The Journal of Phytopharmacology 2017; 6(4): 239-246
Online at:www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2017; 6(4): 239-246
July- August
Received: 08-08-2017
Accepted: 26-09-2017

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Antiinflammatory, analgesic and antipyretic effects of dichloromethane stem bark extract of *Acacia mellifera*

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ABSTRACT

The present study was designed to assess the *in vivo* antiinflammatory, analgesic and antipyretic effect of the dichloromethane stem bark extract of *Acacia mellifera* on experimental animal model at three different dose levels of 50, 100 and 150mg/kg body weight respectively. In addition, phytochemical screening of the extract was done. The inflammatory effect was evaluated by use of carragenaan-induced paw edema in mice, pain was determined using formalin-induced writhing in mice and pyrexia was determined by turpentine-induced pyrexia in rats. The activity of the herbal extract was compared to diclofenac sodium and aspirin. The phytochemical analysis showed the presence of alkaloids, cardiac glycosides, flavonoid, saponins, steroids, terpenoids, tannins and phenolics. The plant extract produced marked anti-inflammatory effect in carrageenan-induced mice paw edema assay, suppressed licking response of animals in both phases of the formalin- induced test and exhibited appreciable antipyretic effects in turpentine-induced pyrexia in rats. The study thus supports the folklore use of the plant in the treatment and management of inflammation, pain and pyrexia.

Keywords: Antiinflammatory, analgesic, antipyretic, dicloromethane, *Acacia mellifera*.

INTRODUCTION

Inflammation is a bodily protective reaction towards an injury caused by irritants (chemical, physical or an infective agent) that disrupts tissue homeostasis [1].It is a processthat involves: recognition of pathogen or injury that leads to activation and release of enzymes and inflammatory mediators which in turn enhance the rapidrecruitment and migration of cells to the inflammatory site. These cellsneutralize and eliminate the injurious stimuli and initiate the healing process that results in tissue and organ restoration. Inflammation may persist due to the body's inability to eradicate the irritant ordysregulation of mechanisms of the resolution phase. This leads to chronic inflammation which is associated with diseases such as atherosclerosis, asthma and rheumatoid arthritis [2].

Pain is a very personal, unpleasant sensation associated with potential or actual tissue damage [3]. Pain is not only a symptom used to diagnose several diseases and conditions but alsohas a protective function. The organism's ability to detect noxious stimuli and engage in appropriate protective behaviours against these stimuliis essential for its survival and wellbeing.Unrelieved pain may cause suffering and inability to perform daily activities hence imposing high health costs and economic losses to the victim and society [4, 5].

Fever or pyrexia is a common medical symptom that involves increase in body temperature outside the normal range which is usually from 36.0°C to 37.5°C (97.0°F to 99.5°F). The elevated temperature creates an environment where infectious agents or damaged tissues cannot survive. Fever is caused or induced by substances called pyrogens such as microbial infections, trauma, drugs and chemicals. These pyrogens trigger the formation of cytokines like interleukins, tumor necrosis factor and interferon. These cytokines enhance the synthesis of prostaglandin E_2 (PGE_2) next to the pre-optic hypothalamus region hence elevating the body temperature through promoting heat generation mechanism and decreasing heat loss.Fever is usually accompanied by symptoms, such as sweating, chills and sensation of cold. It is exhibited in many illnesses for example; malaria, typhoid and arthritis [6, 7].

Non-steroidal anti-inflammatory drugs (NSAIDs) besidesanalgesics, antipyretic andanti-inflammatory drugs are used throughout the world to treat and manage inflammation, pain and fever. However most of these drugs are associated with side effects such as nausea,respiratory depression, gastrointestinal bleeding and addictive potential. Moreover,they are toxic to the brain cortex, hepatocytes, cardiac

muscles and glomeruli. Therefore, there is need for newanti-inflammatory, analgesics and antipyretic drugs with improved efficacy and safety [8, 9, 10].

As alternative or/and complementary interventions herbs have been used to ameliorate various diseases. The useof herbs as medicine is gradually becoming popular throughout the world. Developing nations use herbal medicine for primary health care due to its natural origin,relatively easier availability and stronger therapeutic activities with fewer (if any) side effects [11]. In addition, medicinal plants posses numerous phytocompounds that might become leads for the discovery of new drugs which may be used in the prevention andmanagement of diseases [12]. One such plant with various medicinal uses is *Acacia mellifera*.

Acacia mellifera, also known as black thorn or hook thorn, is a species of *Acacia* which is widely distributed in Kenya and other parts of Africa. In Kenya, shows *Acacia mellifera* is traditionally used to treat stomach problems [13, 14], indigestion [13], pneumonia [15], malaria [14], syphilis [16], back ache, chronic joint pain [15, 17]and in circumcision rites among the Maasai community [14]. Recent scientific studies have shown *Acacia mellifera* possesses antimicrobial, antileshmanial, hepatoprotective and antiviral activities [18, 19, 20]. However, there is no scientific data on its antiinflammatory, analgesic and antipyretic potential. Therefore, this study aimed to scientifically conform the traditional use of A*cacia mellifera* in the management of inflammation, pain and fever.

MATERIALS AND METHODS

Sample Collection

Fresh stem barks of *Acacia mellifera* were collected from Siakago Division, inMbeere North Sub County, Embu County, Kenya. The local traditional medicinal practitioners assisted in identifying the plant. The sample was collectedfrom its natural habitat. They were sorted out, cleaned, and transported in burlap sacks to the Biochemistry and Biotechnology laboratories of Kenyatta University. The plant sample was provided to an acknowledged taxonomist for botanical authentication and a voucher (001/04/2016) was deposited at the Kenyatta University Herbarium.

Sample processing and extraction

The collected stem barks were chopped into small pieces and air dried in the shade for two weeks until they were properly dried. The dried sample was then ground into fine powder by an electric mill. The powdered sample material weighing 400g was soaked in one litre of dichloromethane for 24 hours with occasional swirling to facilitate the extraction process. Whatman's filter paper No.1 was used to filter the extract and the filtrate concentrated using a rotary evaporator at about 40˚C under reduced pressure and vacuum.

Experimental animals

The male and female Swiss albino mice aged 5–6 weeks old (weighing 18–25 g) were used for both antiinflammatory and analgesic test while adult Wistar albino rats of either sex and aged 2-4 months (115-120g body weight) were used for antipyretic test. All experimental animals were housed in the animal house department of Biochemistry and Biotechnology in Kenyatta University. Ethical guidelines and procedures were adhered to when handling experimental animals [21]. The animals were kept in cages at room temperature under standard laboratory conditions. They were fed on standard pellet diet and water *ad libitum.* All the tests were carried out during the daytime in a quiet laboratory setting with ambient illumination.

Determination of Antiinflammatory activity

The antiinflammatory activities of the plant extract was determined through carragenaan-induced paw edema test. Adult albino mice were grouped into six groups comprising 5 animals each. The Group I mice (normal control) was administered with 10% dimethyl sulfoxide (DMSO) (vehicle). Group II mice (negative control) was administered with 10% DMSO. Group III mice (positive control) received the standard drug (diclofenac sodium) at a dose of 15 mg/kg body weight. Groups IV, V and VI mice (experimental groups) were treated with DCM stem bark extract of *A. mellifera* at dose levels of 50, 100 and 150 mg/kg body weight respectively. Thirty minutes post treatment, inflammation was induced by injecting 0.05 ml of 1% (w/v) carrageenan into the sub-plantar region of the right hind paw of the mice in all groups except normal control group mice. A vernier calliper was used to measure the diameter of paw edema immediately before and hourly up to 4 hours after carragenaan administration. The formula below was used to calculate the paw edema inhibitory activity [22].

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

Where;
Ct = Paw diameter at 1 hour after carrageenan administration (control)
Tt = Paw diameter after Treatment

Determination of analgesic activity

Formalin-induced writhing test was used to assess the analgesic activities of the plant extract. Adult albino mice were divided into six groups of five animals each. Group I mice (normal control) was administered with 10% dimethyl sulfoxide (DMSO) only. Group II mice (negative control) was treated with 10% DMSO, while Group III mice (positive control) received the standard drug (diclofenac sodium) at a dose of 15mg/kg body weight.Groups IV, V, and VI mice were given DCM stem bark extract of *A. mellifera* at dose levels of 50, 100 and 150 mg/kg body weight respectively. Thirty minutes post treatment, nociception was induced in mice by administration of 0.05 ml of 2.5% formalin into the left dorsal hind paw of mice in all groups except normal control group mice. A transparent glass chamber was used; the mice were individually placed in it for observation. The duration the mice spent licking or biting the injected paw was recorded [23]. Two distinct periods (early and late phases) of intensive licking/biting activity were identified and recorded separately. The early phase was recorded 1-5 minutes after administering formalin and the late phase recorded 15-30 minutes after formalin injection. The percentage licking time inhibition was then calculated using the formula below [24].

$$\%\ licking\ time\ Inhibition = \frac{C - T}{C} X100$$

Where;
C–the vehicle treated control group value for each phase
T–the treated group value for each phase

Determination of Antipyretic activity

The antipyretic activity of the plant extract was evaluated using turpentine-induced pyrexia. Healthy adult wistar albino rats were grouped into six groups of five rats each. Group I (normal control) was administered with 10% DMSO only. Group II (negative control) had induced pyrexia and was administered with 10% DMSO. Group III (Positive control) had induced pyrexia and was administered with aspirin (200 mg/kg body weight).Groups IV, V and VI had induced pyrexia and received DCM stem bark extract of *A. mellifera* at dose levels of 50, 100 and 150 mg/kg body weight respectively. Pyrexia was induced in rats by administration of 20% turpentine. All the treatments were administered intraperitoneally. The rats had their body temperatures measured rectally using a digital thermometer (MODE: YB-009). A well lubricated digital thermometer was inserted about 3cm into the rectum of the animals. Rats that showed an increase in rectal temperature of at least 0.8^0C were considered pyretic. One hour post treatment, the body temperature for each rat in groups II-VI was recorded. The mean body temperature of Wistar albino rats was recorded at 20 minutes intervals over the first hour before turpentine injection and at hourly intervals up to fourth hour after turpentine injection. Rectal temperature before and after treatment were compared, the percentage change in rectal temperature was calculated using the formula below [25, 26].

$$\%\ change\ in\ rectal\ temperature = \frac{B - Cn}{B} \times 100$$

Where;
B - Rectal temperature at 1 hour after turpentine administration
C_n - Rectal temperature after drug administration

Qualitative Phytochemical Screening

The plant extract was subjected to preliminary qualitative phytochemical screening to determine the active principles present using a standard procedure [27, 28]. The phytochemicals tested included flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, steroids and terpenoids as they have been generally associated with antiinflammatory, antinociceptive and antipyretic properties.

Data Collection and Statistical Analysis

Quantitative data on the licking time, change in the diameter of the paw and change in rectal temperatures was obtained, recorded and tabulated on a broad sheet using MS excel. The raw data was later exported to Minitab statistical software package, version 17.0 where it was subjected to descriptive statistics and expressed as mean ± standard error of mean (SEM). The quantitative data was then analysed by Analysis of Variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison and separation of means. Statistical significance was set at 95% confidence level ($P \leq 0.05$). The qualitative data on phytochemical screening was tabulated and presented in a table.

RESULTS

Antiinflammatory effects of DCM stem bark extract of *Acacia mellifera* on carrageenan-induced edema in mice models

In the first hour after treatment, the extract at doses of 50 and 100mg/kg body weight showed antiinflammatory effect by reducing inflamed hind paw diameter by 1.59% and 3.72% respectively. However, the dose level of 150mg/kg body weight did not exhibit any antiinflammatory effect (Figure 1; Table 1).The antiinflammatory activity exhibited by theextractswas statistically insignificant (p>0.05) (Table 1)compared to the control groups.

Conversely, the three experimental doses of DCM stem bark extract of *A. mellifera* (50, 100 and 150mg/kg body weight) demonstrated antiinflammatory activities by reducing the inflamed paw diameter by 4.39%, 5.40% and 2.76% respectively in the second hour (Figure 1; Table1). However, the antiinflammatory effects exhibited by the three dose levels of the stem bark extract were not statistically significant from each other as well as the normal and positive controls (p>0.05; Table 1).

Inflammation decreased in a reverse dose-dependent manner in the third hour. This trend persisted through to the fourth hour of the test period (Table 1). Treatment of the mice models with DCM stem bark extract of *A. mellifera*, at the doses of 50, 100 and 150 mg/kg body weight, reduced the paw edema diameter by 10.56%, 8.95% and 4.57% respectively (Figure 1; Table 1).Notably, the antiinflammatory effects of the three dose levels did not vary from each other significantly (p>0.05) and were comparable to the standard conventional drug (Diclofenac), which decreased the carrageenan-induced paw edema by 5.23%. The DCM stem bark extract of *A. mellifera*, at the dose level of 150 mg/kg body weight, reduced the paw edema diameter as to normal control group with comparable efficacy with Diclofenac (Table 1).

In the fourth hour, the DCM stem bark extract, at the three dose levels (50, 100 and 150 mg/kg body weight), reduced the paw diameter by 11.05%, 9.91% and 8.27% respectively (Figure 1; Table 1).Indeed, the three extract doses were as effective as the standard drug (Table 1).

Table 1: Antiinflammatory effects of DCM stem bark extract of *Acacia mellifera* on carragenaan-induced inflammation in mice models

Group	Treatment	1 hr	2 hr	3 hr	4 hr
I (Normalcontrol)	DMSO	99.91±0.09^{a} (0.09)	99.92±0.16^{ab} (0.08)	99.91±0.16^{bc} (0.09)	99.91±0.28^{b} (0.09)
II (Negative control)	DMSO+ Carragenaan	101.72±0.69^{a} (-1.72)	102.81±0.61^{b} (-2.81)	103.25±0.91^{c} (-3.25)	104.68±1.40^{b} (-4.68)
III (Positive control)	Diclofenac + Carragenaan	99.91±0.63^{a} (0.10)	98.40±0.84^{ab} (1.61)	94.77±0.78^{ab} (5.23)	91.22±0.79^{a} (8.78)
IV ((Experimental A)	*A. mellifera* extract (50mg/kg bw)+ Carragenaan	98.41±0.32^{a} (1.59)	95.61±1.61^{a} (4.39)	89.44±2.67^{a} (10.56)	88.95±2.74^{a} (11.05)
V (Experimental B)	*A. mellifera* extract (100mg/kg bw)+ Carragenaan	96.28±2.28^{a} (3.72)	94.60±2.29^{a} (5.40)	91.05±1.82^{a} (8.95)	90.09±1.93^{a} (9.91)
VI(Experimental C)	*A. mellifera* extract (150mg/kg bw)+ Carragenaan	100.97±2.11^{a} (-0.97)	97.24±2.47^{ab} (2.76)	95.43±2.62^{ab} (4.57)	91.73±2.05^{a} (8.27)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with different superscript are significantly different by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Figures in parenthesis indicate percentage paw edema inhibition. Carrageenan = 1%; DMSO acted as the vehicle; Diclofenac = 15 mg/kg.

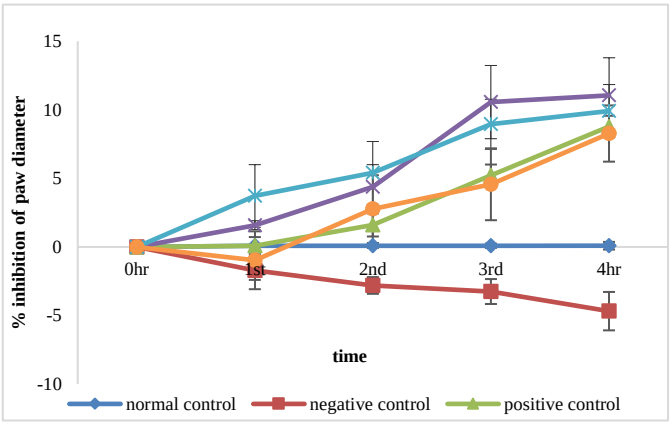

Figure 1: Percent paw edema inhibition by DCM stem bark extract of *Acacia mellifera* in carrageenan-induced inflammation in mice

Analgesic activity of dichloromethane stem bark extracts of *Acacia mellifera* on formalin-induced pain in mice models

In the early phase, the percentage inhibition of paw licking time upon administration of DCM stem bark extracts of *A. mellifera* at the three dose levels (50, 100 and 150 mg/kg body weight) was 48.26%, 1.38% and 22.96% respectively (Figure 2; Table 2) The analgesic activity of the DCM stem bark extract at the dose levels of 100 and 150mg/kg body weight wasstatistically insignificant compared to the reference drug (Diclofenac) ($p > 0.05$) (Table 2). In addition, the antinociceptive effectiveness of the two dose levels (100 and 150 mg/kg body weight) was statistically significant compared to the extract at the dose level of 50mg/kg body weight ($p < 0.05$) (Table 2).

In the late phase, the DCM stem bark extracts of *A. mellifera*, atdose levels of 50, 100 and 150 mg/kg body weight, reduced formalin-induced pain in mice by 28.45%, 57.37% and 83.90% respectively (Figure 2; Table 2).The effectiveness of stem bark extract of *A. mellifera*, at the three dose levels (50, 100 and 150 mg/kg body weight), wasstatistically significant from each other ($p < 0.05$), with the highest tested dose (150 mg/kg bw) being as effective as the reference analgesic (Diclofenac) ($p < 0.05$) (Table 2).

Table 2: Analgesic effects of DCM stem bark extract of *Acacia mellifera* on formalin-induced pain in mice model

Group	Treatment	1 st phase	2nd phase
I (Normal control)	DMSO	0.0±0.0^{a} (100)	0.0±0.0^{a} (100)
II (Negative control)	DMSDO+ Formalin	100±0.0^{a} (0.0)	100±0.0^{e} (0.0)
III (Positive control)	Diclofenac+ Formalin	87.80±7.43^{c} (12.20)	19.80±2.27^{b} (80.20)
IV (Experimental A)	*A. mellifera* extract (50mg/kg bw)+ Formalin	51.74±3.33^{b} (48.26)	71.55±1.72^{d} (28.45)
V (Experimental B)	*A. mellifera* extract (100mg/kg bw)+ Formalin	98.62±7.91^{c} (1.38)	42.63±2.52^{c} (57.37)
VI (Experimental C)	*A. mellifera* extract (150mg/kg bw)+ Formalin	77.04±6.13^{c} (22.96)	16.10±1.93^{b} (83.90)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with different superscript are significantly different by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Figures in parenthesis indicate percent paw licking inhibition. Formalin = 2.5%; DMSO acted as the vehicle; Diclofenac = 15 mg/kg.

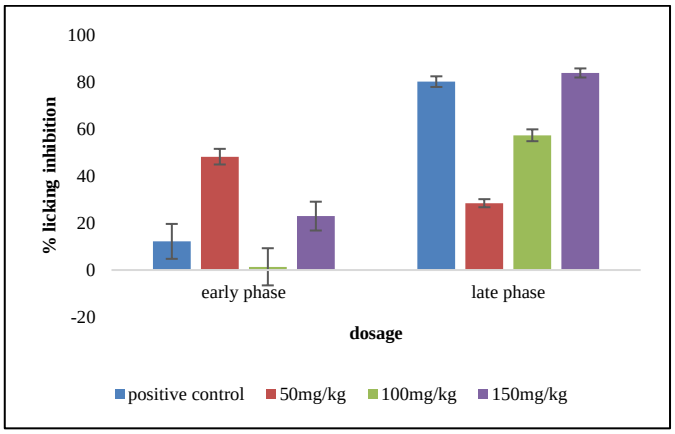

Figure 2: Percent paw licking inhibition by DCM stem bark extracts of *Acacia mellifera* in formalin-induced pain in mice.

Antipyretic activity of DCM stem bark extracts of *Acacia mellifera* on turpentine-induced pyrexia in rats

During the first hour of the test period, the DCM stem bark extracts of *A. mellifera*, at a dose level of 100mg/kg body weight, decreased the elevated rectal temperature by 0.16% (Figure 3; Table 3). However, the other two doses of the extract did not exhibit any antipyretic activity. The antipyretic effect of the three doses of DCM stem bark extract of *A. mellifera* was not statisticallysignificant compared to the three control groups ($p > 0.05$). In addition, the positive control was highly antipyretic at this hour (Figure 3; Table 3).

In the second hour, the DCM extract of *A. mellifera*, at all the three dose levels, demonstrated antipyretic action by decreasing pyrexia by 1.58%, 1.03% and 2.38% respectively (Table 3). The antipyretic effectiveness of the herbal extract at the three dose levels was statistically insignificant compared to the positive control group ($p > 0.05$) (Table 3). However, the antipyretic action of the extract at the doses of 50 and 100 mg/kg body weight was not statistically significant compared to the normal control group ($p > 0.05$) (Table 3).

In the third hour of the bioscreening period, the three doses of the extract lowered the turpentine-induced pyrexia by 2.05%, 1.65% and 2.99% respectively (Table 3). The antipyretic activity of the three

doses of the herbal extract was comparable to that of the positive control (p>0.05) (Table 3). However, the antipyretic effectiveness of the herbal extract, at the dose level of 100 mg/kg body weight, was not statistically significant compared to the normal control group (p > 0.05) (Table 3).

A similar trend was observed in the fourth hour with the three extract doses exhibiting antipyretic action by lowering pyrexia by 2.61%, 3.24% and 3.95% respectively (Table 3). However, the antipyretic activity of the three doses of the extracts was statistically insignificant compared to each other and aspirin (p<0.05) (Table 3).

Table 3: Effects of intraperitoneal administration of DCM stem bark extract of *Acacia mellifera* in turpentine-induced pyrexia in rat model

Groups	Treatment	1hr	2hr	3hr	4hr
I (Normal control)	DMSO	100.06+0.22^{ab} (-0.06)	100.11+0.25^{bc} (-0.11)	100+0.1^{b} (-0.002)	99.90+0.14^{b} (0.11)
II (Negative control)	Turpentine+DMSO	101.53+0.84^{b} (-1.53)	102.02+0.73^{c} (-2.05)	102.25+0.49^{c} (-2.25)	101.42+0.76^{b} (-1.42)
III (Positive control)	Turpentine+ Aspirin	98.49+0.19^{a} (1.52)	97.08+0.59^{a} (2.92)	96.61+0.41^{a} (3.39)	96.41+0.35^{a} (3.60)
IV (Experimental A)	Turpentine+ *A. mellifera* extract (50mg/kg bw)	100.21+0.15^{ab} (-0.21)	98.418+0.37^{ab} (1.58)	97.96+0.36^{a} (2.05)	97.39+0.31^{a} (2.61)
V (Experimental B)	Turpentine+ *A. mellifera* extract (100mg/kg bw)	99.84+0.3^{ab} (0.16)	98.97+0.51^{ab} (1.03)	98.36+0.47^{ab} (1.65)	96.76+0.45^{a} (3.24)
VI (Experimental C)	Turpentine+ *A. mellifera* extract (150mg/kg bw)	100.41+0.19^{b} (-0.41)	97.62+0.42^{a} (2.38)	97.01+0.57^{a} (2.99)	96.05+0.86^{a} (3.95)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with different superscript are significantly different by one-way ANOVA followed by Tukey's post hoc test (p< 0.05). Turpentine =20%; Aspirin = 100 mg/kg body weight and DMSO used as the vehicle.

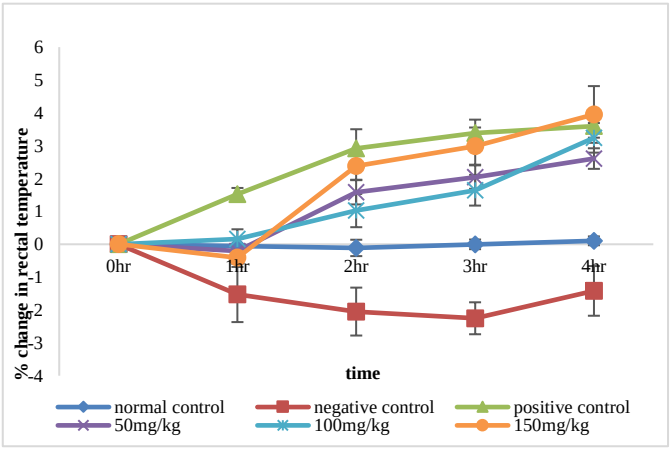

Figure 3: The percent change in rectal temperature by DCM stem bark extracts of *Acacia mellifera* in turpentine-induced pyretic rats

Phytochemical screening

Qualitative phytochemical screening of the DCM stem bark extracts of *A. mellifera* revealed presence of alkaloids, cardiac glycosides, saponins, flavonoids, phenolics, terpenoids, and steroids (Table 4).

Table 4: Phytochemical composition of DCM stem bark extracts of Acacia mellifera

Phytochemicals	Presence/absence
Phenolics	+
Cardiac Glycosides	+
Alkaloids	+
Steroids	+
Terpernoids	+
Tannins	+
Saponins	+
Flavonoids	+

Key: Presence of phytochemical is denoted by (+) sign and absent of phytochemical is

DISCUSSION

The present study assessed the *in vivo* antiinflammatory, analgesic and antipyretic effects of dichloromethane stem bark extract of *Acacia mellifera*.The antiinflammatory activities of the plant extract was evaluated using carrageenan-induced pawedema test. The edema induced by carrageenan corresponds to the acute phase of inflammationin which various mediators operate to produce the inflammatory response. Carragenaan- induced paw edema occurs in two phases (early and late phase), the early phase begins after the administration of the irritant and lasts for an hour. It is characterized by the release of serotonin, histamine, and bradykinins. Nonsteroidal antiinflammatory drugs for example indomethacin or aspirin do not inhibit this phase. The late phase occurs from the second to the fourth hour after the administration of the irritant and is ascribed to the release of prostaglandins, oxygen-derived free radicals, lysosome enzymes and proteases.Most drugs show their anti-inflammatory response at this phase.Moreover, carrageenan-induced edema test is a standard experimental model for assessing the antiinflammatory activities of natural products as well as synthetic chemical compounds [30, 31].

The DCM stem bark extract of *A. mellifera* slightly inhibited inflammation in the early phase and significantly inhibited it in the late phase, with the highest antiinflammatory effect of 3.72% and 11.05% respectively. The herbal extract's ability to reduce paw edema in both phases of the carrageenan-induced edema in mice suggests the involvement of the herbal extract's active principles in inhibiting the release or action of the early and late phase mediators of inflammation thereby suppressing edema. The active principles with good antiinflammatory potential include flavonoids, terpenoids, saponins and tannins. Flavonoids and saponins may have act synergistically to reduce inflammation by inhibiting key enzymes such as cyclooxygenase, lipoxygenase and nitric oxide synthase which are involved in the production of inflammatory mediators and metabolism of arachidonic acid [30]. The inflammatory process is accompanied by the production of free radicalsespecially in the late phase of carrageenan test [32]. Flavonoids, tannins and terpenoids are antioxidants that scavenge these free radicals through their redox properties that allows them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators. Additionally, tannins play an essential role in promoting wound healing [33].

The extract in the first and second hour was less effective compared to the third and fourth hour where it had stronger antiinflammatory activity. This suggests that the active components in the extract perhaps had to undergo biotransformation to have antiinflammatory activity. In addition, *Acacia mellifera* demonstrated reverse dose-dependent antiinflammatory response especially from the third hour, with the highest antiinflammatory effect at the dose of 50 mg/kg body weight. The reverse dose-dependent activities may be due to saturation of the receptors with the active principles in the higher dose level or it took a longer time to deliver the high dose across the peritoneum hence delaying its activity.

The reverse dose-dependent antiinflammatory activity was observed in the methanolic extracts of *Sargassum swartzii* on carrageenan-induced edema in rats [34]. Moreover, dichloromethane: methanolic leaf extracts of *Maytenus obscura* demonstrated significant reverse dose-dependent antiinflammatory effects on carrageenan-induced edema in mice [35]. Similar results were observed in work done by [36] on the methanolic extract of garden egg (*Solanum aethiopicum*) on egg albumin-induced edema in rats.

The evaluation of analgesic properties of DCM stem bark extract of *A. mellifera* was performed using formalin-induced paw licking test. Pain induced by formalin is similar to clinical pain and it takes place in two distinct phases. The early phase begins immediately after formalin injection and last for five minutes, and is probably due to the activation of nerve fibers (nociceptors). The late phase is initiated 15–30 minutes after formalin administration and is mainly mediated by histamine, prostaglandins, bradykinins and serotonin. Centrally acting analgesics inhibit both the early and late phases while peripheral drugs such as NSAIDs and steroids have the ability to interfere with the pain mediators in the late phase [10, 37].

The DCM stem bark extract of *A. mellifera* exhibited antinociceptive effect as it reduced formalin-induced paw licking time in both phases, with significant activity in the second phase. This phenomenon could be attributed to inhibition of either the synthesis and/or release of pain mediators or through nociceptor blockage by analgesic principles. Plants containing organic acid, saponins,alkaloids and flavonoids are known to show significant analgesic activities. Flavonoids and alkaloids inhibit prostaglandins involved in pain perception [38, 39].

In the early phase, the stem bark extract of *A. mellifera* exhibited a non-dose dependent behavior and a dose-dependent effect in the late phase of the formalin-induced paw licking test. The dose dependent behavior might be due to fast metabolism and clearance of the lower dose or the lower dose had an insufficient concentration of the bioactive constituents or inactivation of the active principles in the lower concentration. The non-dose dependent behavior may be because it took the higher concentration a longer time to be absorbed across the peritoneum cavity.

These findings related with previous studies that assessed the analgesic activities of medicinal plants. A study done by [40] on the aqueous stem extract of *Cynanchum viminale* (L) showed that the extract had non dose-dependent analgesic activity in both phases of formalin-induce pain in mice. However, a study on antinociceptive activity of methanolic stem bark extract of *Harrisonia abyssinica* showed dose-dependent activity in both acute and chronic phases of the formalin-induced pain model in mice [41].

The stem bark extract of *A. mellifera* was also investigated for antipyretic effects using turpentine-induced pyrexia in rats. Turpentine is an exogenous pyrogen that induces fever indirectly by initiating the synthesis and release of endogenous pyrogens such as pro-inflammatory cytokines from the host phagocytic cells. These pyrogens, in turn, act by increasing the concentration of prostaglandins E_2 on the thermoregulatory center in the hypothalamus thus raising the core body temperature [42].

The DCM stem bark extract of *A. mellifera* showed appreciable antipyretic activity after four hours of the test period, with 150 mg/kg body weight dose demonstrating the highest reduction in rectal temperature. The ability of the studied extract to reduce the elevated temperature suggests the ability of the extract to cross the brain blood barrier or the action of active principles in the extract which inhibitedprostaglandins biosynthesis or the active principles' ability to stimulate the body to produce its own antipyretic substances such as vasopressin and arginine [43].

Steroids, tannins, alkaloids, flavonoids, saponins and terpenoids are associated with good antipyretic activity. Steroids, tannins, alkaloids and terpenoids are predominant inhibitors of PG synthetase while flavonoids inhibit the production of tumor necrosis factor-α, which stimulates the synthesis of PGE_2 necessary for fever induction. In addition, saponins inhibit the enzymes cyclooxygenase and phospholipase A_2which are involved in the development of pyrexia [44, 45].

The stem bark extract of *A. mellifera* reduced the elevated temperature in a time and dose-dependent manner. The dose-dependent response was evident in the later hours of the test period. These observations could be due tothe factthe lower dose had an insufficient concentration of the bioactive constituents. In addition, the time dependent activity could be attributed to the fact that it took time for bioactive compounds tobe absorbed across the peritoneum cavity thereby causing antipyretic activities. These findings are consistent with a study on the methanolic and ethyl acetate extracts of *Acacia hydaspica* on brewer's yeast-induced pyrexia in Sprague Dawley rats [46]. The dichloromethane: methanolic root bark extracts of *Carissa edulis* showed a dose-dependent antipyretic activity on turpentine-induced pyrexia in rats [42]. Similarly dose dependent antipyretic activities were observed inmethanolic extracts of *Kigelia africana* and *Acacia hockii* on turpentine-induced pyrexia in rats [45].

Non-steroidal anti-inflammatory drugs such as aspirin are used to treat and manage fever in routine practice. Aspirin exerts its antipyretic activity by inhibiting both cyclooxygenase and prostaglandin synthetase enzyme within the hypothalamus [47]. The DCM stem bark extract of *A. mellifera* was as effective as aspirin in this study, thus suggesting mimicry of aspirin action by the bioactive components of the extract. In addition, a study on some *acacia* species showed that the DCM stem bark extract of *A. nubica, A. nilotica* and *A. senegal* had high selective COX-2 inhibition properties [48]. Therefore, it is possible that the action of the DCM extract of *A. mellifera* may be due to the inhibition of cyclooxygenase and/or prostaglandin synthetase, but other possible mechanisms for blocking fever cannot be ruled out.

The therapeutic benefits of traditional medicine are believed to be due to a combination of active principles [49]. The qualitative phytochemical screening of dichloromethane stem bark extract of *A. mellifera* indicated the presence of flavonoids, steroids, alkaloids, saponins, cardiac glycosides, phenolics, terpenoids and tannins. These phytochemicals are associated with good antiinflammatory, analgesic and antipyretic potential [33, 38, 39, 44, 49]. Therefore, the antiinflammatory, analgesic and antipyretic activities of the stem bark extract of *A. mellifera* could be due to the overall effect of the plant constituents.

CONCLUSION

The dichloromethane (DCM) stem bark extract of *Acacia mellifera* showed antiinflammatory, antinociceptive, and antipyretic activities. Stem bark extract of *A. mellifera* displayed significant antiinflammatory effect in acute inflammation, and significantly inhibited pain sensation through both peripheral and central mechanisms. Moreover, the herbal extract also exhibited appreciable antipyretic effects. Therefore, this study scientifically confirms the traditional use of *Acacia mellifera* for the management of inflammation, pain and pyrexia. It may also serve as a more effective alternative and complementary treatment strategy to the conventional interventions.

Acknowledgement

The authors acknowledge Dr. John K. Mwonjoria, Mr. Daniel Gitonga and Mr. James Adino for their technical support.

Conflict of Interest

The authors declare no conflict of interest.

Funding

The study received no funding.

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HOW TO CITE THIS ARTICLE
Veronica SA, Cheruiyot KS, Bosibori MJ, Munene IM, Murugi NJ, Piero NM. Antiinflammatory, analgesic and antipyretic effects of dichloromethane stem bark extract of *Acacia mellifera*. J Phytopharmacol 2017;6(4):239-246.