Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 3, 2015

Original Article

ANTIINFLAMMATORY PROPERTIES OF DICHLOROMETHANE: METHANOLIC LEAF EXTRACTS OF *CAESALPINIA VOLKENSII* AND *MAYTENUS OBSCURA* IN ANIMAL MODELS

MWANGI B. MAINA[1*], GITAHI S. MAINA[1], NJAGI J. MURIITHI[1], MWORIA J. KIAMBI[1], ALIYU UMAR[1], NJOROGE W. ANN,[1] MWONJORIA K. JOHN[1], NGUGI M. PIERO[1], MBURU N. DAVID[1]

[1]Department of Biochemistry and Biotechnology, Kenyatta University, P. O Box 43844-00100, Nairobi
Email: mwangi.bonifacemaina@gmail.com

Received: 31 Mar 2015, Revised and Accepted: 15 May 2015

ABSTRACT

Objective: Inflammation is the reaction to injury of the living tissues. Conventional medication of inflammation is expensive and arguably associated with various severe adverse effects hence the need to develop herbal agents that are effective as alternative. *Caesalpinia volkensii* and *Maytenus obscura* are plants that grow in Mbeere County of Eastern region of Kenya. This study was designed to evaluate the anti-inflammatory activity of *C. volkensii* and *M. obscura* plants.

Methods: Experimental animals were divided in to four groups; normal group, diseased negative control group, diseased reference group and diseased experimental groups. Inflammation was inducted into the mice using carrageenan. The experimental groups were treated with leaf extracts of the plants at the concentration of 50 mg/kg, 100 mg/kg and 150 mg/kg. Anti-inflammatory activities in rats were compared with diclofenac (15 mg/kg) as the standard conventional drug.

Results: The leaf extracts of *C. volkensii* reduced paw edema by between 6.50%-13.42% while the extracts of *M. obscura* reduced it by between 4.94%-22.36%. Diclofenac reduced paw edema by between 4.11%-10.47%.

Conclusion: The phytochemical screening results showed that the extracts of *C. volkensii* had flavonoids, steroids and phenolics while the leaf extracts *M. obscura* had phenolics, terpenoids and saponins. Flavonoids, saponins and phenolics have been associated with anti-inflammatory activities. Therefore, the study has established that the DCM: methanolic leaf extracts of *Caesalpinia volkensii* and *Maytenus obscura* are effective in management of inflammation.

Keywords: *Caesalpinia volkensii*, *Maytenus obscura*, Leaf extracts, Edema, Carrageenan.

INTRODUCTION

Inflammation is the response of living tissue to noxious stimuli, such as pathogens and irritant agents, which involves changes in blood flow, increased vascular permeability and leucocytes migration to the inflamed site [1]. Although a considerable number of anti-inflammatory drugs are available for the treatment of inflammation, there is a continuous search for new compounds as therapeutic alternatives, because these drugs exert a wide range of side effects and low efficacy, especially for chronic diseases [2]. Natural products have been one of the most successful sources for the discovery of new therapeutic agents to benefit those afflicted by inflammatory diseases [3] NSAIDs are amongst the most commonly used anti-inflammatory agents and they act to inhibit COX enzymes and reduce the formation of prostaglandins. NSAIDs can cause liver damage [4] renal failure [5], aseptic meningitis [6] and can interfere with bone fracture healing [7].

A study done in the Washambaa community of Tanzania reported twenty two plants as being routinely used by the community for the treatment of pain and inflammation [8]. Not only, that plant-derived drug offers a stable market worldwide, but also plants continue to be an important source for new drugs. The study aims at providing preliminary information of producing standardized herbal formulation more effective in the treatment of pain and inflammation that are arguably less toxic, less costly and easily accessible than conventional antinociceptive and anti-inflammatory drugs.

MATERIALS AND METHODS

Collection and preparation of plant materials

The plants were collected from Siakago division, Mbeere North Sub County, Embu County, Kenya. The fresh leaves of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) were identified and collected with the help of local traditional medicinal practitioners. The samples were collected with acceptable bio-conservation methods, sorted out, cleaned, and transported in polythene bags to the Biochemistry and Biotechnology laboratories of Kenyatta University for studies. The plant samples were provided to an acknowledged taxonomist for botanical authentication and a voucher specimen deposited at the Kenyatta University Herbarium.

Sample processing and extraction

The leaves of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) were chopped into small pieces and air dried at room temperature for two weeks until they were properly dry. They were then ground into fine powder using an electric mill followed by sieving through the mesh sieve. For each sample, 200 grams of powder were weighed, soaked separately in a cold 1:1 mixture of methanol and DCM and stirred for six hours to obtain the extract. The extract was filtered using whatman filter papers and the filtrate concentrated under reduced pressure and vacuum using rotary evaporator. The concentrate was put in air tight container and stored at-4 °C before use in bioassay studies.

Experimental design

Laboratory animals

Wister mice of either sex, between 2-3 months old weighing15-35g were used in the study [9]. The animals breeding colonies were acquired and bred in the animal breeding and experimentation facility of the department of Biochemistry and Biotechnology, Kenyatta University. The animals were be kept in the standard cages and maintained under the standard laboratory conditions of ambient room temperature with 12 h lights followed by 12 h dark cycle throughout the experiments. They were fed on a standard rodent pellets diet and supplied with water *ad libitum* [10].

The animals were allowed to acclimatize for 48h before beginning the experiment. The ethical guidelines and procedures for handling

experimental animals were followed and all experimental protocols were in compliance with the institutional ethics committee on research in animals as well as internationally accepted principles for laboratory animal use and care throughout the study. All the tests were carried out during the daytime in a quiet laboratory setting with ambient illumination and temperature similar to those of the animal house.

Evaluation of anti-inflammatory activity

The animals were selected 24h prior to experimentation. The experimental animals were divided into six groups of five (n=5) and treated as shown in table 1.

Table 1: Treatment protocol for evaluation of antiinflammatory activities

Group	Status	Treatment
I	Normal control	Carrageenan only
II	Negative control	Carrageenan+10% DMSO
III	Positive control	Carrageenan+15 mg/kg diclofenac
IV	Experimental group A	Carrageenan+50 mg/kg+10% DMSO
V	Experimental group B	Carrageenan+100 mg/kg+10% DMSO
VI	Experimental group C	Carrageenan+150 mg/kg+10% DMSO

Carrageenan = 100 µg DMSO =10%

The paw edema/Inflammation was induced by carrageenan (0.0l ml, 1% w/v in normal saline) into sub-plantar tissue of right hind paw [11]. The linear paw circumference was measured at hourly interval for 4 hours [12]. The edema was measured prior to the carrageenan injection and compared with the circumference of the same paw after carrageenan injection.

Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using protocols described by [13, 14] and standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for include; flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, steroids and terpenoids.

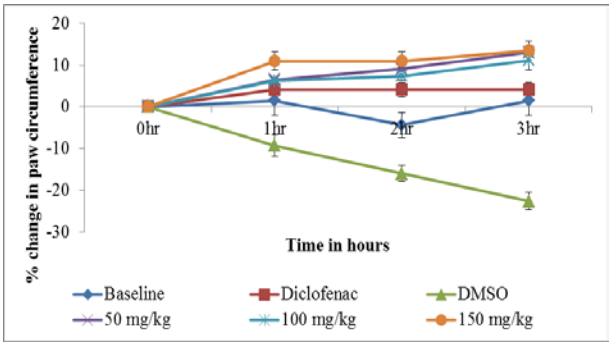

Fig. 1: Effects of DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms on carrageenan induced inflammation in mice

Data management and statistical analysis

The experimental data on the increase in the diameter of the paw were obtained from all the animals in different groups, recorded and tabulated on a broad sheet using MS Excel program. The results were expressed as mean±standard error of mean (SEM) for analysis. Statistical significance of difference among group was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests to separate the means and obtain the specific significant differences among the different groups. Unpaired student t-test was done to compare the mean activities of leaf extract of Caesalpinia volkensii and leaf extracts of Maytenus obscura. The value of $P \leq 0.05$ was considered significant. Analysis of the data was done using Minitab statistical software.

Table 2: Effects of DCM: methanolic leaf extracts of *Caesalpinia volkensii* Harms on carrageenan induced inflammation in mice

Group	Treatment	Percent change in paw circumference after drug administration			
		0h	1h	2h	3h
Baseline	None	100±0.00	98.790±3.63^{b}	104.00±3.05^{b}	98.790±3.63^{b}
Negative control	Carrageenan+DMSO	100±0.00	109.36±2.67^{a}	116.14±1.91^{a}	122.83±2.04^{a}
Posive control	Carrageenan+Diclofenac+DMSO	100±0.00	95.810±1.71^{bc}	95.810±1.71^{bc}	95.810±1.71^{b}
DCM: methanolic leaf extract	Carrageenan+50 mg/kg	100±0.00	93.500±0.10^{bc}	90.920±1.57^{c}	87.000±0.20^{b}
	Carrageenan+100 mg/kg	100±0.00	93.810±0.15^{bc}	92.640±1.11^{c}	88.880±2.24^{b}
	Carrageenan+150 mg/kg	100±0.00	98.120±2.15^{c}	89.120±2.15^{c}	86.620±2.25^{b}

All values are expressed as mean±SEM for five animals per group. Statistical comparison was made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$), Carrageenan = 100 µg; DMSO = 10%; Diclofenac = 15 mg/kg.

RESULTS

Anti-inflammatory effects of DCM: Methanolic extract of Caesalpinia volkensii Harms on carrageenan induced edema in mice.

The three dose levels (50, 100 and 150 mg/kg body weight), of the DCM: Methanolic leaf extracts of C. volkensii reduced carrageenan induced inflammation in mice. This was indicated by the reduction of the hind paw circumference.

In the first hour, the DCM: Methanolic leaf extracts of C. volkensii at all the doses levels (50, 100 and 150 mg/kg body weight) reduced the paw circumference by 6.5%, 6.19% and 10.98%, respectively (fig. 1). In this hour, the anti-inflammatory activity of the DCM: Methanolic leaf extracts of C. volkensii at all the three doses levels (50, 100 and 150 mg/kg body weight) was not significantly different compared to the positive control groups (p>0.05; table 2). However, the reduction of paw circumference by DCM: Methanolic leaf extracts of C. volkensii at the dose level of 50 and 100 mg/kg body weight and diclofenac was not significantly different compared to baseline (p>0.05; table 2).

In the second hour, the DCM: Methanolic leaf extracts of C. volkensii at all the doses levels reduced the paw circumference by 9.1%, 7.41% and 10.98%, respectively (fig. 1). In this hour, the anti-inflammatory activities of DCM: Methanolic leaf extract of C. volkensii at all the three dose levels (50 mg/kg, 100 mg/kg and 150 kg/kg body weight) were significantly different compared to baseline and negative control groups (p<0.05; table 2). Besides, in this hour, the anti-inflammatory activity of extracts at all the dose levels (50 mg/kg, 100 mg/kg and 150 kg/kg body weight) was comparable to the standard drug (diclofenac) (p>0.05; table 2).

In the third hour, the DCM: Methanolic leaf extracts of C. volkensii at the dose levels of 50, 100 and 150 mg/kg body weight reduced in paw circumference of mice to be 87.00%, 88.88% and 86.62%, respectively (table 2). At this hour, the group of mice treated with 150 mg/kg of the herbal extract exhibited the highest anti-

inflammatory effect (fig. 1; table 2). Although anti-inflammatory activities of DCM: Methanolic leaf extracts of C. volkensii at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) was not significantly different compared to baseline (p>0.05; table 2), they were comparable to diclofenac (reference drug) (p>0.05; table 2). In addition, the anti-inflammatory activities of the extracts were significantly different compared to the negative control group (p<0.05; table 2).

Effects of DCM: Methanolic leaf extracts of *M. obscura* on carrageenan induced inflammation in mice

In general, the DCM: Methanolic leaf extract of *Maytenus obscura* (A. Rich.), at all the three dose levels (50, 100 and 150 mg/kg body weight), inhibited carrageenan induced inflammation in mice demonstrated by the reduction in the paw circumference.

In the first hour of the test period, the DCM: Methanolic leaf extract of *M. obscura* at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) reduced paw circumference to 93.11%, 88.96% and 95.15%, respectively (table 3). In this hour, Although the anti-inflammatory activities of DCM: methanolic leaf extracts of *C. volkensii* at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) was not significantly different compared to baseline (p>0.05; table 3), they were comparable to diclofenac (reference drug) (p>0.05; table 3). However, the anti-inflammatory activities of the extracts were significantly different compared to the negative control group (p<0.05; table 3).

In the second hour, the percent paw circumference reduction by the DCM: Methanolic leaf extracts of *M. obscura* at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) were 11.77%, 18.52% and 11.12%, respectively (fig. 2). At this hour, the group of mice treated with 100 mg/kg of the herbal extract exhibited the highest anti-inflammatory effect (fig. 2; table 3). The DCM: methanolic leaf extracts of *M. obscura* at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) significantly reduced the carrageenan-induced paw edema compared to the baseline and negative control groups (p<0.05;Table 3) but were comparable to diclofenac (p>0.05;Table 3).

In the third hour, the DCM: methanolic leaf extracts of *M. obscura* at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) reduced the paw circumference to 77.89%, 80.11% and 87.63%, respectively (table 3). In this hour, the anti-inflammatory effects of the DCM: Methanolic leaf extract of *M. obscura* at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) were significantly different from the baseline and negative control groups (p<0.05; table 3). The anti-inflammatory effects of the DCM: Methanolic leaf extracts of *M. obscura* at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) was comparable to the reference drug. However, the group of mice treated with 50 mg/kg was even better than the other dose levels as well as diclofenac for it reduced paw circumference by 22.36% while diclofenac reduced it by 10.47% (fig. 2).

Table 3: Effect of DCM: Methanolic leaf extracts of *M. obscura* on carrageenan induced inflammation in mice

Group	Treatment	Percent change in paw circumference after drug administration			
		0h	1h	2h	3h
Baseline	None	100±0.00	98.830±3.91^{b}	103.75±4.30^{b}	98.720±3.12^{b}
Negative control	DMSO	100±0.00	112.57±5.14^{a}	119.33±5.09^{a}	120.76±5.42^{a}
Posive control	Diclofenac	100±0.00	91.890±1.37^{b}	89.600±1.05^{c}	89.600±1.05^{bc}
DCM: methanolic	50 mg/kg	100±0.00	93.110±2.06^{b}	88.530±2.29^{c}	77.890±2.99^{c}
leaf extract	100 mg/kg	100±0.00	88.960±1.11^{b}	81.360±2.28^{c}	80.110±1.71^{c}
	150 mg/kg	100±0.00	95.150±1.22^{b}	88.960±1.11^{c}	87.630±0.29^{bc}

All values are expressed as mean±SEM for five animals per group. Statistical comparison were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test (p > 0.05). Carrageenan = 100 µg DMSO = 10%; Diclofenac = 15 mg/kg.

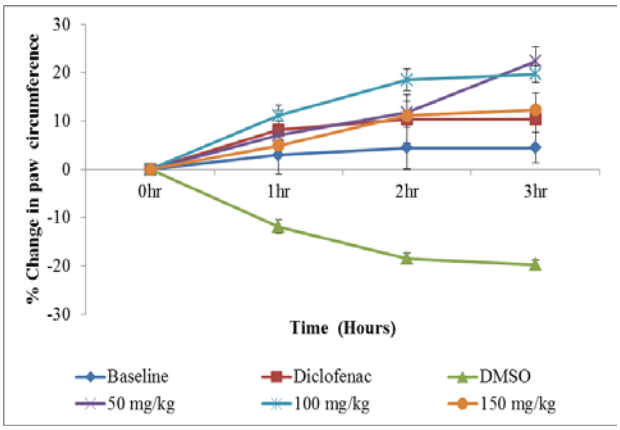

Fig. 2: Effects of *Maytenus obscura* (A. Rich.) on the percent change in carrageenan induced inflammation in mice

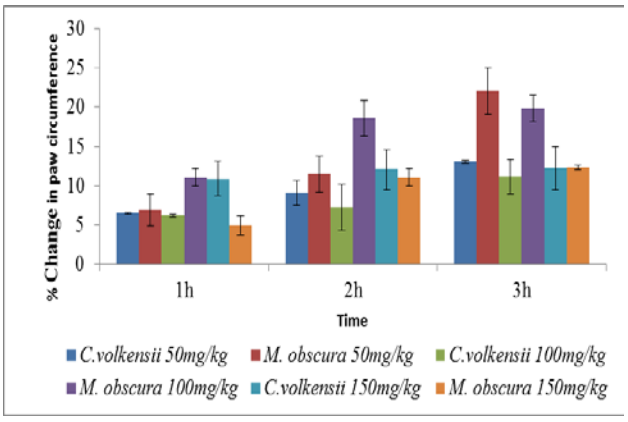

Fig. 3: Comparison of the percent change in carrageenan induced inflammation DCM: Methanolic leaf extracts of *C. volkensii* and *M. obscura* at various hours of the test period

In comparison, the DCM: Methanolic leaf extract of *Caesalpinia volkensii* Harms exhibited more anti-inflammatory effect than DCM: Methanolic leaf extract of *M. obscura* at all dose levels in the third hour of the test period. However, DCM: Methanolic leaf extract of *Maytenus obscura* (A. Rich.) at the dose level of 150 mg/kg body weight had the most effective anti-inflammatory effect than *Caesalpinia volkensii* Harms in the first and second hours (fig. 3).

Phytochemical screening

As table 4 shows, *Caesalpinia volkensii* contained flavonoids, steroids and phenolics whereas, alkaloids, terpenoids, saponins and cardiac glycosides were absent. On the other hand, *Maytenus obscura* contained phenolics, trepenoids and saponins. However, alkaloids, flavanoids, steroids and cardiac glycosides were absent (table 4).

Table 4: Phytochemistry of the *Caesalpinia volkensii* and *Maytenus obscura*

Classes of compounds	*Caesalpinia volkensii*	*Maytenus obscura*
Alkaloids	-	-
Flavonoids	+	-
Steroids	+	-
Phenolics	+	+
Terpenoids	-	+
Saponins	-	+
Cardiac glycosides	-	-

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign.

DISCUSSION

The present study was designed to evaluate the anti-inflammatory properties of the DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.). The evaluation of antiinflammation properties of the extracts was done using carregeenan induced edema in mice. Carrageenan-induced hind paw edema, cotton pellet induced granuloma and Freund's adjuvant are the standard experimental models for acute, sub-acute and chronic inflammation respectively. Carrageenan is a generic name for a family of gel-forming and viscosifying polysaccharides, which are obtained by extraction from certain species of red seaweeds. Carrageenan is often used for the testing of anti-inflammatory agents [15]. Carrageenan has been widely used as a harmful agent capable of inducing experimental inflammation for the screening of compounds possessing anti-inflammatory activities. This phlogistic agent, when injected locally into the rat paw, produces a severe inflammatory reaction, which is perceptible within thirty minutes [16, 17]. Carrageenan induced rat paw edema is a suitable *in vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting acute inflammatory mediators [18]. Carrageenan-induced hind paw edema in mice is a biphasic event. The early phase (90-180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270-360 min) is associated with the activation of kinin-like substances such as, prostaglandins, proteases and lysosome [19, 20]. Carrageenan was, therefore, selected as a phlogistic agent in this study.

After three hours of the test period, the DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) produced appreciable anti-inflammatory activity against carrageenan-induced edema in mice. The DCM: methanolic extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) demonstrated the greatest edema reduction activity by 13.42% and 22.36% at dose levels of 150 mg/kg and 50 mg/kg body weight, respectively, which compare well with diclofenac that caused a reduction of between 4.11%-10.47%. These findings are in agreement with the effects of other medicinal plants in laboratory animals. Similar work carried out by [21] demonstrated effective anti-inflammatory activity of ethanolic and aqueous extracts of *Kalanchoe pinnata* (Lam.) Pers on carrageenan induced edema in rats. In addition, related results were also observed by [22] who demonstrated anti-inflammatory activity against carrageenan induced edema in laboratory animals injected with methanolic extracts of dried leaves of *Tithornia diversifolia*.

The NSAIDs like diclofenac help reduce inflammation and associated pain. The NSAIDs block enzyme cyclooxygenase (COX) which catalyze prostaglandin biosynthesis [23]. There are two COX enzymes; COX-1 and COX-2. Both enzymes catalyze synthesis of prostaglandins which promote inflammation, pain, and fever. Non steroidal anti-inflammatory drugs (NSAIDs) block the COX enzymes and reduce prostaglandins throughout the body. As a consequence, ongoing inflammations are reduced. In general, non steroidal anti-inflammatory drugs produce their anti-inflammatory actions, through inhibition of prostaglandin biosynthesis. Therefore, it is apparent that the anti-inflammatory action of DCM: Methanolic extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) were related to inhibition of prostaglandin synthesis.

The significant inhibitory activity shown by the DCM: Methanolic extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* at all dose levels (50, 100, and 150 mg/kg body weight) over a period of 3h in carrageenan-induced inflammation was quite similar to that exhibited by the group treated with diclofenac. The highest percentage paw edema inhibition activity by DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms was 13.42% at the dose level of 150 mg/kg body weight. Previous studies with some other plants like *Plumeria acuminate* [24] showed the same effect in this model. These results indicate that the extract acts in later phase in a dose dependent manner, probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization. This dose dependent anti-inflammatory effect in later phase could be explained by passive diffusion of active principles across the cell membrane in the peritoneal cavity.

Phytochemical screening done on the DCM: Methanolic leaf extract of *Caesalpinia volkensii* Harms indicated that it contains flavonoids, phenolics and steroids. The observed anti-inflammatory effect might be due to the presence of flavonoids. Flavonoids have been reported to inhibit prostaglandin synthesis [25]. It is ubiquitously known that flavonoids have a great potential as anti-inflammatory agents[26]. Therefore, it is postulated that flavonoids in the extract may correlate appropriately for the present activities.

The dose ranges used in this study were within the dose ranges used by [27], who used doses of 50, 100 and 150 mg/kg body weight while evaluating analgesic and anti-inflammatory studies of the methanolic extract of *Anisopus manii* (N. E. Br) in rodents. [28]also used levels of 50, 100 and 200 mg/kg body weight while evaluating the antinociceptive antipyretic effects of DCM: Methanolic leaf extracts of Solanum incanum (Linneaus) in animals models. However, [29] while examining the anti-inflammatory activities of methanolic leaf extract of *Mimusops elengi* L. used dose levels of 100, 200 and 300 mg/kg body weight in rats.

The reduction in the paw circumference in case of treatment with *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) extracts were comparable to that of diclofenac administration. The leaf extracts of *C. volkensii* reduced the paw edema by between 6.50%-13.42% while the extracts of *M. obscura* reduced it by between 4.94%-22.36%. Diclofenac reduced the paw edema by between 4.11%-10.47%. Therefore, the extracts can be considered as suitable anti-inflammatory agents comparable to the standard drug (diclofenac) at all dose levels.

That the dose level of 150 mg/kg body weight of the DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms was more effective than diclofenac suggests a possible better blockage of prostaglandins biosynthesis or mimicry of diclofenac action by the active principles in the extracts.

CONCLUSION

In conclusion, the present study has demonstrated the anti-inflammatory potential of DCM: Methanolic leaf extract of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) in animal models. The significant reduction of paw circumference in mice when treated with standard drugs as well as different doses of extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) reflects that they are endowed with potent anti-inflammatory properties.

Therefore, the DCM: Methanolic leaf extract of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) might help in preventing pain complications and serve as good bio-resources for generating a readily available herb formulation that is more effective in the treatment of conditions, which is cheaper than the conventional synthetic drugs. This study, scientifically confirms and supports the traditional use of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) for management of inflammatory conditions.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the technical support availed to them by Daniel Gitonga Mwaniki and James Adino.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Amaral AJ, Kestens HHD, Megens HJ, Heuven HCM, Dibbits B, DEN dunmen J, *et al*. Application of massive parallel sequencing to whole genome SNPdiscovery in the porcine genome. BMC Genomics 2009;10:374.
2. Amaral JF, Silva MI, Neto MR, Neto PF, Moura BA, de Melo CT, et al. Antinociceptive effect of the monoterpene R-(+)-limonene in mice. Biol Pharm Bull 2007;30:1217-20.
3. Harvey LA, J Batty, J Crosbie, S Poulter, RD Herbert. Arandomized trial assessing the effects of 4 weeks of daily stretching on ankle mobility in patients with spinal cord injuries. Arch Phys Med Rehabil 2000;81:1340-7.
4. Purcell P, Henry D, Melville G. Diclofenac hepatitis. J Gut 1991;32:1381-5.
5. Fored CM, Ejerblad E, Lindblad P, Fryzek JP, Dickman PW, Signorello LB, *et al*. Acetaminophen, aspirin, and chronic renal failure. N Engl J Med 2001;345:1801-8.
6. Nguyen HT, Juurlink DN. Recurrent ibuprofen-induced aseptic meningitis. Ann Pharmacother 2004;38:408-10.
7. Wheeler P, Batt ME. Do non-steroidal anti-inflammatory drugs adversely affect stress fracture healing? Br J Sports Med 2005;39:65-9.
8. Schalage WK, Bulles H, Friedridrichs D, Kuhn M, Teredesai A. Cytokeratin expression patterns in rat respiratory tract as markers of epithelial differentiation in inhalation toxicology. Toxicol Pathol 1999;26:324-43.
9. Khan H, Saeed M, Gilani AH, Muhammad N, Haq IU, Ashraf N. Antipyretic and anticonvulsant activity of Polygonatum verticillatum: comparison of rhizomes and aerial parts. Phytother Res J 2013;27(3):468-71.
10. Vogel HG. Drug discovery and evaluation pharmacological assays. Springer-Verlag Berlin Heidelberg New York; 2002;1408:2-716.
11. Winter CA, Risky EA, Nuss GW. Carragenan-induced Edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;111:544-7.
12. Bamgbose SOA, Noamesi BK. Studies on cryptolepine inhibition of carrageenan-induced oedema. J Planta Med 1981;42:392-6.
13. Harbone JB. Phytochemical methods: a guide to modern techniques of plants analysis. Chapman & Hal Publishers: London, UK; 1998;3:60-6.
14. Kotake CK. Practical Pharmacognosy. Vallabh Prakashan, New Delhi: India; 2000;4:107-11.
15. Zacharopoulos VR, Phillips DM. Vaginal formulations of carrageenan protect mice from herpes simplex virus infection. Clin Diagn Lab Immunol 1997;4:465–8.
16. John H, Nodine MD. Chicago: Year Book Medical. Publishers Inc; 1999. p. 492.
17. Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A, Aouni M. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthisfrom* southern Tunisia. J Ethnopharmacol 2010;128:15-9.
18. Morebise O, Fafunso MA, Makinde JM, Olajide OA, Awe EO. Antiinflammatory property of the leaves of *Gongronema latifolium*. Phytother Res 2002;16:S75-S77.
19. Erdemoglu N, Turan NN, Akkol EK, Sener B, Abacioglu N. Estimation of anti-inflammatory, antinociceptive and antioxidant activities on *Arctium minus* (Hill) Bernh. ssp. minus. J Ethnopharmacol 2009;121:318-23.
20. Thomazzi SM, Silva CB, Silveira DCR, Vasconcellos CLC, Lira AF, Cambui EVF. Antinociceptive and anti-inflammatory activities of *Bowdichia virgilioides* (sucupira). J Ethnopharmacol 2010;127:451-6.
21. Shashank J Levy, I Fishbain B, Broday D. Application of distributed sensor networks for estimating exposures to air pollution in urban areas. Basel. SN; 2013.
22. Victor BO, Caleb OW, Ayodele OS, Samuel BO. Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. J Ethanopharm 2003;90:317-21.
23. Blandizzic C, Ippolito C, Segnanic C, De Giorgio R. Quantitative evaluation of myenteric ganglion cells in normal human left colon: Implications for histopathological analysis. J Cell Tissue Res 2009;336:191-201.
24. Gupta AK, Misra N. Hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxicated albino rats. Am J Pharmacol Toxicol 2006;1:17-20.
25. Alcaraz MJ, Ferrandiz ML. Modification of arachdonic metabolism by flavonoids. J Ethnopharmacol 1987;21:209-29.
26. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. Trop J Pharm Res 2008;7:1089-99.
27. Musa AM. Phytochemical and some pharmacological studies of the *Indigofera pulchra* Willd. PhD Thesis, Ahmadu Bello University, Zaria; 2008.
28. Mwonjoria JK, Ngeranwa JJ, Kariuki HN, Githinji CG, Sagini MN, Wambugu SN. Ethno medicinal, phytochemical and pharmacological aspects of solanum incanum (lin.). Int J Pharmacol Toxicol Sci 2014;2(2):17-20.
29. Ganesh M, Vasudevan M, Kamalakannan K, Kumar AS, Vinoba M, Ganguly S, *et al.* Antiinflammatory and analgesic effects of pongamia glabra leaf gall extract. Pharmacology online 2008;1:497-512.