (0.5 mL) consisted of 0.45 mL of bovine serum albumin (BSA) aqueous solution and 0.05 mL of MREGK at different concentrations (10, 50, 100, 300, 500, and 1000 $\mu\text{g/mL}$)

The control solution (0.5 mL) consisted of 0.45 mL of an aqueous solution containing 5% bovine serum albumin (BSA) and 0.05 mL of distilled water.

The reference solution (0.5 mL) included 0.45 mL of 5% BSA aqueous solution and 0.05 mL of sodium diclofenac solution at different concentrations (10, 50, 100, 300, 500, and 1000 µg/mL).

The pH of all solutions was adjusted to 6.3 using 1N HCl solution. Subsequently, the samples were incubated at 37°C for 20 minutes, after which they were heated to 57°C to maintain this temperature for 3 minutes. Following cooling, 2.5 mL of phosphate-buffered saline solution (0.1M; pH 6.3) was introduced to each solution, and absorbance readings were taken at 416 nm using a UV-visible spectrophotometer. The extent of inhibition of protein denaturation was determined as follows:

$$\% \text{ Inh} = \frac{OD(\text{control}) - OD(\text{test})}{DO(\text{control})} \times 100$$

OD = optical density

The control represents 100% denatured proteins.

Assessment of the *in vivo* anti-inflammatory activity: carrageenan-induced paw edema

Male mice weighing 25 and 30 g were distributed into 6 groups of 5 animals each and treated as follows:

Three groups were treated with MREGK (25, 50, and 100 mg/kg, *p.o.*);

A positive control group received diclofenac (5 mg/kg, *p.o.*);

Two control groups (normal and negative) received 10 mL/kg, *p.o.* of distilled water each.

One hour post-treatment administration, mice received a 50 µL injection of a freshly prepared carrageenan suspension (1%) in 0.9% saline solution into the sub-plantar tissue of the right hind paw. The normal control group received an injection of an equal volume of physiological saline²³. Paw thickness was measured hourly for 6 hours using a Vernier caliper²⁴. The percentage inhibition of edema for each group was standardized against the negative control group. This inhibition percentage was computed using the formula provided below.

$$\% \text{ Inh} = \frac{D(\text{negative control}) - D(\text{test group})}{D(\text{negative control})} \times 100$$

D = diameter of the paw

Acute toxicity test

Two groups (n = 3) of female rats (Wistar) weighing 150-200 g were utilized. One group served as a control and received distilled water (10 mL/kg), while another group received a high dose (2000 mg/kg) of MREGK. The rats were closely monitored at 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours, 48 hours, and 14 days for any abnormal behavior or signs of toxicity. Monitored parameters included physical appearance, grooming behavior, salivation, trembling, lethargy, convulsions, diarrhea, coma, startle response, stool characteristics, changes in body weight, and mortality.

Statistical analysis

The results of the total phenolic content, antioxidant, and *in vitro* anti-inflammatory activities assays were presented as means ± SD of three replicates (n = 3). Statistical analysis was conducted using one-way analysis of variance (ANOVA) with Dunnett's post hoc tests for multiple comparisons. Statistical significance was considered at *p* < 0.05. GraphPad Prism 8.01 software was employed for data analysis.

Results

Phytochemical characteristics of MREGK

Extraction yield, total phenolic compounds, flavonoids, and tannins contents

The MREGK extraction yield was 22.33%. Various MREGK phytoconstituents including total phenolic compounds, flavonoids, and tannins are depicted in Table 1.

Antioxidant activities of MREGK

The result displayed in Table 2 revealed that MREGK exhibits a strong ability to reduce iron, with a reducing power of 75.05 mg.vitCE/100 gDE. Furthermore, the extract analyzed in this study demonstrates significant antiradical activities, with high Trolox equivalent concentrations of 426.36

Table 1 — Ferric reducing potential and scavenging activity of methanolic root extract of *G. kraussiana*

	FRAP (mg.vitC.Eq/100 g DE)	DPPH (mM.T.Eq/100 g DE)	ABTS (mM.T.Eq/100 g DE)
MREGK	75.05 ± 3.87	426.36 ± 10.17	349.14 ± 5.72

The values were expressed as means ± SEM, (n = 3) for a total of three samples. MREGK: methanolic root extract of *G. kraussiana*; ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid; DPPH: 2,2-diphenylpicrylhydrazyl; FRAP: ferric reducing antioxidant potential; mg.vitC.Eq: milligram equivalent of Vitamin C; mM.T.Eq: millimol of Trolox equivalent; DE: dry extract.

Table 2 — Percentage of inhibition of protein denaturation as a function of methanolic root extract of *G. kraussiana* (MREGK) and diclofenac concentrations.

Concentration (µg/mL)	10	50	100	300	500	1000
Diclofenac (%)	57.08 ± 2.12 ^a	68.46 ± 3.02 ^a	71.62 ± 3.08 ^a	72.88 ± 3.13 ^a	87.13 ± 4.03 ^a	93.35 ± 5.24 ^a
MREGK (%)	50.27 ± 1.92 ^b	61.67 ± 2.67 ^a	65.09 ± 1.82 ^a	71.74 ± 2.89 ^a	80.66 ± 3.60 ^a	91.82 ± 3.73 ^a

The values were represented as means ± SEM, (n = 3). MREGK: methanolic root extract of *G. kraussiana*
Mean values with different superscript letters in the same column are significantly different (p < 0.05).

Table 3 — Effect of the methanolic root extract of *G. kraussiana* (MREGK) on the percentage inhibition of edema induced by Carrageenan compared to diclofenac.

Time (hours)	1	2	3	4	5	6
Diclofenac (5 mg)	21.67%**	22.72%**	23.93%**	26.75%**	27.46%**	29.25%**
25 mg/kg	0.1166	18.68%*	15.69%*	17.98%*	20.98%**	23.61%**
MREGK 50 mg/kg	26.11%**	29.79%**	25.38%**	28.07%**	34.46%***	36.52%***
100 mg/kg	23.33%**	27.27%**	25.87%**	35.08%***	40.41%***	43.14%***

Mean values, (n = 5), * p < 0.05; ** p < 0.01; and *** p < 0.001 compared to the Negative Control.

mmol.TE/100 gDE and 349.14 mmol.TE/100 gDE respectively, against the DPPH and ABTS radicals.

Effect of MREGK on protein denaturation inhibition

According to Table 3, the inhibition rate of protein denaturation increased with higher concentrations of both MREGK and Diclofenac. At a concentration of 10 µg/mL, an antithermodenaturing activity was noted with MREGK (50.27%) compared to that of diclofenac (57.08%). At the concentration of 100 µg/mL, an antithermodenaturing activity noted with MREGK and diclofenac was 65.09% and 71.62% respectively. At the concentration of 1000 µg/mL, a strong antithermodenaturing activity was noted with MREGK (91.82%) compared to that of diclofenac (93.35%)

Effect of MREGK on carrageenan-induced paw edema

The results obtained from this study on the anti-inflammatory effect of the MREGK, conducted on the acute paw edema model induced by carrageenan in mice are depicted in Figure 1. According to these results, paw edema significantly progressed (p < 0.001) in group of negative control, reaching a maximum value of 3.7 mm at the 5th hour, compared to the normal control group which measured an average diameter of 1.7 mm. In the groups treated with diclofenac (5 mg/kg) and MREGK (25, 50, and 100 mg/kg), a notable reduction in paw edema was observed compared to the negative control group from the 1st hour through the 6th hour (Figure 1), with statistical significance noted (p < 0.001; p < 0.01).

In order to highlight the anti-inflammatory effect of these extracts, the calculated inhibition percentages (Table 4) were reported relative to the negative

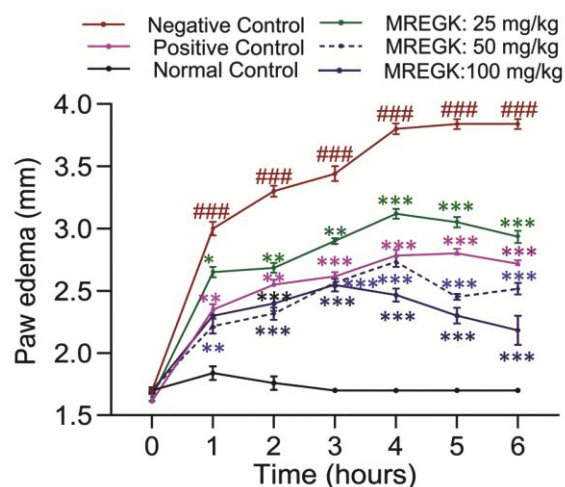


Figure 1 — Effect of MREGK on the evolution of carrageenan-induced edema in mice

The values are presented as means ± SD, (n = 5). Significant differences compared to the control groups are presented 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. MREGK: methanolic root extract of *Gnidia kraussiana*

control, and compared to that of the standard. As early as the 1st hour, MREGK (50 and 100 mg/kg) showed paw edema inhibition percentages of 26.11% and 23.33%, similar to that observed with diclofenac (21.67%). From the 4th hour onwards, MREGK (50 and 100 mg/kg) exhibited a higher paw edema inhibition rate compared to diclofenac (5 mg/kg).

Acute toxicity of the MREGK

According to Table 4, rats administered 2000 mg/kg of MREGK exhibited moderate signs of toxicity four hours after administration, such as drowsiness, isolation, and hypoactivity. Doughy stools were

Table 4 — Results of acute toxicity tests of methanolic root extract of *G. Kraussiana* in rats.

Clinical Sign	Period Dose	D1				D2	D4	D6	D8	D10	D12	D14
		1H	2H	3H	4H							
Grooming	MEGK (2 g/kg)	N	N	N	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Fur appearance	MEGK (2 g/kg)	N	N	N	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Trembling	MEGK (2 g/kg)	N	N	N	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Coma	MEGK (2 g/kg)	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Distilled water	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Salivation	MEGK (2 g/kg)	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Distilled water	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Convulsion	MEGK (2 g/kg)	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Distilled water	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Startle response	MEGK (2 g/kg)	R	R	R	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Stool appearance	MEGK (2 g/kg)	N	N	N	P	P	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Mortality	Distilled water and	0	0	0	0	0	0	0	0	0	0	0
	MEGK(2 g/kg)											

N = Normal; P = Pasty; R = Reduced; Abs = Absent.

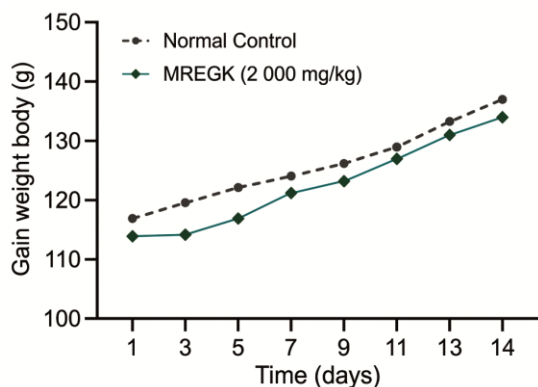


Figure 2 — Effect of MREGK on the body weight gain of rats
MREGK: methanolic root extract of *Gnidia Kraussiana*.

observed on the second day. From the 3rd until the 14th day of the experiment, all animals behaved like to the normal control group.

Moreover, there were no notable changes observed in the weight progression of the animals studied during the 14-day observation period. This is depicted in Figure 2.

Discussion

The objective of the current study was to assess the anti-inflammatory effects of MREGK. This was assessed both *in vitro* on protein denaturation inhibition and *in vivo* using the carrageenan-induced paw inflammation model.

Phytochemical analyses of medicinal plants are important for further recognizing the therapeutic

efficacy of the plant. Analysis of MREGK's phytochemical composition revealed the presence of phenolic compounds, tannins, and flavonoids, known for their robust antioxidant properties that could support various therapeutic benefits²⁵. These compounds provide protection against disorders caused by free radicals while inhibiting the action of enzymes producing these free radicals such as xanthine oxidase^{25,26}. Protection is better ensured when the anti-radical activity in ABTS and DPPH assays is high, and when antioxidant activity in the FRAP test is significant. The present study showed that MREGK exhibited high antioxidant potential *in vitro*, particularly in terms of radical scavenging activity (DPPH and ABTS) and iron-reducing power. Compounds with antioxidant properties are capable of preventing protein denaturation, which is one of the causes of inflammatory diseases. The automatic generation of antigens in this disorder could be due to protein denaturation. Protein denaturation involves modifications of electrostatic, hydrophobic, and disulfide bonding^{22,27}.

In the present study, MREGK significantly inhibited protein thermal denaturation in the *in vitro* assay. Consequently, it is suggested that MREGK's prevention of protein denaturation and auto-antigen production may be responsible for its anti-arthritis effect²⁸. Thus, it has been reported that a compound with a percentage inhibition of protein denaturation greater than 20 % is expected to possess anti-

inflammatory properties²⁹. These effects are likely attributed to certain phenolic compounds (flavonoids and tannins) present in MREGK. Phenolic compounds have the ability to interact with the aliphatic region around lysine or threonine residues on the albumin protein, thus preventing protein denaturation^{28,30}.

The results from *in vitro* studies suggest that MREGK could potentially provide protective effects in live organisms against oxidative damage and free radicals implicated in various health conditions such as tumors, cancers, diabetes, sickle cell disease, and rheumatoid arthritis.^{31,32} Articular cartilage is primarily composed of proteins whose denaturation leads to the development of inflammation³³. Several agents, including substances such as carrageenan, can cause harm to articular cartilage^{33,34}.

Carrageenan induces local edema through tissue damage, reaching its peak after 4 hours. Injection of carrageenan subcutaneously induces a localized inflammatory response characterized by five well-known signs: swelling, redness, heat, loss of function, and local hypersensitivity^{35,36}.

The development of edema following carrageenan injection is a biphasic process. The initial phase occurs immediately after injection, resulting in the release of histamine and serotonin by mast cells. These factors induce vascular changes that lead to plasma exudation²³, followed by a second phase of swelling, caused by increased release of arachidonic acid metabolites such as prostaglandins and leukotrienes in the inflammatory area³⁷. This stage is responsive to prostaglandin synthesis antagonists and natural or synthetic anti-inflammatories such as glucocorticoids³⁸. Non-steroidal anti-inflammatory drugs also inhibit cyclooxygenase enzymes involved in prostaglandin synthesis²³.

In this study, the validity of the method was first assessed by testing diclofenac, a non-steroidal anti-inflammatory drug known to be effective against carrageenan-induced oedema³⁹. The increase in paw diameters observed in the edematous control group up to the 6th hour suggests that any reduction in edema during the 6-hour period would be attributed to the effects of the administered treatments. Throughout the study, diclofenac significantly decreased ($p < 0.01$) edema formation starting from the first hour and continuing for the duration of the experiment. MREGK (25, 50, and 100 mg/kg) significantly exhibited inhibitory effects on edema formation at all evaluation time points, similar to that observed in the diclofenac-treated group. The maximal activity of

MREGK observed from the 3rd hour of experimentation, coinciding with the release of prostaglandins at the inflammatory site, which could be attributed to the presence of cyclooxygenase inhibitors in these extracts that may lead to the inhibition of prostaglandin synthesis. This finding suggests that MREGK possesses an anti-inflammatory mechanism of action similar to that of nonsteroidal anti-inflammatory drugs, possibly due to the presence of bioactive compounds such as total phenolic compounds, flavonoids, and tannins³⁹. Many studies have shown that polyphenols and their metabolites inhibit enzymatic activities involved in arachidonic acid metabolism and reduce the production of inflammation mediators such as prostaglandins and leukotrienes^{31,40}. The outcomes of our investigation are in agreement with previous studies that validate the anti-inflammatory activity and lysosomal enzymes release inhibition potential of *Gnidia kraussiana* release of strongly implicated in the inflammatory response¹⁴.

The acute toxicity study of MREGK focused on observing physiological changes in female rats. At the end of the 14-day observation period, the parameters studied showed no signs of toxicity. No mortality was recorded, resulting in an LD₅₀ greater than 2000 mg/kg. Therefore, following Hodge and Stenner's⁴¹ toxicity scale, MREGK falls into the non-toxic classification. This result corroborates those of other researchers⁴² who found similar results in their work.

Conclusion

Based on the study's results, the methanolic root extract of *Gnidia kraussiana* shows strong scavenging activity against DPPH and ABTS radicals and effectively reduces ferric ions *in vitro*, indicating significant antioxidant activity. Moreover, the methanolic root extract of *Gnidia kraussiana* demonstrates remarkable anti-inflammatory efficacy both *in vitro* and *in vivo* due to various bioactive compounds endowed with antioxidant potentials responsible for its pharmacological effectiveness. Additionally, the studied plant extract showed no signs of acute oral toxicity in experimental rats at a high dose of 2000 mg/kg. Further research aimed at evaluating chronic toxicity and other models of chronic inflammation is necessary.

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Conflict of interest

Authors have declared that no competing interests exist.

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