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# Anti-Inflammatory Activity of Dichloromethanolic Root Extract of *Clutia abyssinica* in Swiss Albino Mice

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## Abstract

Various diseases and injuries are always presented with inflammation. Conventional drugs that are prescribed for management of inflammation pose adverse effects after long term use leading to their limited use in clinical settings. Herbal medicines from medicinal plants have been used by mankind to treat diseases and ailments for centuries. Herbal medicines are believed to have secondary metabolites capable of healing diseases and infections. *Clutia abyssinica* is a shrub that is found in East Africa, Central Africa, and South Africa and it has been used traditionally to cure several ailments including malaria, chest pain, gonorrhoea, fever, infertility, pain, inflammation, skin diseases, cancer, liver pains and kidney problems. The aim of the project was to evaluate the anti-inflammatory activity of DCM root extract of *C. abyssinica* in Swiss albino mice. Thirty Swiss albino mice were randomly divided into six groups: normal control, negative control, positive control and three experimental groups. Swiss albino mice were administered with DCM root extract at concentration of 50, 100 and 150 mg/kg body weight. Inflammation was induced by injection 1% carrageenan solution into the right hind paw. The DCM extracts of *C. abyssinica* reduced paw edema by between 0.88% and 5.34%, while diclofenac reduced paw edema by between 2.21 and 5.35%. Phytochemical screening of the plant extract revealed bioactive compounds associated with anti-inflammatory activities. The present study therefore, confirms the folklore use of the medicinal plant to manage inflammation.

**Keywords:** Anti-inflammatory; *Clutia abyssinica*; Herbal; Dichloromethanolic

## Introduction

Inflammation is considered a normal process that provide protective role to tissue injury brought about by microbiologic agents, physical trauma and noxious chemicals [1]. Inflammation is an innate immune response to tissue damage and helps in repair and regeneration of injured tissue [1]. Inflammation process involves tissue-destroying process in which blood-derived products such as plasma proteins, fluids and leukocytes are recruited into perturbed tissues [2]. A number of processes are involved in inflammation for example mediator release, cell migration, tissue breakdown, enzyme activation, regeneration and repair [3]. Increased temperature, pain, loss of function, swelling, and redness are the cardinal signs of inflammation [4].

Inflammation is characterised by a number of components that result from tissue injury and this include; leukocyte infiltration, edema formation and granuloma formation [5,6]. For edema to occur, synergism between various inflammatory mediators are involved which result in increased vascular permeability and blood flow into the damaged tissue [7]. Inflammation can either be acute or chronic inflammation. Acute inflammation is the initial response to tissue injury, while chronic inflammation occurs as a result of failure in management of acute inflammation and an autoimmune response to a self-antigen [8]. Inflammation can be triggered by a number of diverse inflammatory mediators for example prostaglandins, leukotrienes, neuropeptides, eicosanoids, nitric oxide, histamine, serotonin, kinins, biological oxidants and platelet activating factors [9].

The inflammatory process is considered a complex process in which once stimulated by injury or harmful stimuli leads to production of pro-inflammatory mediators in a sequential manner whose early effect is pain, tissue destruction then followed by healing and recovery [10]. To relieve inflammation, NSAIDs such as diclofenac, ibuprofen and indomethacin are highly prescribed around the world [11]. However, these drugs only provide asymptomatic relief and the greatest

disadvantage lies in their toxicity to liver, kidney and reappearance of symptoms after discontinuation [12].

According to a study by WHO, a world population of between 75-80% still depends on herbal medicines [13]. Therefore, medicinal plants are assuming important role in their wellbeing [14]. Although the root extract and leaf extract of *Clutia abyssinica* is widely used by the Kalenjin community to treat inflammation there is no scientifically documented data about its aforementioned folklore use [15]. The present study was therefore designed to evaluate the anti-inflammatory activity of DCM root extract of *Clutia abyssinica* in animal models.

## Materials and Methodology

### Plant sample collection and preparation

Sample roots of *C. abyssinica* were sourced from Kaptebee village, Turbo sub-county in Uasin Gishu county Kenya from its natural habitat under accepted bio-conservation methods with the help of local herbalist in January 2016, a period in which the herbal plant is believed to have its maximum healing activity. A sample of fresh twigs was botanically authenticated by an acknowledged taxonomist and a sample voucher deposited at the National Museum of Kenya herbarium with the voucher number (NMK/BOT/CTX1/2). The sample roots

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were washed with clean water to remove stuck soil and chopped into small pieces, air dried for 14 days. The samples were then packed into paper envelopes and transported to Kenyatta University Biochemistry laboratory where they were milled into coarse powder.

### Extraction

A quantity of 400 g pulverized root sample was soaked in 1300 ml of DCM for 48 h with occasional shaking within the first 8 h on hourly basis to uniformly mix the sample. DCM has a lower boiling point, dissolves most non polar compounds in the extract hence it was chosen as appropriate solvent. Whatman's filter paper No. 1 was used to filter the extract. Rotary evaporator was used to concentrate the extract under reduced pressure at a temperature of 41°C and the concentrate (4 g) stored in closed containers at 4°C until use in bioassays.

### Experimental design

**Laboratory animals:** Swiss albino mice aged between 6-7 weeks, weighing between 19-24 g were utilized for screening anti-inflammatory effects of the extract. The animals were kept in standard laboratory cages in animal house at Kenyatta University in the Department of Biochemistry and Biotechnology. The experimental animals were fed on rodent pellets and allowed access to water *ad libitum*. Standard procedures and ethical guidelines for handling animals in the study were followed [14].

**Determination of anti-inflammatory effect:** Edema was induced by injecting 0.05 ml of 1% (w/v) carrageenan solution into the sub-plantar region of right hind paw of the Swiss albino mice according to procedure described by Jia et al. [16]. Thirty Swiss albino mice (male) were grouped into 6 groups of 5 animals each and treated as follows; each mouse in group I (normal control) was administered with the vehicle (10% DMSO) only but inflammation was not induced. All the mice in Group II (negative control) were induced with inflammation and administered with the vehicle (10% DMSO) while those in group III (positive control) were induced with inflammation and administered with diclofenac (15 mg/kg body weight) thirty minutes prior to administration of 1% carrageenan solution into the sub-plantar region of right hind paw of Swiss albino mice. Animals in groups IV-VI were induced with inflammation and administered with the plant extract at 50 mg/kg, 100 mg/kg and 150 mg/kg body weight respectively, thirty minutes prior to administration of 1% carrageenan solution. The reference drug and the plant extracts were administered intraperitoneally. This design is summarized in Table 1

Measurement of the hind paw diameter was carried out using a Vernier calliper in order to find out the diameter of right hind paw immediately before and after 1 h, 2 h, 3 h and 4 h following carrageenan injection. Percentage inflammation inhibition was calculated according to the formula described by Winter et al. [17].

$$\% \text{ paw edema inhibition} = \frac{Ct - Tt}{Ct} \times 100$$

Where:

Ct: Paw diameter at 1 h after carrageenan administration (control);

Tt: Paw diameter after treatment.

**Qualitative phytochemical screening:** Qualitative phytochemical screening was performed on the plant extract to determine presences or absences of bioactive compounds following a protocol described by Kokate [18]. The following phytochemicals compounds associated with

Group	Status	Treatment
I	Control	DMSO
II	Negative control	Carrageenan+DMSO
III	Positive control	Carrageenan+15 mg/kg/bw diclofenac
IV	Experimental group A	Carrageenan+DMSO+50 mg/kg/bw extract
V	Experimental group B	Carrageenan+DMSO+100 mg/kg/bw extract
VI	Experimental group C	Carrageenan+DMSO+150 mg/kg/bw extract

Carrageenan=1%; DMSO=10%

**Table 1:** Treatment protocol for evaluation of anti-inflammatory activity.

anti-inflammatory activities were tested saponins, flavonoids, alkaloids, terpenoids, phenolics, cardiac glycosides and steroids (Tables 2 and 3).

**Data management and statistical analysis:** Data on hind paw edema diameter was obtained from the laboratory animals, recorded and entered into Microsoft excel program. It was then exported to Minitab statistical software version 17.0 for descriptive statistics and inferential analysis. Results were expressed as Mean  $\pm$  Standard error of the mean. One way ANOVA was used to analyse for statistical significance among the groups followed by Tukey's post hoc test for separations of means.

### Results

#### Anti-inflammatory activity of DCM root extract of *C. abyssinica*

Generally, the DCM root extract, at the three doses demonstrated an anti-inflammatory activity on carrageenan-induced paw edema in Swiss albino mice. This was indicated by reduction in the diameter of the hind paw after administration of the three extract doses (Table 2 and Figure 1). In the first hour, the DCM root extract of *C. abyssinica*, at 50 mg/kg, 100 mg/kg and 150 mg/kg body weight, reduced the inflamed hind paw diameter by 0.88%, 1.88% and 3.30% respectively while diclofenac reduced by 2.21% (Table 2; Figure 1). The anti-inflammatory activities of DCM root extract of *C. abyssinica*, at all the three extract doses were significantly different ( $P < 0.05$ ; Table 2). However, the anti-inflammatory activities of DCM root extract of *C. abyssinica*, at 100 mg/kg body weight and reference drug (diclofenac) were not significantly difference ( $P > 0.05$ ; Table 2).

In the 2<sup>nd</sup> h, the DCM root extract of *C. abyssinica*, at 50 mg/kg, 100 mg/kg and 150 mg/kg body weight and diclofenac reduced the inflamed hind paw diameter by 1.47%, 3.04%, 3.76% and 3.57% respectively thereby demonstrating anti-inflammatory activities (Table 2; Figure 1). The anti-inflammatory effects of the extract at the doses of 100 mg/kg and 150 mg/kg body weight as well as diclofenac (reference drug) showed no significant difference ( $P > 0.05$ ; Table 2). However, the anti-inflammatory activity of the extract at the dosage of 50 mg/kg body weight was significantly different from the extract at the dosages of 100 mg/kg and 150 mg/kg body weight and diclofenac (reference drug) in the 2<sup>nd</sup> h ( $P < 0.05$ ; Table 2).

In the 3<sup>rd</sup> h, the extract at 50 mg/kg, 100 mg/kg and 150 mg/kg body weight and reference drug reduced the inflamed hind paw diameter by 1.61%, 3.59%, 4.64% and 5.01% respectively (Table 2; Figure 1). The anti-inflammatory effects of the extract at dose of 100 mg/kg body weight and 150 mg/kg body weight were not significantly different ( $P > 0.05$ ; Table 2). The anti-inflammatory activity of the extract at 150 mg/kg body weight and diclofenac showed no significant differences ( $P > 0.05$ ; Table 2 and Figure 1).

In the 4<sup>th</sup> h, the extract at the doses of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight reduced inflamed hind paw diameter by

Group	Treatment	Percentage change in paw diameter in (mm)				
		0 h	1 h	2 h	3 h	4 h
Normal Control	10% DMSO	100.00 ± 0.00	99.92 ± 0.08 <sup>d</sup>	99.92 ± 0.08 <sup>c</sup>	99.84 ± 0.16 <sup>d</sup>	99.92 ± 0.08 <sup>c</sup>
		0	0.08	0.08	0.09	0.08
Negative Control	Carrageenan	100.00 ± 0.00	102.40 ± 0.24 <sup>e</sup>	103.46 ± 0.42 <sup>d</sup>	104.34 ± 0.34 <sup>e</sup>	104.73 ± 0.32 <sup>d</sup>
		0	(-2.4)	(-3.46)	(-4.35)	(-4.74)
Positive Control	Carrageenan+Diclofenac (15 mg/kg bw)	100.00 ± 0.00	97.79 ± 0.09 <sup>b</sup>	96.43 ± 0.24 <sup>a</sup>	94.98 ± 0.30 <sup>a</sup>	94.64 ± 0.35 <sup>a</sup>
		0	2.21	3.57	5.01	5.35
DCM <i>C. abyssinica</i> Extract	Carrageenan+50 mg/kg bw	100.00 ± 0.00	99.12 ± 0.18 <sup>c</sup>	98.41 ± 0.28 <sup>b</sup>	98.39 ± 0.56 <sup>c</sup>	98.16 ± 0.6 <sup>b</sup>
		0	0.88	1.47	1.61	1.84
	Carrageenan+100 mg/kg bw	100.00 ± 0.00	98.12 ± 0.05 <sup>b</sup>	96.96 ± 0.22 <sup>a</sup>	96.41 ± 0.23 <sup>b</sup>	95.70 ± 0.22 <sup>a</sup>
		0	1.88	3.04	3.59	4.3
	Carrageenan+150 mg/kg bw)	100.00 ± 0.00	96.70 ± 0.14 <sup>a</sup>	96.24 ± 0.29 <sup>a</sup>	95.35 ± 0.21 <sup>ab</sup>	94.64 ± 0.18 <sup>a</sup>
		0	3.3	3.76	4.64	5.34

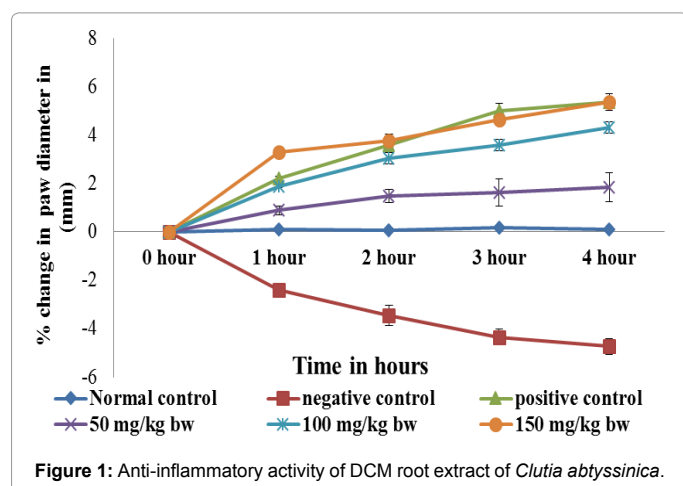
Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript letter are not significant different (one way ANOVA followed by Tukey's test) (p>0.05). Percentage reduction in the size of the edema is given within parenthesis

**Table 2:** Anti-inflammatory activities of DCM root extracts of *Clutia abyssinica* on carrageenan-induced inflammation in Swiss albino mice.

Phytochemicals	DCM root extract of <i>Clutia abyssinica</i>
Alkaloids	+
Flavonoids	+
Steroids	+
Saponins	+
Cardiac glycosides	+
Phenolics	-
Terpenoids	+

Present phytochemicals denoted by (+), absent phytochemicals denoted by (-)

**Table 3:** Qualitative phytochemical composition of DCM root extract of *Clutia abyssinica*.



**Figure 1:** Anti-inflammatory activity of DCM root extract of *Clutia abyssinica*.

1.84%, 4.30% and 5.34% respectively. The diclofenac (reference drug) reduced inflamed hind paw edema diameter by 5.35% (Table 2; Figure 1). The anti-inflammatory activities of extract at 150 mg/kg body weight and 100 mg/kg body weight were not significantly different and were comparable to diclofenac (P>0.05; Table 2). However, the anti-inflammatory effect of the extract at 50 mg/kg body weight was significantly different from 100 and 150 mg/kg body weight and reference drug (diclofenac) (P<0.05; Table 2).

## Discussion

Although during the past years there has been remarkable progress in medical science in management of inflammation using conventional

drugs [19], there is need to seek alternative methods to manage these ill indicators of health. Conventionally used anti-inflammatory drugs such as NSAIDs pose adverse effects after long term use like cardiac abnormalities, peptic ulcers, prolonged bleeding, hepatic failure and renal failure among others effects leading to their limited use in clinical settings [20]. Therefore, due these limitations and other associated problems of these conventional drugs, search of newer drugs from medicinal plants with anti-inflammatory activities is essential. In this regard, alternative medicines from natural sources such as medicinal plants are an important option into discovery of novel drugs because currently available conventional drugs are derived from ethnomedicine [21].

Evaluation of anti-inflammatory activities of DCM root extract of *C. abyssinica* was performed using carrageenan induced-inflammation in Swiss albino mice. It is the most used primary test for screening new anti-inflammatory agents and constitute a simple and routine animal model for evaluation of inflammation [22,23]. Carrageenan is obtained from a sea weed known as carrageen moss. It is a sulphated polysaccharide [24].

Carrageenan induces severe inflammatory reaction when injected into the hind paw of rats/mice and usually induces paw edema in two phases which are dependent on age and weight of the experimental animal [25,26]. The first phase (0-2 h) is due to release of inflammatory mediators such as serotonin and histamine, resulting in sensitization of central nociceptor neurons. The lysosome, protease, prostaglandins and bradykinin are released majorly in the second phase [27]. The second phase (2.5-5 h) of edema is sensitive to clinically used anti-inflammatory drugs. It is also used to access anti-inflammatory effect of natural product [28]. During the second phase prostaglandins play a major role in inflammatory reaction and can stimulate the nociceptors to induce pain [29].

The DCM root extract of *C. abyssinica* demonstrated significant anti-inflammatory activities against carrageenan-induced edema in Swiss albino mice by reducing inflamed hind paw diameter. There were correlations, between results obtained from this study and other studies done on evaluation of anti-inflammatory activity of medicinal plants using animal models. Similar study conducted on *in vivo* anti-inflammatory studies of methanolic bark extract of *Rumex vesicarius* linn using carrageenan-induced edema in Wistar albino rats exhibited anti-inflammatory activities [30]. In addition, a study conducted on anti-inflammatory properties of dichloromethane:methanolic leaf

extract of *Caesalpinia volkensi* and *Mytenus obscura* in animal models demonstrated strong anti-inflammatory activities [31]. Therefore, it is possible that DCM root extract of *C. abyssinica* inhibited production of prostaglandins that are responsible for induction of inflammation. The dose ranges (50 mg/kg, 100 mg/kg and 150 mg/kg) used in this study for bioscreening of anti-inflammatory activities of the plant extract were similar to dose range [32-34].

The DCM root extract of *C. abyssinica*, demonstrated a dose dependent response and therefore an increase in dose lead to an increase the anti-inflammatory activity. The dose dependent response was observed from the first hour to the fourth hour. The four hour recorded the highest reduction in the size of the hind paw edema followed by the third, second and the first hours respectively. The differences in percentage inhibition in the fourth hour and the first hour might be attributed to absorption, distribution and metabolism of bioactive compounds. The plant extract at 150 mg/kg body weight exhibited higher anti-inflammatory effects than the other two dose levels in the entire experimental period. This can be explained in terms of sufficient quantity of bioactive compounds in the dose level than the other two doses. Another factor is small concentration of active phytochemicals in the other two doses might have been quickly metabolized and excreted. The dose dependent response in this study was similar to studies by Mwangi et al.; Nthiga et al. [31,35].

The anti-inflammatory activity of plant extract might be attributed to one or more bioactive compounds present in the extract. Alkaloids, flavonoids, cardiac glycosides and sterols have been reported to inhibit prostaglandin pathway [36]. Moreover, flavonoids can specifically inhibit cyclooxygenase-2, phospholipase, TNF- $\alpha$  and lipo-oxygenase enzyme of arachidonic acid metabolism [37,38]. Saponins have also been shown to inhibit inflammation activity [39]. It is therefore believed that flavonoids, alkaloids, terpenoids and saponins present in the extract might have acted synergistically to bring forth anti-inflammatory activities.

## Conclusion

The DCM root extract of *C. abyssinica* showed anti-inflammatory activity by reducing inflamed paw diameter of Swiss albino mice, indicating disruption in the synthesis of the pro-inflammatory mediators. These findings imply that *C. abyssinica* may be a potential candidate to obtain an anti-inflammatory agent in management of inflammation. The present study therefore validates the traditional use of the plant the Kalenjin community.

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