Medicinal & Aromatic Plants

Safari et al., Med Aromat Plants 2016, 5:2
http://dx.doi.org/10.4172/2167-0412.1000237

Research Article Open Access

Anti-pyretic, Anti-inflammatory and Analgesic Activities of Aqueous Leaf Extract of *Urtica Dioica* (L.) in Albino Mice

Safari VZ*, Ngugi MP, Orinda G and Njagi EM

Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya

Abstract

Urtica dioica has been used to manage several diseases including pain, inflammation and fever. However, its efficacy has not been scientifically validated. The aim of this study therefore is to investigate the analgesic, antipyretic and anti-inflammatory activities of its aqueous extracts. The plant extract was collected from Loita division, Narok County in Kenya. A total of 96 albino mice with an average weight of 20 g were used for this study. Analgesic activity was determined by use of formalin−induced writhing test. A writhe was recorded by a stopwatch following the stretching of the abdomen and/or stretching of at least one hind limb. Anti-inflammatory activity was established by a formalin induced inflammation test. Hourly changes in paw sizes and reduction of edema around the paw was determined using a venier calipers. Antipyretic activity was carried out using Brewer's yeast induced pyrexia. Temperature of each mouse was determined rectally by thermal probe thermometer. The aqueous leaf extracts of *Urtica dioica* reduced pain, inflammation and fever mostly at dose 150 mg/kg body weight. Based on these findings it was concluded that the present study has demonstrated the analgesic, anti-inflammatory and antipyretic potential of aqueous leaf extracts of *Urtica dioica* in albino mice and will serve as good bio-resource for generating readily available herbal formulations that are more effective in the treatment of pain, inflammation and fever conditions which are cheaper than the conventional synthetic drugs and have no side effects.

Keywords: *Urtica dioica*; Pyrexia; Antinociceptive activity; Antiinflammatory activity

Introduction

Urtica diocia L (family Urticaceae) commonly known as stinging nettle has, for a long time, been used as herbal remedy to a vast array of diseases [1]. The most common ailments treated by this species are bites and stings from insects and burns. The plant is an annual shrub that grows to 0.6 m tall and commonly occurs in the temperate zones of Asia, America, Europe and parts of Africa [1]. The shrub is covered with stinging trichomes that contain histamine, acetylcholine and formic acid that cause irritation and blistering of the skin. For this reason, it has been undervalued by most communities [1]. Despite this limitation, the stinging nettle has been extensively used for medicinal purposes [2-4]. For instance, fresh juice from the plant has been reported to stimulate the digestive system and flow of milk in nursing mothers, provide temporary relief from pain as well as treating fever and diabetes [2,3]. It has also been shown to counter symptoms of allergies [5] and increase thyroid function [1]. Although this shrub has been used from the ancient to modern times with great success achieve, the mode of preparation of the medicinal compounds and administration (popularly cooked and orally taken) could lead to less efficiency. It is therefore important to scientifically determine the right dosage as well as the time of action in vitro through extraction of active compounds and testing on experimental animals.

Materials and Methods

Collection and preparation of plant materials

Fresh leaf material of *U. dioica* was collected from Loita division, Narok county in Kenya. This plant is believed by the locals to have medicinal value against wounds and diabetes. The plant material was identified and authenticated with help from the Department of Botany, Kenyatta University. Preparation of plant extract was carried out using a protocol as described by [6]. The powdered materials were kept at room temperature away from direct sunlight in closed dry khaki paper bags.

Extraction

The powdered material was separately extracted with single distilled water at 125 g/L on a 60°C water bath for 6 hours. The solvent extract was then concentrated to dryness under reduced pressure and the residue preserved at 4°C for future use. About 375 g of *Urtica dioica* was dissolved in 3 L of single distilled water in a conical flask and the mixture put on the water bath. Decantation and filtration processes through a No.1 Whatman filter paper were repeated until the sample became clear. The filtrate was freeze-dried, weighed and stored in an airtight plastic bag and refrigerated until it was used for bioassay. This procedure gave 76 g of freeze-dried *Urtica dioica*.

Preparation of reagents and extracts used for bioassay

The plant extract for determination of analgesic, anti-inflammatory and antipyretic activities were prepared in the following manner. 0.1 ml normal saline was used as a control on each laboratory animal and for preparation and dissolving of reagents, standard drug and each plant extract and was obtained from freshly dissolved 0.85 g Nacl in 100 ml distilled water. 0.05 ml of 2.5% formalin used to induce pain and also inflammation on each laboratory animal was obtained from 2.5 ml of 40% formaldehyde raised with 100 ml normal saline. 50 mg/kg, 100 mg/kg and 150 mg/kg dose plant extract were prepared from dissolving 0.005 mg, 0.01 mg and 0.015 mg respectively of freeze-dried

***Corresponding author:** Safari VZ, Department of Biochemistry and Biotechnology, P.O Box 43844-00100, Nairobi, Kenya, Tel: +254-728670104; E-mail: vickylennox@yahoo.com

Received December 04, 2015; **Accepted** February 08, 2016; **Published** February 11, 2016

Citation: Safari VZ, Ngugi MP, Orinda G, Njagi EM (2016) Anti-pyretic, Anti-inflammatory and Analgesic Activities of Aqueous Leaf Extract of *Urtica Dioica* (L.) in Albino Mice. Med Aromat Plants 5: 237. doi:10.4172/2167-0412.1000237

plant material in 0.5 ml normal saline each and 0.1 ml of prepared plant extract was used per animal as treatment. 10 ml/kg of 15% w/v yeast subcutaneously injected to induce pyrexia in each mouse was prepared by dissolving 0.03 g in 0.2 ml of normal saline. Paracetamol was the reference drug for fever and was prepared by dissolving 0.286 mg of paracetamol tablet in 0.1 ml normal saline and given orally to each mouse. 75 mg/ 3 ml Sodium diclofenac was the reference drug administered to the standard group to treat inflammation and was prepared by adding 10 µl of diclofenac to 0.1 ml normal saline and administered per mouse. The diclofenac sodium for treatment of pain was prepared from 12 µl of 75 mg/ 3 ml Sodium diclofenac added to 0.1 ml normal saline and injected per mouse.

Animal models

Swiss albino mice of average weight of 20 g were used in this study. These animals were maintained in the experimental room at the Animal House, Department of Biochemistry and Biotechnology, Kenyatta University. The room was set at controlled conditions of 25 ± 2°C temperature, 55% humidity and 12 hr light/12 hr darkness photoperiod regime to acclimatize the animals. The mice were kept in a cage and fed with standard laboratory food and water *ad libitum*.

Experimental design

Determination of analgesic activity: To determine the analgesic activity of the plant extract, a formalin–induced writhing test was carried out using a method described by [7]. The mice were individually placed in a glass beaker and observed for writhing. The number of stretches per animal was recorded for the following 30 minutes. A writhe was recorded following the stretching of the abdomen and/or stretching of at least one hind limb according to [8] (Table 1).

Determination of anti-inflammatory activity: To determine the anti-inflammatory effect of the extract in mice, a formalin induced inflammation test was carried out as described by [8]. Inflammation was induced by intraperitoneal injection of 0.05 ml of 2.5% formalin into the left hind paw of each mouse (Table 2). Hourly changes in paw sizes and reduction of edema around the paw was determined using a venier calipers.

Group	Status	Treatment
I	Control	Normal saline (0.1 ml)+Formalin (0.05 ml of 2.5% formalin)
II	Baseline	Formalin (0.05 ml of 2.5% formalin)
III	Standard	Diclofenac (12 µl of 75 mg/3 ml diclofenac sodium+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)
IV	Test-1	50 mg/kg extract (0.001 g+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)
V	Test-2	100 mg/kg extract (0.002 g+0.1ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)
VI	Test-3	150 mg/kg extract (0.003 g+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)

Table 1: Treatment protocol for the determination of analgesic activity for the aqueous leaf extract of *Urtica dioica*.

Group	Status	Treatment
I	Control	Normal saline (0.1 ml)+Formalin (0.05 ml of 2.5% formalin)
II	Baseline	Formalin (0.05 ml of 2.5% formalin)+Formalin (0.05 ml of 2.5% formalin)
III	Standard	Diclofenac (10 µl of 75 mg/3 ml diclofenac sodium+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)
IV	Test-1	50 mg/kg extract (0.001 g+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)
V	Test-2	100 mg/kg extract (0.002 g+0.1ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)
VI	Test-3	150 mg/kg extract (0.003 g+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)

Table 2: Treatment protocol for the determination of anti-inflammatory activity for the aqueous leaf extract of *Urtica dioica*

Determination of antipyretic activity: The antipyretic activity of the plant extract was evaluated using Brewer's yeast induced pyrexia as described by [9]. According to the protocol, 15% aqueous suspension of Brewer's yeast was first prepared using normal saline (Table 3). Temperatures of each mouse was then determined by thermal probe thermometer rectally at hourly interval for three hours after extract and drug administration.

Results

Aqueous leaf extract of *Urtica dioica* reduced acute pain significantly in a dose dependent manner (Figure 1 and Table 4). At the dose level of 150 mg/kg body weight, the aqueous leaf extracts exhibited significant

Group	Status	Treatment
I	Control	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast+0.2 Normal saline)+Normal saline (0.1 ml)
II	Baseline	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast+0.2 Normal saline)
III	Standard	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast+0.2 Normal saline)+Paracetamol (0.286 mg in 0.1 ml normal saline)
IV	Test-1	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast+0.2 Normal saline)+50 mg/kg extract (0.001 g+0.1 ml Normal saline)
V	Test-2	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast+0.2 Normal saline)+100 mg/kg extract (0.002 g+0.1ml Normal saline)
VI	Test-3	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast+0.2 Normal saline)+150 mg/kg extract (0.003 g+0.1 ml Normal saline)

Table 3: Treatment protocol for the determination of antipyretic activity for the aqueous leaf extract of *Urtica dioica*.

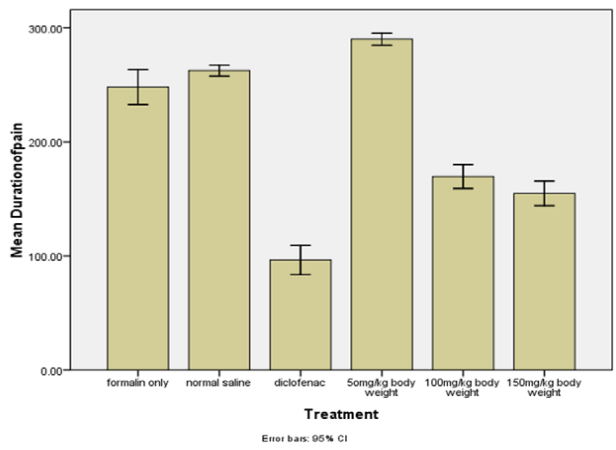

Figure 1: Analgesic effect of *Urtica dioica* aqueous extract on acute pain.

Group	Treatment	Mean paw-licking time (sec) ± SD
1 Control	Normal saline	262.4 ± 3.84^{c}
2 Baseline	Formalin	248.0 ± 12.20^{c}
3 Standard	Diclofenac	96.4 ± 10.35^{a}
4 Test-1	50 mg/kg	290.0 ± 4.18^{d}
5 Test-2	100 mg/kg	169.6 ± 8.44^{b}
6 Test-3	150 mg/kg	154.8 ± 8.64^{b}

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey's post hoc test (p>0.05). n=5

Table 4: Analgesic effect of *Urtica dioica* aqueous extract on acute pain.

analgesic effect compared with the control and baseline groups ($p<0.05$; Table 4). Diclofenac reduced pain significantly compared to the control and baseline groups ($p<0.05$; Table 4). Dose level of 100 mg/kg body weight was as effective as that at dose 150 mg/kg body weight. In late phase, aqueous leaf extract of *Urtica dioica* showed analgesic activity against formalin induced pain but not in a dose dependent manner (Figure 2 and Table 5). The group treated with plant extract at dose level of 100 mg/kg body weight showed decreased paw licking time and was statistically different from the control ($p>0.05$; Table 5). Diclofenac did reduce pain significantly compared to the control ($p>0.05$; Table 5). Treatment of mice with leaf extracts of *Urtica dioica* showed some anti-inflammatory activity against formalin-induced edema, which was indicated by reduction in paw edema (Figure 3 and Table 6). In the first hour, the paw diameter of the group of mice which had been treated with the leaf extracts of *U. dioica* at doses of 50, 100 and 150 mg/kg body weight recorded slightly lower paw diameters (Table 6). The anti-inflammatory effectiveness at dose level of 150 mg/kg was better compared to the other doses as well as the reference drug as it reduced inflammation by 88.94% (Table 6). In the second hour, the anti-inflammatory activity of *U. dioica* at all dose level (50, 100 and 150 mg/kg body weight) was found to lower the elevated paw diameter (Figure 3 and Table 6). At this hour, the mice treated with the herbal extract at dose 150 mg/kg body weight exhibited greater anti-inflammatory effect by 75.38% (Table 6). Three hours after drug administration, *U. dioica* at all dose levels (50, 100 and 150 mg/kg body weight) was found to lower the formalin-induced inflammation (Figure 3 and Table 6). The group of mice treated with 100 mg/kg showed better anti-inflammatory effect than the other dose levels by reducing paw edema to 72.88%. However, the group treated with diclofenac had greater anti-inflammatory activities for it lowered the paw diameter to 70.38% (Table 6). In the fourth hour, *Urtica dioica* exhibited anti-inflammatory activity in a dose dependent manner with dose of 150 mg/kg body weight reducing paw diameter by 61.82%. Diclofenac performed better than the other treatments as it lowered the paw diameter to 56.30% (Table 6). Treatment of mice with leaf extracts of *Urtica dioica* showed some antipyretic activity against brewer's yeast induced pyrexia, which was indicated by reduction in rectal temperature (Figure 4 and Table 7). In the first hour after treatment, plant extract at dose of 100 mg/kg body weight showed the highest antipyretic activity among the extract dosages as it reduced fever to 97.89% (Table 7). Aqueous extract of *U. dioica* exhibited antipyretic activities but not in a dose dependent manner and was seen no to be better in reducing fever than the reference drug at dose 100 mg/kg body weight (Figure 4 and Table 7). In the second hour, plant extract at dose of 100 mg/kg body weight showed the highest effectiveness in reducing the rectal temperature to 93.95% compared

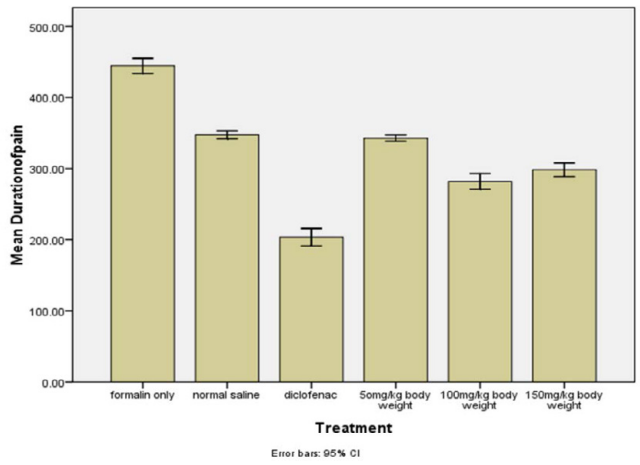

Figure 2: Analgesic effect of *Urtica dioica* aqueous extract on chronic pain.

Group	Treatment	Mean paw-licking time(sec) ± SD
1 Control	Normal saline	347.4 ± 4.39^{d}
2 Baseline	Formalin	444.4 ± 8.50^{e}
3 Standard	Diclofenac	203.6 ± 9.86^{a}
4 Test-1	50 mg/kg	342.8 ± 3.56^{d}
5 Test-2	100 mg/kg	282.0 ± 8.80^{b}
6 Test-3	150 mg/kg	298.4 ± 7.70^{c}

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey's post hoc test ($p>0.05$). n=5

Table 5: Analgesic effect of *Urtica dioica* aqueous extract on chronic pain.

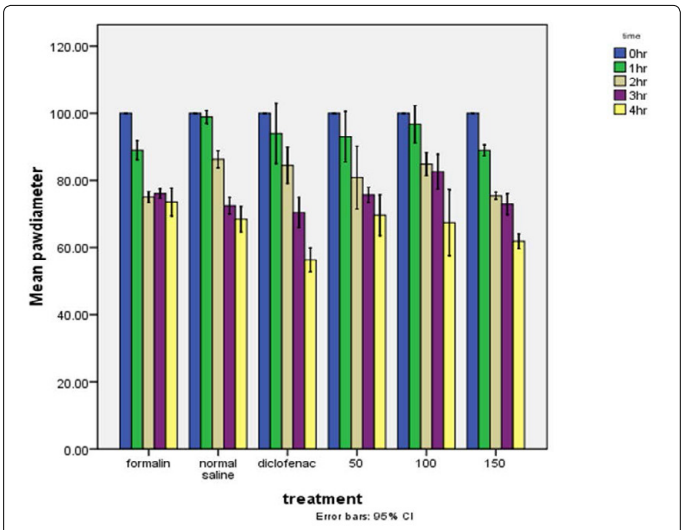

Figure 3: Anti-inflammatory effect of *Urtica dioica* aqueous extract on albino mice.

		Percent change in paw diameter (mm) after drug administration				
Group	**Treatment**	0 hr	1 hr	2 hr	3 hr	4 hr
Control	Normal saline	100.00 ± 0.00^{Ecd}	98.87 ± 1.54^{Dcd}	86.24 ± 2.06^{Ccd}	72.44 ± 1.98^{Bcd}	68.42 ± 3.04^{Acd}
Baseline	Formalin	100.00 ± 0.00^{Ebc}	88.98 ± 2.28^{Dbc}	75.01 ± 1.32^{Cbc}	76.00 ± 1.14^{Bbc}	73.54 ± 3.32^{Abc}
Standard	Diclofenac	100.00 ± 0.00^{Eab}	93.96 ± 7.23^{Dab}	84.45 ± 4.35^{Cab}	70.38 ± 3.62^{Bab}	56.30 ± 2.9^{Aab}
Test-1	50 mg/kg	100.00 ± 0.00^{Ebcd}	92.97 ± 6.05^{Dbcd}	80.80 ± 7.54^{Cbcd}	75.66 ± 1.85^{Bbcd}	69.61 ± 4.89^{Abcd}
Test-2	100 mg/kg	100.00 ± 0.00^{Ed}	96.71 ± 4.50^{Dd}	84.81 ± 2.71^{Cd}	82.54 ± 4.18^{Bd}	67.40 ± 7.94^{Ad}
Test-3	150 mg/kg	100.00 ± 0.00^{Ea}	88.94 ± 1.32^{Da}	75.38 ± 0.85^{Ca}	72.88 ± 2.50^{Ba}	61.82 ± 1.78^{Aa}

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey's post hoc test ($p>0.05$). n=5

Table 6: Anti-inflammatory effect of *Urtica dioica* aqueous extract on albino mice.

		Percent change in rectal temperature (°C) after drug administration			
Group	**Treatment**	0 hr	1 hr	2 hr	3 hr
Control	Normal saline	100.0 ± 0.00^{Ba}	94.49 ± 0.55^{Aa}	93.70 ± 0.84^{Aa}	95.28 ± 0.85^{Aa}
Baseline	Yeast	100.0 ± 0.00^{Bde}	98.10 ± 0.01^{Ade}	99.09 ± 0.21^{Ade}	99.91 ± 0.70^{Ade}
Standard	Paracetamol	100.0 ± 0.00^{Bd}	98.50 ± 0.61^{Ad}	98.96 ± 2.04^{Ad}	99.03 ± 2.20^{Ad}
Test-1	50 mg/kg	100.0 ± 0.00^{Be}	99.19 ± 0.01^{Ae}	101.44 ± 0.82^{Ae}	98.75 ± 0.77^{Ae}
Test-2	100 mg/kg	100.0 ± 0.00^{Bb}	97.89 ± 0.01^{Ab}	93.95 ± 0.02^{Ab}	95.26 ± 0.02^{Ab}
Test-3	150 mg/kg	100.0 ± 0.00^{Bc}	98.72 ± 0.10^{Ac}	97.75 ± 0.21^{Ac}	96.43 ± 0.21^{Ac}

Table 7: Antipyretic effect of *Urtica dioica* aqueous extract on albino mice.

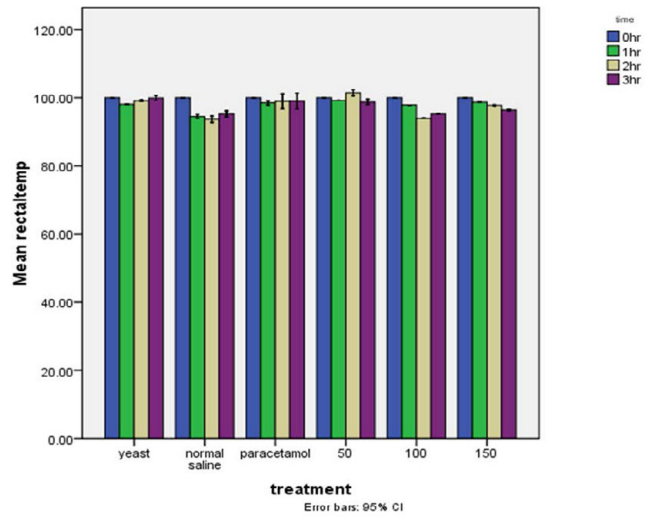

Figure 4: Antipyretic effect of *Urtica dioica* aqueous extract on albino mice.

to the reference drug which had a pyretic effect at this hour (Table 7). *U. dioica* exhibited an antipyretic effect though not in a dose dependent way (Figure 4, Table 7). Plant extract at dose 50 mg/kg showed a pyretic effect instead as the rectal temperature was increased to 101.44% at this hour compared with the value at the first hour after treatment (Table 7). However the rectal temperature of the group treated with herbal medicine at dose 150 mg/kg showed a slight decrease in temperature to 97.75% compared to the first hour after treatment (Figure 4 and Table 7). In the third hour, plant extract at dose 100 mg/kg was more effective compared to the reference drug and plant extract at the other dosages as it lowered fever to 95.26%, the antipyretic effect of the herbal medicine on fever was not in a dose dependent manner (Figure 4 and Table 7). The reference drug was found to increase fever instead showing an increase to 99.03% at this hour compared to the control (Table 7).

Discussion

The search for bioactive components which can be used as non-conventional analgesics, NSAIDs and antipyretics has received considerable attention in recent times because of the increasing worldwide development of lasting solutions to pain, inflammation and fever which are safe to human and with no side effects as seen with modern medicine. Thus, this study was oriented to evaluate the curative capacity of aqueous leaf extract of *Urtica dioica* against pain, inflammation and fever. The evaluation of analgesic, anti-inflammatory and antipyretic properties of the leaf extracts was done by formalin induced pain and inflammation and brewer's yeast induced pyrexia in albino mice. Subcutaneous injection of a dilute aqueous formalin (formaldehyde) solution into the dorsal surface of the rat or mouse hind paw elicits two distinct quantifiable nociceptive behaviors, i.e. flinching / shaking and licking / biting of the injected paw [10,11]. This formalin-induced nociceptive behavior shows an early and a late phase. The early phase, which starts immediately following injection of formalin, only lasts approximately 5 min and is probably due to direct chemical stimulation of nociceptors (acute pain). The second phase, which lasts 20 to 40 min, starts approximately 15 to 30 min following formalin injection and experimental data suggest that peripheral, inflammatory processes are involved [11]. The formalin test differs from most other nociceptive tests, such as the hot plate, tail flick and tail pinch tests, in that it enables evaluation of analgesic activity towards moderate, continuous pain generated by injured tissue. As a result, it has been suggested that this test provides a more valid model been suggested that this test provides a more valid model such as the hot plate and tail flinch tests [10,12,13]. The two distinct phases in formalin test are due to direct effect of formalin on nociception and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons [10,11,14]. Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain [15]. In this study, aqueous leaf extract of *Urtica dioica* showed the highest analgesic effect at dose of 150 mg/kg for acute pain and dose 100 mg/kg and 150 mg/kg body weight for chronic pain. These findings suggest both direct analgesic effects on the nociceptor blockage and an inhibition of the synthesis and/or release of inflammatory pain mediators such as prostaglandins. These results are similar to other previous studies on evaluation of analgesic activities of medicinal plant extracts. That the aqueous extracts of *Urtica dioica* demonstrated a reduction in the formalin-induced paw licking time in both phases is consistent with [16] who observed analgesic activity of hydroalcoholic extract of *Marrrubium parviflorum* against formalin-induced pain in mice. Similarly, the methanolic leaf extract of *Securinega virosa* demonstrated related analgesic effect in acetic acid induced writhing test and formalin test models [17].

That the aqueous leaf extract of *Urtica dioica* produced a dose dependent analgesic activity is related to a study by [18] who observed the analgesic effect of *B. coriacea* extract an acetic acid induced writing reflex method and showed that the extract at the doses used reduced the mean number of abdominal constrictions or writhing in a dose dependent manner when compared to the negative control group. The aqueous leaf extract of *Urtica dioica* at the lower dose level of 50 mg/kg body weight was not as effective as the two higher doses (100 and 150 mg/kg body weight) in both phases. These findings may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principle. It's also likely that at the lower dose there is simply not a sufficient concentration of the active principle(s). The analgesic effect of *Urtica dioica* can be attributed to one or more groups of the phytoconstituents observed in the extracts. Several studies have shown the analgesic activity of such compounds. Phytochemical screening of methanolic leaf extract of *Securinaga virosa* revealed the presence of flavonoids, saponins, tannins, glycosides,

alkaloids and steroids [18]. A study on the phtochemical composition of *Urtica dioica* has revealed presence of saponins, tannins, flavonoids, alkaloids and phenols [19]. Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannins [20]. Flavonoids such as quercetin are known to be effective in acute inflammation [21]. There are also reports on the analgesics effects of alkaloids, essential oils and saponins [22-24]. The analgesic and anti-inflammatory effect of the extracts in this study may therefore, be due to the presence of flavonoids, tannins, alkaloid or saponins. Flavonoids are widely shown to target prostaglandins which are involved in the pain perception through moderating opioidergic mechanism. These findings strongly recommend that these medicinal plants have peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential. The profound analgesic activity of these medicinal plants may be due to the interference of their active principle(s) with the release of pain mediators. Tissue damage and injury are always associated with pain and inflammation. In this formalin test, the mice used were treated with several treatments to reduce inflammation. Formalin test is a biphasic response where first phase is the direct effect of formalin which involves neurogenic pain. The pain is usually initiated when harmful mechanical, thermal or chemical stimuli agitate the peripheral terminals of particular main afferent neuron named nociceptors [25].

The second phase is involved in the inflammatory reactions. In this study, it was noticed that exposure of formalin induced inflammation to various treatments resulted in a significant inhibition of inflammation. The aqueous leaf extract of *Urtica dioica* was found to significantly suppress the inflammation when treated with different concentrations. The aqueous extract of *Urtica dioica* exhibited greater anti-inflammatory activity at dose 150 mg/kg. Lower dose of 50 mg/kg was not as effective and may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principles or the lower dose was an insufficient concentration of the active principles. The association of both analgesic activity and moderate anti-inflammatory effect observed with the extracts has also been shown in non-steroidal anti-inflammatory drugs (NSAIDs). It is a well-established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of cyclo-oxygenase activity [26]. The anti-inflammatory effects of the extracts may be due to their content of flavonoids, tannins, alkaloids and saponins. Several studies have shown the analgesic activity of such compounds. A study by [27] showed that the *Viola betonicifolia* methanolic extract was found to contain alkaloids, saponins, flavonoids, tannins, proteins, and phenolic compounds where the anti-inflammatory activity of *V. betonicifolia* was attributed to these groups of chemical compounds. The anti-inflammtory effect of the four medicinal plants extracts was not evident in every concentration of the extracts as early as the first hour of formalin injection but maximum inhibition was during the fifth hour. They did not maintain the suppression of the inhibition throughout the duration of the study. These findings could have been due to the fact that the active principles in the extracts required biotransformation so as to have an anti-inflammatory effect.

Brewer's yeast was used to induce fever in albino mice. Fever was recorded 19hrs after yeast injection since yeast takes a total of about 19hrs to cause the elevation of body temperature [28]. Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect [29,30]. Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins [31]. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibitions of these mediators are responsible for the antipyretic effect [32].

The oral administration of *Urtica dioica* significantly attenuated rectal temperature of yeast induced albino mice. Thus it can be postulated that *Urtica dioica* contained pharmacologically active principle(s) that interfere with the release of prostaglandins. After three hours of the test period, the aqueous leaf extract of *Urtica dioica* produced appreciable antipyretic activity against brewer's yeast induced pyrexia in albino mice. Dose of 150 mg/kg body weight demonstrated the greatest rectal temperature lowering activity for all medicinal plants. These findings were in agreement with the effects of other medicinal plants in laboratory animals. Similar work carried out by [33] showed that the hydro alcoholic extract of *Rosa alba* plant possessed a significant antipyretic effect in yeast induced elevation of body temperature in experimental rats. It was revealed that the extract showed dose dependent antipyretic activity. At a dose of 200 mg/kg it showed significant antipyretic activity. Non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus. Work done by [33] showed that the antipyretic activity of hydro alcoholic extract of *Rosa alba* is probably by inhibition of prostaglandin synthesis in hypothalamus. Therefore it is possible that the antipyretic action of aqueous extracts of *Urtica dioica* was related to the inhibition of prostaglandin synthesis in hypothalamus. However, other alternative mechanisms for blocking fever cannot be ruled out. Further hydro alcoholic extract of *Rosa alba* was found to contain carbohydrates, alkaloids, glycosides, flavonoids and tannins, through preliminary photochemical screening. Qualitative phytochemical screening in this study revealed that the aqueous leaf extract of *Urtica dioica* contain tannins, saponins, phenolics, alkaloids and flavonoids. A number of these phytochemicals have been shown to exhibit inhibitory action on cyclooxygenase enzyme and, as a result, produce antipyretic activity by preventing the formation of prostaglandins or by increasing the concentration of body's own antipyretic components [34]. Flavonoids are known to target prostaglandins which are involved in the pyrexia. Hence the presence of flavonoids in the aqueous leaf extract of *Urtica dioica* plant may be contributory to its antipyretic activity. The presence of alkaloids in these extracts could also be responsible for the antipyretic activity. For instance, according to [35] while evaluating on antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica,* reported that alkaloids also possesses antipyretic effects. The antipyretic activity of the aqueous leaf extract of *Urtica dioica* may also be attributed to the presence of saponins, which are involved in inhibition of prostaglandin synthesis. According to the study of [36] saponins are suggested to act synergistically to exert antipyretic activity. In a related study, the antipyretic effect of ethanolic root extracts of *Asparagus racemosus* on yeast-induced hyperthermia in rats was attributed to the saponins in the extracts [37]. It was observed that aqueous leaf extract of *Urtica dioica* at lower dose levels of 50 and 100 mg/kg body weight were not as effective as the higher dose of 150 mg/kg body weight, and thus may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principles. It's also likely that at the lower dose there is simply not a sufficient concentration of the active principle(s). The aqueous leaf extract of *Urtica dioica* at all the dose levels, did not lower rectal temperature in the first and second hours as effectively as in the third hour. These findings could have been due to the fact that the active

principles in the extracts required biotransformation so as to become antipyretic. That the dose level of 150 mg/kg body weight of the aqueous leaf extract of *Urtica dioica* was marginally effective than paracetamol, suggests a possible better blockage of prostaglandins biosynthesis or mimicry of paracetamol action by the active principles in the extract. It is also possible that the herbal extracts were efficiently inhibiting alternative mechanisms for blocking fever. The decline in rectal temperature in case of treatment with the medicinal plants extracts was not as sudden as that of paracetamol administration. Therefore, the extracts offer some advantage over the standard drug (paracetamol).

Conclusion

In conclusion, the present study has demonstrated the analgesic, anti-inflammatory and antipyretic potential of aqueous leaf extracts of *Urtica dioica* in albino mice. The aqueous leaf extracts of *Urtica dioica* were able to inhibit pain sensation of both phases. It is, therefore, possible to find opioid analgesics as well as analgesics in aqueous leaf extracts of *Urtica dioica* that act by inhibition of inflammatory pathways responsible for pain. Furthermore, the classes of phytochemicals in aqueous leaf extract of *Urtica dioica* have previously been observed to contribute to antipyretic and analgesic activities. The aqueous leaf extract of *Urtica dioica* have potent anti-inflammatory activity in rats in a dose dependent manner. The mechanism of anti-inflammation by aqueous leaf extract of *Urtica dioica* might be related with the compounds of bioactive and phytochemicals present in the plants. Therefore, these medicinal plants have the prospect to be used as herbal remedy for inflammation. The significant reduction in pyrexia in mice when treated with standard drugs as well as different doses of extracts, reflect that aqueous leaf extracts of *Urtica dioica* are endowed with potent antipyretic properties. Therefore, the aqueous leaf extract of *Urtica dioica* might help in preventing pain, inflammation and fever complications and serve as good bio-resource for generating a readily available herbal formulation that is more effective in the treatment of pain, inflammation and fever conditions which is cheaper than the conventional synthetic drugs and has no side effects. However, the mode of analgesic, anti-inflammatory and antipyretic actions of the studied extract is still obscure. The present study, therefore, scientifically confirms and supports the traditional use of aqueous leaf extract of *Urtica dioica* for management of fever and painful conditions.

Acknowledgments

The authors acknowledge Mr. James Adino and Mr. Wycliffe Wenwa for their technical support.

Conflict of Interest

The authors declared no conflict of interest.

Funding

This study received no funding.

References

1. Bisht S, Snehlata B, Bisht NS (2002) Urtica dioica (L): an undervalued, economically important plant. Agricultural Science Research Journals 2: 250-252.
2. Alford L (2007) The use of nettle stings for pain. Altern Ther Health Med 13: 58.
3. Randall C, Meethan K, Randall H, Dobbs F (1999) Nettle sting of Urtica dioica for joint pain--an exploratory study of this complementary therapy. Complement Ther Med 7: 126-131.
4. Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, et al. (1997) Phytotherapy of hypertension and diabetes in oriental Morocco. J Ethnopharmacol 58: 45-54.
5. Roschek B Jr, Fink RC, McMichael M, Alberte RS (2009) Nettle extract (Urtica dioica) affects key receptors and enzymes associated with allergic rhinitis. Phytother Res 23: 920-926.
6. Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 30: 379-384.
7. Wheeler-Aceto H, Porreca F, Cowan A (1990) The rat paw formalin test: comparison of noxious agents. Pain 40: 229-238.
8. Loux JJ, DePalma PD, Yankell SL (1972) Antipyretic testing of aspirin in rats. Toxicol Appl Pharmacol 22: 672-675.
9. Dubuisson D, Dennis SG (1977) The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4: 161-174.
10. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K (1992) The formalin test: an evaluation of the method. Pain 51: 5-17.
11. Haley JE, Dickenson AH, Schachter M (1989) Electrophysiological evidence for a role of bradykinin in chemical nociception in the rat. Neurosci Lett 97: 198-202.
12. Abbott FV, Franklin KB, Ludwick RJ, Melzack R (1981) Apparent lack of tolerance in the formalin test suggests different mechanisms for morphine analgesia in different types of pain. Pharmacol Biochem Behav 15: 637-640.
13. Alreja M, Mutalik P, Nayar U, Manchanda SK (1984) The formalin test: a tonic pain model in the primate. Pain 20: 97-105.
14. Hunskaar S, Hole K (1987) The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30: 103-114.
15. Gaertner M, Müller L, Roos JF, Cani G, Santos AR, et al. (1999) Analgesic triterpenes from Sebastiania schottiana roots. Phytomedicine 6: 41-44.
16. Khanavi M, Delnavazi MR, Nikoui V, Ostadhadi S, Bakhtiarian A (2012) Asian Journal of plant sciences 11: 96-99.
17. Yerima M, Magaji MG, Yaro AH, Tanko Y (2009) Nigerian Journal of Pharmaceutical Sciences 8: 47-53.
18. Ezeja MI, Ezeigbo II, Madubuike KG (2011) Analgesic activity of the methanolic seed extract of Buchholzia coriacea. Research Journal of Pharmaceutical, Biological and Chemical Sciences.
19. Mukundi MJ, Mwaniki NEN, Piero NM, Murugi NJ, Daniel AS, et al. (2015) In Vivi Anti-diabetic Effects of Aqueous Leaf Extracts of Rhoicissus tridentata in Alloxan Induced Diabetic Mice. Journal of developing drugs 4: 131.
20. Ahmadiani A, Hosseiny J, Semnanian S, Javan M, Saeedi F, et al. (2000) Antinociceptive and anti-inflammatory effects of Elaeagnus angustifolia fruit extract. J Ethnopharmacol 72: 287-292.
21. Rajnarayana K, Sripal Reddy M, Chaluvadi MR (2001) Bioflavanoids Classification, Pharmacological, Biochemical effects and Therapeutic potential. Indian Journal of Pharmacology 33: 2-16.
22. Choi J, Jung HJ, Lee KT, Park HJ (2005) Antinociceptive and anti-inflammatory effects of the saponin and sapogenins obtained from the stem of Akebia quinata. J Med Food 8: 78-85.
23. de Araújo PF, Coelho-de-Souza AN, Morais SM, Ferreira SC, Leal-Cardoso JH (2005) Antinociceptive effects of the essential oil of Alpinia zerumbet on mice. Phytomedicine 12: 482-486.
24. Reanmongkol W, Subhadhirasakul S, Thienmontree S, Thanyapanit K, Kalnaowakul J et al. (2005) Analgesic activity of the alkaloid extract from Kopsia macrophylla leaves in mice. Songklanakarin Journal of Science and Technolology, 27 (Supplement 2): 509-516.
25. Tominaga M, Numazaki M, Iida T, Moriyama T, Togashi K, et al. (2004) Regulation mechanisms of vanilloid receptors. In Symposium on Pathological Pain: From Molecular Clinical Aspects, held at the Novartis Tsukuba Research Institute, Tsukuba, Japan, in collaboration with the Novartis Foundation (Japan) for the promotion of Science, 30 September-2 October 2003. Chichester: John Wiley & Sons. pp. 4-18.
26. Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 231: 232-235.
27. Muhammad N, Saeed M, Qayum M, Khan H (2013) Anti-microbial Screening of Viola betonicifolia. Middle-East Journal of Scentific Research 15: 55-60.
28. Turner RA (1965) Screening method in Pharmacology, AcademicPress, New York & London 268.

29. Khan I, Nisar M, Ebad F, Nadeem S, Saeed M, et al. (2009) Anti-inflammatory activities of Sieboldogenin from Smilax china Linn.: experimental and computational studies. J Ethnopharmacol 121: 175-177.

30. Devi BP, Boominathan R, Mandal SC (2003) Evaluation of antipyretic potential of Cleome viscosa Linn. (Capparidaceae) extract in rats. J Ethnopharmacol 87: 11-13.

31. Moltz H (1993) Fever: causes and consequences. Neurosci Biobehav Rev 17: 237-269.

32. Rawlins M, Karger S (1973) Mechanism of salicylate-induced antipyresis. Biomed Central 1973:311.

33. Pentela B, Thalla S, Chaithanya G, Jansi A, Kumar KT, et al. (2012) International Journal of Chemical and Pharmaceutical Sciences 3.

34. Okokon JE, Nwafor PA (2010) Antiinflammatory, analgesic and antipyretic activities of ethanolic root extract of Croton zambesicus. Pak J Pharm Sci 23: 385-392.

35. Reanmongkol W, Matsumoto K, Watanabe H, Subhadhirasakul S, Sakai S (1994) Antinociceptive and antipyretic effects of alkaloids extracted from the stem bark of Hunteria zeylanica. Biol Pharm Bull 17: 1345-1350.

36. Zakaria ZA, Wen LY, Abdul Rahman NI, Abdul Ayub AH, Sulaiman MR, et al. (2007) Antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of Bauhinia purpurea leaves in experimental animals. Med Princ Pract 16: 443-449.

37. Vasundra DPA, Divya PS (2013) Antipyretic activity of ethanol and aqueous extract of root of Asparagus racemosus in yeast induced pyrexia. Asian Journal of Pharmaceutical and Clinical Research 6: 0974-2441.

Citation: Safari VZ, Ngugi MP, Orinda G, Njagi EM (2016) Anti-pyretic, Anti-inflammatory and Analgesic Activities of Aqueous Leaf Extract of *Urtica Dioica* (L.) in Albino Mice. Med Aromat Plants 5: 237. doi:10.4172/2167-0412.1000237