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Research Article

Anti-inflammatory activity of selected plants used by the Ilkisonko Maasai, Kenya

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Background: The Ilkisonko Maasai are a Kenyan pastoralist community that uses indigenous plants for the management of pain and inflammatory conditions such as arthritis.

Objectives: The purpose of this study was to validate the use of plants for medicinal purposes by the Ilkisonko Maasai through testing for anti-inflammatory activity using the carrageenan-induced rat paw oedema assay.

Methodology: The methanol extracts (400 mg/kg body weight) of *Rhus natalensis* (bark), *Acacia drepanolobium* (bark), *Acacia nilotica* (bark), *Acacia reficiens* (bark), *Acacia robusta* (bark), *Grewia villosa* (bark), *Ximenia americana* (bark and leaves) and *Rhus vulgaris* (leaves) were evaluated for *in vivo* anti-inflammatory efficacy using the carrageenan-induced rat paw oedema assay. Diclofenac (20 mg/kg body weight) was used as the positive control and paw volume was measured by a plethysmometer.

Results: The maximum percentage inhibition (PI) of the extracts was observed as *Grewia villosa* (58.6% at 24 h), *Rhus vulgaris* (57.8% at 24 h), *Acacia nilotica* (55.5% at 1 h), *Ximenia americana* (54.5% at 1 h), *Acacia drepanolobium* (50.9% at 24 h), *Acacia reficiens* (47.6% at 1 h), *Rhus natalensis* (43.8% at 24 h) and *Acacia robusta* (37.4% at 24 h) ($p < 0.05$ for all PI). Diclofenac (20 mg/kg) showed a steady increase in PI from 1 h to 4 h with a maximum PI of 66.2% ($p < 0.05$) at 4 h and the lowest PI of 14.3% at 24 h.

Conclusion: All extracts of the plants assessed exhibited anti-inflammatory activity at early phase of inflammation. Additionally, extracts of five plants, namely *Rhus natalensis*, *Acacia drepanolobium*, *Acacia robusta*, *Grewia villosa* and *Rhus vulgaris* showed anti-inflammatory activity at both early and late phases of inflammation. There is need for further studies to identify phytochemicals with active anti-inflammatory activity.

Key words- Ilkisonko Maasai, carrageenan, inflammation, *Rhus*, *Acacia*, *Grewia* and *Ximenia*

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1. Introduction

An effective inflammatory response is necessary and beneficial in protecting an organism from harmful stimuli. However, an unresolved and prolonged inflammatory response leads to destruction of tissues involved and is evident in a number of pathologies (Warrington et al, 2011). In such cases, non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are some of the conventional drugs used to suppress inflammation. These drugs however, only relieve the inflammation but do not remove the trigger or treat its cause and hence may need long-term administration. Prolonged use of NSAIDs and corticosteroids cause side effects such as nephrotoxicity, hepatotoxicity, peptic ulcers, osteoporosis and Cushing's syndrome (Juthani et

al, 2017). These side effects create a compelling medical need for discovery and development of novel anti-inflammatory agents with a better safety profile. Plants with known ethnomedicinal uses present a potential source of new and potentially safer anti-inflammatory compounds.

The Ilkisonko Maasai are an indigenous pastoralist community living mostly in the southern part of Kenya and northern Tanzania. They have a rich culture which entails the use of traditional plants to preserve health. This includes the dietary use of decoctions made from medicinal plant species prevalent in their locality as food additives or for prevention and treatment of various illnesses (Johns et al, 1999). In a previous study (Kimondo et al, 2015), we documented the use of 30 plant species mainly as adaptogens, antimicrobial agents, and for the management of pain and inflammatory conditions such as arthritis among the Ilkisonko Maasai. Subsequent phytochemical screening showed that methanol extracts of eight of the 30 plant species had high phenolic and flavonoid content, as well as attractive antioxidant potential, all good indicators of biological activity including anti-inflammatory efficacy (Kimondo et al, 2019). This study therefore sought to further investigate the anti-inflammatory activity of the eight plants used by the Ilkisonko Maasai in folk medicine (Kimondo et al, 2015). The plants investigated in this study are *Rhus natalensis*, *Acacia drepanolobium*, *Acacia nilotica*, *Acacia reficiens*, *Acacia robusta*, *Grewia villosa*, *Ximenia americana* and *Rhus vulgaris*. Several plants in the genera investigated in this study, have previously reported *in vitro* or *in vivo* anti-inflammatory activity. Methanol and aqueous root bark extracts of *Grewia asiatica* (Paviaya et al, 2013), methanol extract of *Rhus vulgaris* leaves (Odongo et al, 2017), aqueous extract of *Acacia nilotica* pods (Dafallah and Al-Mustafa, 1996) and leaves and root extracts of *Ximenia caffra* (Mulaudzi et al, 2013) showed anti-inflammatory activity in cell and animal models. This study reports for the first time the anti-inflammatory activity of methanol extracts of *Rhus natalensis*, *Acacia nilotica*, *Acacia drepanolobium*, *Acacia reficiens*, *Acacia robusta*, *Grewia villosa* and *Ximenia americana*. The methanol extracts were used because they had been previously shown to have significantly higher total phenol, total flavonoid content and antioxidant activity than their corresponding aqueous extracts (Kimondo et al, 2019).

2. Methods

2.1 Materials, reagents and equipment

Lambda-carrageenan (22049), diclofenac sodium, methanol and dimethyl sulfoxide (DMSO) were obtained from Sigma Aldrich Co. (St Louis, MO, USA). Normal saline solution was bought from Dawa Limited (Nairobi, Kenya). Büchi Rotavapor R-200 rotary evaporator (Büchi Labortechnik, Flawil) was used to evaporate extracting methanol from the plant extracts. Plethysmometer LE 7500 (Panlab Harvard) was used to measure the rat paw oedema.

2.2 Plant collection and preparation of extracts

The medicinal plants were collected in December 2014, identified, catalogued and voucher specimens deposited at the University of Nairobi herbarium (Kimondo et al, 2015). These were *Rhus natalensis* (**JW2014/02**) root bark; *Acacia drepanolobium* (**JW2014/09**) stem bark; *Acacia nilotica* (**JW2014/10**) stem bark; *Acacia reficiens* (**JW2014/12**) root bark; *Acacia robusta* (**JW2014/15**) stem bark; *Grewia villosa* (**JW2014/18**) root and stem bark; *Ximenia americana* (**JW2014/19**) stem bark and leaves; and *Rhus vulgaris* (**EAO2016/063**) leaves (Odongo et al, 2017; Kimondo et al, 2019).

The appropriate plant parts were dried under shade at ambient temperature and pulverized to a fine powder. Methanol extract was obtained by macerating the powder in six times its weight using 80% v/v methanol at room temperature for 72 h. The extracts were filtered, concentrated under vacuum, and dried further in an oven at 40°C for 24 h.

2.3 Experimental animals

Adult Wistar rats weighing 190-250 grams were obtained from Small Animal Facility for Research and Innovation (SAFARI) of the Jomo Kenyatta University of Science and Technology. They were kept at a maximum of five per cage (435 × 290 × 150 mm) in a well-ventilated animal house with room temperature maintained at 20-25°C with a 12 h light/dark cycle. The animals had *ad libitum* access to rat chow (Unga Feeds™) and clean drinking water. Animal beddings comprising of saw dust, was changed once daily. The rats were allowed to habituate for one week before experimentation. Prior to the carrageenan-induced rat paw oedema test, they were fasted overnight but provided with water *ad libitum*.

2.4 Carrageenan induced rat paw oedema test

Carrageenan induced rat paw oedema was carried out as previously described (Ganga et al, 2012). Ten groups of five adult Wistar rats each, received either methanol plant extracts (400 mg/kg body weight), diclofenac (20 mg/kg body weight), or vehicle control (distilled water) in 3% w/w DMSO orally 1 h before the carrageenan injection (Adedapo et al, 2008). Acute inflammation was induced by the sub-plantar administration of 0.1 ml of 1% w/v carrageenan in normal saline in the right hind paw of the rats. The paw volume was measured (up to the tibio-tarsal junction) at time 0 (immediately before the injection), 1, 2, 3, 4 and 24 h after carrageenan injection by the volume displacement method using a digital plethysmometer. An increase in the volume of the paw was taken as an indication of oedema. The percentage inhibition (PI) of the inflammation was calculated (Pérez González et al, 2013; Meshram et al, 2016).

2.5 Statistical analysis

Data were expressed as mean paw volume. The descriptive statistics were the standard deviation of the mean and the corresponding percentage inhibition of inflammation calculated. The difference in response was analysed using ANOVA followed by the *post hoc* Tukey's test. A p-value of < 0.05 was considered significant.

2.6 Ethical considerations

Ethical approval for this study was obtained from Kenyatta National Hospital-University of Nairobi

Research Ethics Review Committee (**KNH/ERC/A/173**). The animal care and use protocol was approved by the Small Animal Facility for Research and Innovation (SAFARI) Animal Ethics Committee, Jomo Kenyatta University of Agriculture and Technology.

3. Results

Diclofenac (20 mg/kg) showed a steady increase in PI from 1 h to 4 h with a maximum PI of 66.2% at 4 h and the lowest PI of 14.3% at 24 h. All methanol extracts (400 mg/kg) showed significant inhibition of carrageenan rat paw oedema at 1 h compared to the negative control. Most extracts (RN, AD, AB, GV and RV) showed a constant decline in PI from 1 h to 3 h or 4 h except for AN, AR and XA which had their lowest PI at 24 h (14.3%, 17.4% and 17.4% respectively). Though the PI values for AN, AR and XA consistently declined from 1 h to 4 h, only the PIs of AN and XA were significantly different from the negative control at all 4 time points (AN- 55.5%, 45.8%, 32.8% and 27.8%; XA- 54.5%, 45.0%, 44.6% and 50.6%). The maximum PI for the methanol extracts was observed as GV (58.6% at 1 h), RV (57.8% at 24 h), AN (55.5% at 1 h), XA (54.5% at 1 h), AD (50.9% at 24 h), AR (47.6% at 1 h), RN (43.8% at 24 h) and AB (37.4% at 24 h). The following methanol extracts showed their highest PIs of inflammation at 1 h and 24 h, respectively; RN (41.9% and 43.8%), AD (40.3% and 50.9%), AB (37.2% and 37.4%), GV (58.6% and 55.1%) and RV (35.9% and 57.8%) (**Table 1**).

Table 1: Anti-inflammatory effects of the select plant extracts on carrageenan-induced rat paw oedema.

	Mean paw volume in mL ± SD (% inhibition, n=5) at the respective times					
CODE	**0 h**	**1 h**	**2 h**	**3 h**	**4 h**	**24 h**
C	0.86±0.11	1.24±0.21	1.38±0.22	1.45±0.22	1.56±0.22	1.39±0.14
DC	0.92±0.07	1.10 ±0.08* (52.9)	1.17±0.07* (52.7)	1.20±0.08* (54.1)	1.16±0.08* (66.2)	1.38±0.06 (14.3)
RN	0.78±0.06	1.00±0.06* (41.9)	1.16±0.06 (28.6)	1.28±0.06 (15.5)	1.29±0.09 (27.3)	1.08±0.07* (43.8)
AD	1.08±0.08	1.31±0.08* (40.3)	1.46±0.06 (27.1)	1.53±0.04 (23.7)	1.57±0.03* (30.4)	1.34±0.06* (50.9)
AN	0.95±0.12	1.12±0.12* (55.5)	1.23±0.11* (45.8)	1.35±0.14* (32.8)	1.46±0.16* (27.8)	1.40±0.11 (14.3)
AR	0.91±0.12	1.11±0.12* (47.6)	1.28±0.15* (30.5)	1.36±0.15 (24.7)	1.47±0.17 (20.7)	1.35±0.15 (17.4)
AB	0.87±0.08	1.11±0.08* (37.2)	1.23±0.05 (31.7)	1.32±0.09 (24.7)	1.32±0.09* (36.7)	1.20±0.04* (37.4)
GV	1.09±0.13	1.25±0.13* (58.6)	1.41±0.17* (39.3)	1.52±0.19 (28.4)	1.62±0.19 (25.6)	1.33±0.16* (55.1)
XA	0.90±0.04	1.08±0.06* (54.5)	1.19±0.06* (45.0)	1.23±0.06* (44.6)	1.25±0.06* (50.6)	1.34±0.04 (17.4)
RV	0.86±0.06	1.08±0.07* (35.9)	1.18±0.07* (33.7)	1.27±0.06 (26.4)	1.29±0.07* (32.9)	1.03±0.08* (57.8)

Key: C- negative control; DC- positive control (diclofenac); RN- *Rhus natalensis*; AD- *Acacia drepanolobium*; AN- *Acacia nilotica*; AR- *Acacia reficiens*; AB- *Acacia robusta*; GV- *Grewia villosa*; XA- *Ximenia americana*; RV- *Rhus vulgaris*. * Statistically significant $p < 0.05$.

4.0 Discussion

An injection of carrageenan has been shown to induce biphasic paw oedema in rats (Patrono and Baigent, 2014). Mediators such as serotonin and histamine are responsible for the swelling, redness and pain experienced during the early phase of inflammation. These mediators then induce cyclooxygenase (COX) 2 and its products, which start the second phase of inflammation (Fernando et al, 2005). Diclofenac showed significant anti-inflammatory activity, which consistently rose from the first to the fourth hour, with the highest PI at 66.2% (4 h). This is in agreement with a previous study showing a similar PI after oral administration of diclofenac (20 mg/kg) (Sakat et al, 2014). Diclofenac has a short half-life (Schweitzer et al, 2009) as illustrated by its weak activity at 24 h with a PI of 14.3%.

Acacia nilotica and *Ximenia americana* significantly inhibited paw oedema from 1 to 4 hours (AN- 55.5, 45.8, 32.8 and 27.8; XA- 54.5, 45.0, 44.6 and 50.6). This is in concurrence with previously observed anti-inflammatory activity of phytochemicals from these two plants. Niloticane, a diterpene from the bark of *Acacia nilotica*, has been reported to exhibit good COX-2 inhibitory activity (Eldeen et al, 2010) while polysaccharide rich fractions from *Ximenia americana* have anti-inflammatory and anti-nociceptive activity (Da Silva-Leite et al, 2017). Also, in Brazil, a topical ointment of *Ximenia americana* branch extract,

exhibited significant anti-inflammatory activity (Neto Júnior et al, 2019).

All methanol extracts of the plants assayed had anti-inflammatory activity during the early phase, potentially indicating the presence of compounds that inhibit biological actions of serotonin and histamine. As previously reported, the plants assayed in this study had high polyphenol content (Kimondo et al, 2019). Polyphenols have been shown to reduce the levels of pro-inflammatory markers in mice and slow down the progression of inflammation (Aboura et al, 2017). Gallic acid, a phenolic acid common in all genera evaluated in the current study, was previously reported to reduce expression of COX-2 and pro-inflammatory cytokines *in vitro* (BenSaad et al, 2017). Umbelliferone, a flavonoid from *Acacia* species, was found to reduce the production of inflammatory markers and increase apoptosis of abnormal cells, processes that can promote resolution of inflammation (Muthu et al, 2016). The good anti-inflammatory activity exhibited by *Rhus natalensis*, *Acacia drepanolobium*, *Acacia robusta*, *Grewia villosa* and *Rhus vulgaris* extracts during the early and delayed phases may be an indicator of the presence of such compounds acting during both phases. It could also mean the presence of more active metabolites 24 h after administration of the extract. Further, flavonoids in wine have been found to retain anti-inflammatory activity even after metabolism as compared to diclofenac metabolites which are only weakly active (Davies and Anderson, 1997; Fernandes et al, 2017). Apart from the phenolic compounds, other phytochemicals such as the triterpenoids from the *Acacia* and *Grewia* genera (lupenone and lupandiol, respectively) significantly decrease acute and subacute inflammation in mice as well as interlukin-1β and interferon-γ levels (Ahmadu et al, 2010; Xu et al, 2019).

5.0 Conclusion

The methanol extracts of all the plants assayed showed anti-inflammatory activity at the early phase of inflammation. Plant extracts with anti-inflammatory activity in both early and late phases were *Rhus natalensis*, *Acacia drepanolobium*, *Acacia robusta*, *Grewia villosa* and *Rhus vulgaris.* However, for a better understanding of the differences in efficacy, there is a need for further studies with different doses of each extract in order to get a dose-response relationship as well as determine the 50% inhibitory doses (ID_{50}).

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Conflict of Interest declaration

The authors declare no conflict of interest.

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