



## Research Article

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## Anti-inflammatory Effects of *Carissa spiranum* Mediated via Attenuation of Leucocyte Migration

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**ABSTRACT**

Inflammation is a physiological process vital for pathogen elimination and tissue repair. However, left untreated, it can cause undesirable effects. There are several methods for management of the condition, including traditional remedies from plant sources that are thought to have few deleterious effects on the body, as is the case with steroids and NSAIDs. One of the plants widely used is *Carissa spiranum*, though little has been done to determine the effects of purified extracts on the condition and their mode of action. The present study investigated the effects of purified plant extract and its flavonoid-rich fraction on inflammation and leucocyte migration in mice. Formalin and carrageenan were used as the phlogistic substances in inflammation induction and leucocyte migration, respectively. While diclofenac sodium and dexamethasone were used as standard drugs. The diameter of the paw edema induced in the left hind paw of mice was measured and quantified as the edema developed in mice, while the number of leucocytes in peritoneal fluid lavage after pretreatment with carrageenan and determined with improved Neubauer chamber was used in the determination of the extent of leucocyte migration. The extracts exhibited a significant ( $p < 0.05$ ) reduction in paw edema diameter and delayed leucocyte migration compared to the vehicle. These observations suggest that the plant extracts may contain compounds that exert their anti-inflammatory effects via attenuation of leucocyte migration.

**Keywords:** Inflammation, *Carissa spiranum*, Anti-inflammatory activity.

**INTRODUCTION**

Since ancient times, plant extracts have been used to curb or treat many diseases. One of the most utilized plants in herbal preparations is *Carissa spiranum*, often called conkerberry-English, tamurkeyat-Nandi, mukawa-Kikuyu, lamuriak - Maasai). It is used as a remedy for fever, hepatitis, venereal diseases, respiratory and gastrointestinal diseases, some microbial infections, diabetes, cancer, malaria, pneumonia, infertility, ulcers, and snake bites [1]. The fruits are edible, while the roots are used to flavor soups [2]. *C. spiranum* possesses antioxidant [3], antimicrobial [4], antidiabetic [5], anti-tumor [6], anti-viral [7], antibacterial [3], anticonvulsant [8], antiarthritic [9], antileishmanial [10], antiplasmodial [11], antipyretic [12], antihelminthic [13], analgesic [14], cardio and hepatoprotective activities [15], antidepressant [16], aphrodisiac [17], wound healing [18], anti-venom, teratogenic [19] and anti-inflammatory [20]. Several phytochemicals isolated from the *Carissa* genus include carissanol, olivil, carandinol, ursolic acid, carissone, scopoletin, naringin, lupeol, carissol, carissaeduloside A, D, J, sarhamnoside, and 3 $\beta$ -hydroxyolean-11-en-28 [21]. Though crude *Carissa spiranum* extract has been shown to exhibit anti-inflammatory effects, there are no reported studies on the impact of more purified extracts of the same and the probable anti-inflammatory mode of action. Therefore, this study aimed to determine the anti-inflammatory effect of the plant extracts and the possible mechanism of action using animal model.

**MATERIALS AND METHODS****Collection and preparation of plant materials**

The roots of *Carissa spiranum* were obtained from the Juja area of Kiambu County, Kenya. They were cleaned, chopped, and dried away from direct sunlight in the biochemistry laboratory for three weeks. They were then ground into powder using an electric grinding mill.

**Preparation of plant extract**

150 g of the root powder was weighed and then defatted using petroleum ether prior to extraction with methanol. Then, the powder was soaked, stirred, and allowed to stand for 2 hours before decanting. Then re-soaked and procedure was repeated twice i.e. for the next 24 and 48 hours. The supernatant was

filtered using a Whatman No. 1 filter paper, and the filtrate was concentrated at reduced pressure using a rotary evaporator to obtain the extract.

### Preparation of Flavonoids-rich Fractions

Flavonoids were extracted using the method described by Houghton and Roman [22] and used by Wambugu *et al.* [23] as follows: 200 g of dried powdered plant material was weighed and defatted using petroleum ether and extracted with methanol. The extract was then treated with 1N hydrochloric acid (HCl) and then partitioned with diethyl ether. The ether fraction was dried to obtain the flavonoids.

### Experimental Animals

Male and female Swiss Albino mice weighing 18-24g, 6-8 weeks old, were used for this experiment. The mice were placed in cages in the animal house in a room maintained at room temperature one week before the beginning of the experiment to allow acclimatization. Water and food were provided *ad libitum*. Experimental animals' mice were randomly allocated into six groups (n=6). The experiment was conducted per the guidelines for laboratory animal use and care [24], and the permit was granted by the Kenyatta University Animal Care and Use Committee and the National Commission for Science and Technology (NACOSTI).

### Drugs and chemicals

This experiment used petroleum ether, methanol, normal saline, formalin, carrageenan, hydrochloric acid, diethyl ether, diclofenac sodium, and dexamethasone.

### Bioassays

#### Anti-inflammatory Assay

This assay was conducted as described by Hunskaar and Hole [25] and used by Wambugu *et al.* [23] with modifications. Mice were injected intraperitoneally with diverse test doses, normal saline, and diclofenac

(15mg/kg). Fifty microliters of 5% formalin was injected into the mice's left hind paw 30 minutes after intraperitoneal administration of the diclofenac (15mg/kg), experimental test drugs, flavanoid-fraction doses, and vehicle. The initial paw diameter was taken before formalin injection, and the rest were measured after 1, 2, 3, and 4 hours following formalin injection. The paw diameter before formalin injection was compared to the diameter after formalin administration.

### Leukocyte migration Assay

Leukocyte migration was done as described by Ferrandiz and Alcaraz [26] and used by Mwonjoria *et al.* [27]. Approximately 0.25ml of 1% carrageenan was injected intraperitoneally 30 minutes following subcutaneous administration of 5mg/kg of dexamethasone and intraperitoneal administration of extracts and vehicle. Four hours later, the mice were euthanized using a cotton soaked with chloroform in a small jar. About 2 ml of normal saline containing EDTA was injected into the peritoneal cavity, followed by peritoneal lavage. The total number of white blood cells from the lavaged fluid was counted using the Improved Neubauer chamber.

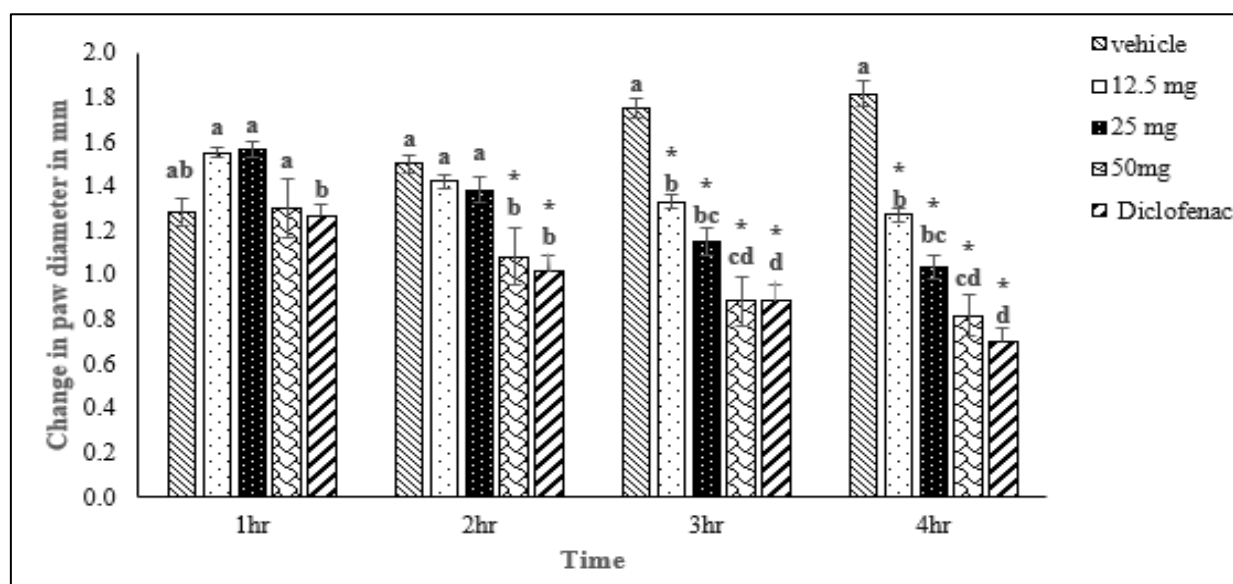
### Statistical analyses

Data was expressed as means and standard error of the means and was analyzed using one-way ANOVA and Tukey's *post hoc* test. The significance level was set at value of  $p < 0.05$ .

## RESULTS

### Effects of *Carissa spiranum* extract on formalin-induced inflammation in mice

All the doses of methanol *C. spiranum* exhibited significant ( $p < 0.05$ ) edema reduction compared to the negative control. However, this effect was observed at different intervals depending on dose levels. The 50 mg dose results were comparable to the diclofenac after 2 hours. The 12.5 and 25 mg doses significantly reduced paw edema 3 hours following formalin injection Fig 1.

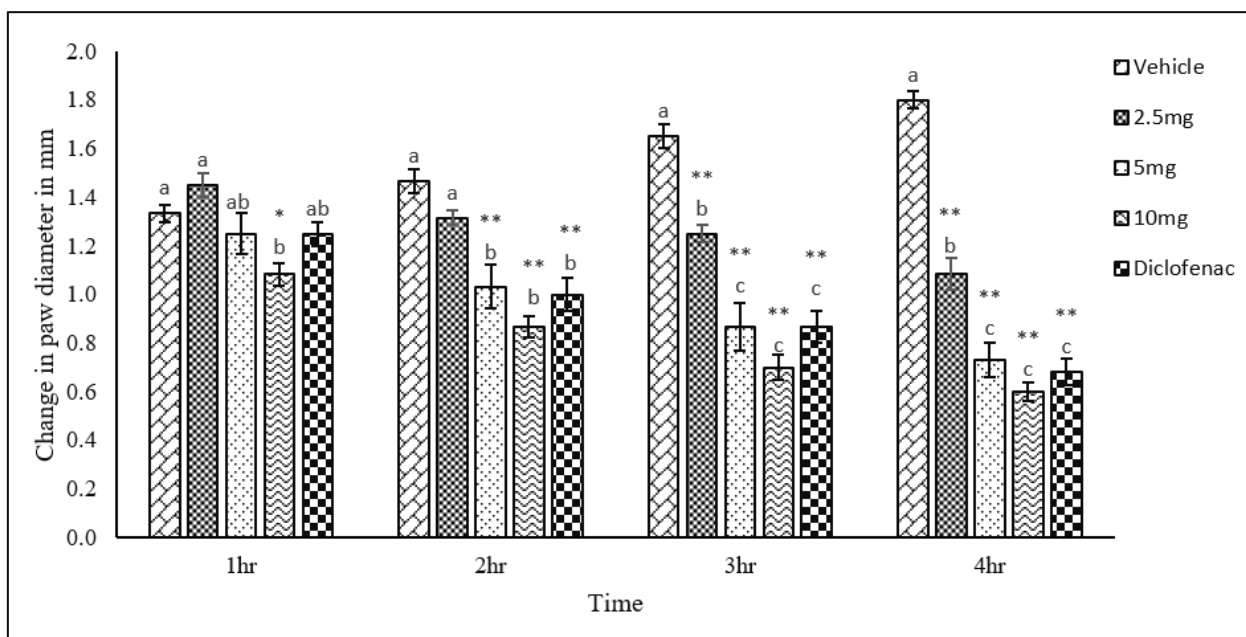


**Figure 1:** Effects of *Carissa spiranum* on formalin-induced inflammation in mice. \* $P < 0.05$  against the vehicle. Means that share letters are not significantly different.

### Effects of *Carissa spiranum* extract on formalin-induced inflammation in mice

On the other hand, administration of the flavonoid fraction of *C. spiranum* showed a higher activity than the crude extract. All the

doses showed a higher significant activity, which was similar to diclofenac after 2 hours of formalin injection.

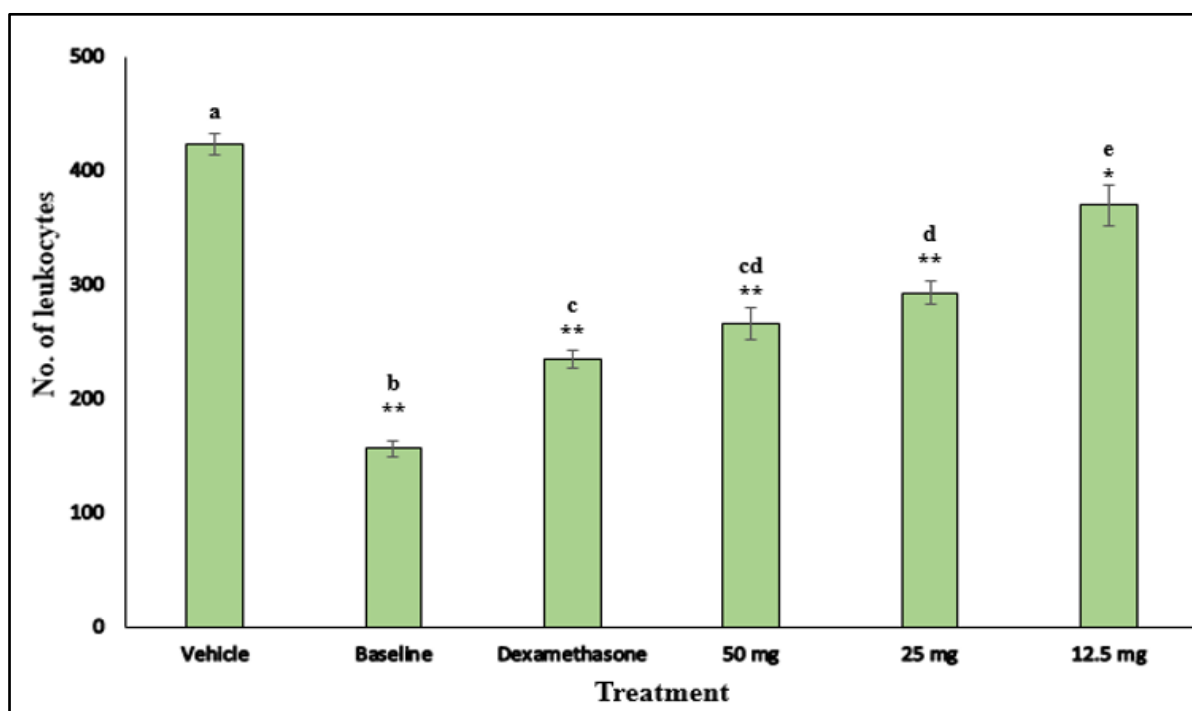


**Figure 2:** Effects of flavonoid-fraction of *Carissa spiranum* on formalin-induced inflammation in mice. \*P<0.05, \*\*p<0.001 against vehicle. Means that share letters are not significantly different.

### Effects of *Carissa spiranum* on Carrageenan-induced leukocyte

The leukocyte migration assay results showed that all doses of *C. spiranum* inhibited carrageenan-induced chemotaxis in the peritoneal

exudates. The effects of plant extract on leukocyte migration inhibition displayed a dose-dependent activity.



**Figure 3:** Effects of *Carissa spiranum* on carrageenan-induced leukocyte migration in mice. \*(p < 0.05), \*\*(p < 0.001) against vehicle. Means that share letters are not significantly different.

### DISCUSSION

One key feature of the inflammatory process is the occurrence of edema, which refers to tissue swelling resulting from the accumulation of tissue fluid in the interstitial space [28]. It may be associated with diseases such as renal, cardiac, or hepatic disorders, which lead to salt retention and expansion of extracellular fluid volume [29]. Other causes include microcirculation alteration that impacts the balance of fluid movement between the blood vessel and

surrounding tissues, leading to fluid accumulation and edema development [30]. Local edema results from the release of vasodilation substances such as histamine, cytokines, prostaglandins, bradykinins, and leukotrienes in the surrounding tissues. In the current study, the methanol extract of *Carissa spiranum* and the flavonoid-rich fraction of the same obliterated the edema induced by formalin, a phlogistic substance. Hence, it can be inferred that the extracts may have exhibited an anti-inflammatory effect. In addition, the inflammatory process is mediated by the cyclooxygenase (COX) system, which

involves the release of prostaglandins<sup>[31]</sup>. Some non-steroidal anti-inflammatory drugs, such as aspirin, block the COX enzyme through permanent enzyme acetylation, hence reducing the amount of prostaglandins, a mechanism that seems to alleviate the inflammatory process<sup>[32]</sup>. It is also possible that these extracts may have utilized the same mechanism in attenuation of the edema in mice, a phenomenon also observed in some plants, such as the willow tree bark, which contain several phytochemicals such as salicylic glycosides, a compound that has the same effect as aspirin during an inflammatory condition<sup>[33]</sup>.

The *Carissa spinarum* extract also significantly reduced leukocyte migration during the inflammatory process. Leukocyte migration, which involves diapedesis and chemotaxis, plays a crucial role in the immune response<sup>[34]</sup>. It recruits leukocytes to the site of injury or infection before degranulation, releasing inflammatory mediators such as histamine, prostaglandins, lipoxins, chemokines, and cytokines<sup>[31]</sup>. Blockage of the inflammatory process can be alienated by antagonistic effects of various remedies to this mediation, such as ondansetron, etc., or by inhibition of the migration and degranulation of leukocytes.

In the study, there was a significant decrease in the migration tendency of the leukocytes, which may have impacted the inflammatory process either directly or perhaps via an antagonistic effect on the inflammatory mediators<sup>[35]</sup>.

## CONCLUSION

From this study, both the methanol extract and flavonoid fraction of *Carissa spinarum* exhibited significant anti-inflammatory effects and attenuated the process of leukocyte migration. Hence, it can be inferred that *Carissa spinarum* roots contain compounds that may possess anti-inflammatory effects via attenuation of migration of white blood cells, which may serve as a source of novel drugs for managing inflammatory conditions.

## Conflict of interest

The authors declared no conflict of interest.

## Financial Support

None declared.

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