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Antinociceptive and anti-inflammatory effects of flavonoids rich fraction of *Solanum incanum* (Lin) root extracts in mice

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ABSTRACT

Solanum incanum (Solanaceae) is a common perennial shrub-like herb that grows up to 0.9-1.2 m high which is a widely used as folklore remedy for ailments such as stomach-ache, headache, painful menstruation, angina, fever, sore throat and other painful conditions, sexually transmitted diseases, skin infections, burns wounds, pneumonia and rheumatism by various African communities. Crude root extracts of the herb were shown to exhibit antinociceptive and ant-inflammatory effect. In spite of all these uses of *S. incanum*, there's no enough science-based information on the effect of purified extracts of the herb on these signs and symptoms. Hence the main objective of the study was to evaluating the antinociceptive and anti-inflammatory effects flavonoids rich fraction from *S. incanum* root in mice. In this study 6.5, 12.5 and 25 mg of flavonoids rich fraction *S. incanum* roots extract, diclofenac and the normal saline (vehicle) was injected subcutaneously in Swiss Albino mice 30 minutes prior to induction of pain and inflammation. Pain and inflammation were induced using dilute formalin solution that was injected in the animal's left hind paw. The time spent in pain behavior (lifting, leaking and biting the injured paw) was measured and recorded for the first 5 minutes and between 15-30 minutes after formalin injection. Acute edema was used as an acute inflammatory model. The paw diameter was measured prior to injection with formalin and then after two hours. Significant analgesic and anti-inflammatory activities ($p < 0.05$) were exhibited by 6.5 and 12.5 mg doses. These findings indicated *S. incanum* root extracts contains flavonoids with antinociceptive and inflammation effects.

Keywords: *Solanum incanum*, Flavonoids, Inflammation, Antinociception, Pain.

INTRODUCTION

Herbal remedies play an important role in management of various disease conditions with about 28% of all modern drugs being of sourced from plants [1]. An example of such plants used as folklore medicine include *Solanum incanum Solanaceae* (Bitter or Sodom apple-English, *Entulele*-Maasai, *Mũtongu/Mũturere*-Gĩkũyũ) which is a perennial shrub like herb that is used traditionally to treat ailments such as stomach-ache, headache, painful menstruation, sore throat, angina, colic, and other painful states such as rheumatism etc are treated with extracts from the plant [2]. Fruit sap in and leaves are used as an eye bath while the fruits are used by control ticks [3].

Extract from the plant exhibited antinociceptive effects [4, 6] and anti-inflammatory effects [6] in animal models. Extract from the seeds exhibited significant increase in hemoglobin, number of erythrocytes and white blood cells as well as that of the platelets [7]. It also showed insecticidal and insect deterrent activities [8] and inhibited the acetylcholinesterase activity [9] in green peach aphids. Alkaloids solarmagine from the plant, caused apoptosis in various tumor cell lines [5]. Other effects observed with the plants extracts include broad-spectrum antifungal [10] and antimicrobial activities [10, 11]. The fruits and the leaves contain dimethylnitrosamine a carcinogenic compound [12]. The various parts of the plant were found to contain saponins, steroids and glycoalkaloids (mainly solasonine) but fruits contain the highest concentrations [13] while the highest levels of alkaloid solasodine were observed in young leaves and stem of *S. incanum* [14]. The flavonoids are major secondary metabolites in plants that are associated with both antinociceptive and anti-inflammatory effects [15]. However, in spite of all these studies, little scientific study has been done to try and elucidate the nature and type of the phytochemicals responsible for these effects. Therefore, this study evaluated both anti-inflammatory & antinociceptive activity of flavonoids rich fraction of *S. incanum* using animal model.

MATERIALS AND METHODS

Plant materials

The *Solanum incanum* roots were collected during the day from Kasarani area in Nairobi metropolis. They were identified in the university of Nairobi herbarium courtesy of a taxonomist, Mr Mutiso and a voucher specimen JM/2013 issued. The roots were washed thoroughly with tap water then chopped in small pieces then air dried under shade away from sunlight for four weeks. They were then crushed to a powder using an electric mill and stored in a cool dry place in air tight containers.

Preparation of flavonoids rich fraction

The preparation of the flavonoid rich fraction was done according to the method described by Houghton & Roman, [16]. About 100 grams of the root powder was defatted using petroleum ether, then, extracted with methanol for 72 hours. The mixture was decanted & then supernatant clarified, using Whatman number 1 filter paper. The filtrate was concentrated using rotor evaporator to obtain the extract which was then treated with 1N hydrochloric acid followed by partitioning with diethyl ether. The ether fraction containing the crude flavonoids was evaporated to obtain the flavonoids rich fraction.

Phytochemical Analysis

To confirm the presence of flavonoids a test described by Hossain *et al*., [17] was carried out, where a few drops of dilute sodium hydroxide were added to 1ml aqueous solution of the extract and a yellow color was observed. A few drops of dilute hydrochloric acid were then added rendering the solution colorless thus indicating the presence of flavonoids. This was followed by Shimoda's test where a few drops of concentrated hydrochloric acid were added to 1ml aqueous solution of the crude extract followed by 0.5 g of Zinc turnings. The tubes were then heated in boiling water bath for five minutes and color changes observed. A reddish pink color was formed confirming the presence of flavonoids [18].

Experimental animals

Adult Swiss Albino mice of both sexes equitably distributed were used for both assays. The mice were divided into groups of six and placed in cages in rooms between 20 to 25ºC and a 12-hour day light/dark cycle. They were allowed seven days to acclimatize prior to commencement of experiments. Mice pellets from Unga feeds Kenya Ltd and tap water were provided throughout the experiments period. The experiments were carried out in accordance to the guidelines on care and use of laboratory animals [19].

Standard chemicals and drugs

The standard drugs and chemicals used in the study included chloroform, diclofenac as sodium salt (CSPC Pharma Co. Ltd), formalin, petroleum ether, methanol, diethyl ether and dimethyl sulfoxide (DMSO).

Antinociceptive activity assay

The animals were injected subcutaneously (s.c.) with 6.5, 12.5 and 25 mg/kg doses of the flavonoids rich fraction (extracts) in 0.2ml normal saline, while the negative control received same volume of normal saline (vehicle) and the positive control got subcutaneously (s.c.) 15mg/kg diclofenac sodium 30 minutes prior to formalin injection. In order to induce pain, 50 micro liters of 5 percent formaldehyde solution was injected in the sub plantar area of left hind paw. The animals were placed individually in a transparent observation chamber (about 270 cm^3) with mirrors placed on the side of the cage for ease of visualization. The lifting, shaking, licking and biting of affected paw was categorized as sign of nociception and time spent in manifestation of these signs was recorded from 0 - 5 minutes (first phase) and then 15 - 30 minutes (second phase) after formalin injection. The first phase pain occurs due to direct action of formalin on the pain receptors while the later phase is due to inflammatory response as well as central sensitization [20].

Anti-inflammatory activity assay

To evaluate the anti-inflammatory effects of *S. incanum*, the method described Hunskaar & Hole [20] was used. The mice were injected subcutaneously (s.c.) with 6.5, 12.5 and 25 mg/kg doses of the flavonoids rich fraction (extracts) in 0.2ml normal saline, while the negative control received same volume of normal saline (vehicle) and the positive control got s.c. 15mg/kg diclofenac sodium 30 minutes prior to formalin injection. Inflammation was induced by administration of 50 micro liters of 5 percent formaldehyde solution in the sub plantar area of left hind paw of mice. The paw circumference prior to injection of formalin was measured using a string and a ruler. Two hours after the injection with formalin, the circumference of the paw was measured. The difference between the initial and the final circumference was used to quantify inflammation as the edema developed in mice. The data obtained for each set of experiment was expressed as a means and their standard errors (SEM). It was then analyzed using one-way ANOVA followed by *Scheffé* as the post Anova. The value of $P < 0.05$ was the limit of significance.

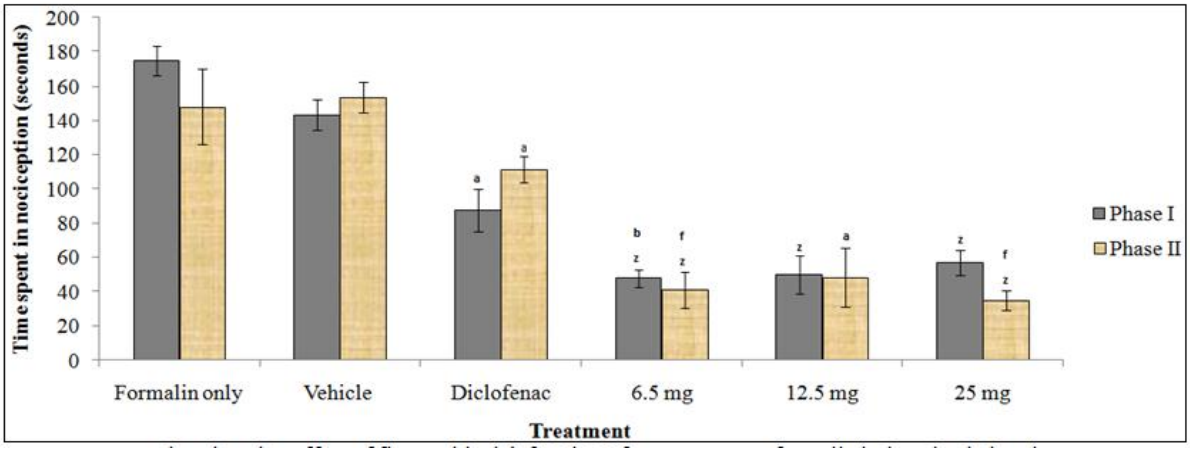
RESULTS AND DISCUSSION

Since flavonoids occur ubiquitously in plants kingdom, they are an integral component of human dietary ingredients. The major sources of flavonoids include vegetables, fruits and wine since they are a major coloring component of flowering plants [21]. Their biological effects have triggered the need to evaluate their structure and functional relationship. Their molecular configuration plays an important role in their metabolism and biological activity as well as their bioavailability. In the study, the flavonoids rich fraction of *S. incanum* root extract exhibited significant antinociceptive effect ($p< 0.05$) compared to that of the vehicle and diclofenac in both phases of nociception (Table1; Fig.1). The flavonoids are believed to exert their antinociceptive activity via several mechanisms which include arachidonic acid metabolic pathway, cyclooxygenase and mitogen-activated protein kinase pathways [22, 23]. They block protein kinase C-α and PKC-ε activation by PMA, mechanical hyperalgesia induced by bradykinin, without affecting similar responses caused by epinephrine and prostaglandin E2 [22]. Therefore, it is likely that the flavonoids in the *S. incanum* extract may have exerted antinociception via one of these mechanisms.

Table 1: The effect of flavonoids rich fraction of *S. incanum* on formalin induced nocicption in mice

Treatment	Phase I	Phase II
Formalin only	174.667	147.833
Vehicle	143.333	153.333
Diclofenac	87.6667 [a]	111.333 [a]
6.5 mg	47.8333 [zf]	41 [zf]
12.5 mg	49.8 [z]	48.4 [a]
25 mg	56.6667 [z]	34.8333 [zf]

z ($p<0.001$) & **a** ($p<0.05$) relative to vehicle while **f** ($p<0.001$) & **b** ($p<0.05$) relative to diclofenac



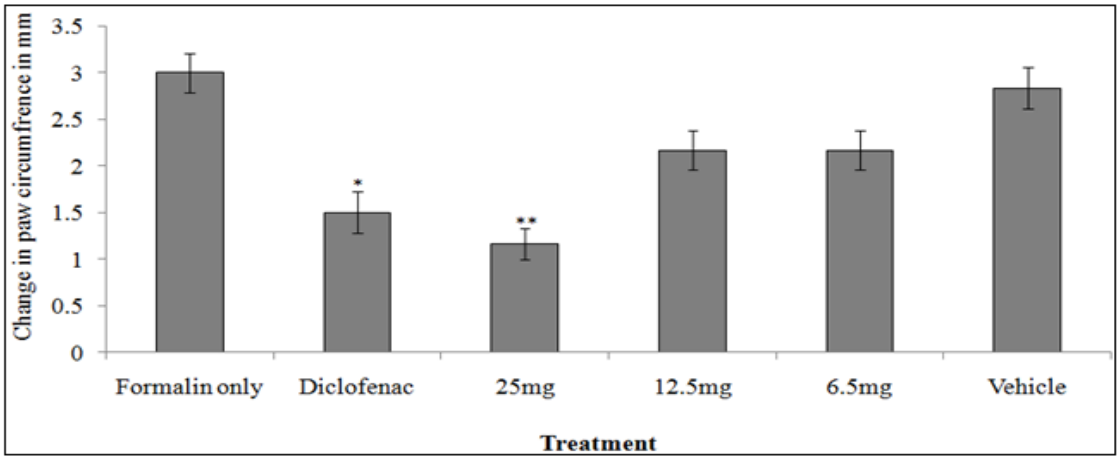
a $p<0.00$ & **z** $p<0.001$ relative to vehicle. **f** $p<0.001$ & **b** $p<0.05$ relative to diclofenac

Figure 1: Antinociceptive effect of flavonoids rich fraction of *S. incanum* on formalin induced pain in mice

Table 2: The effect of flavonoids rich fraction of *S. incanum* on formalin induced edema in mice

Treatment	Mean circumference (mm) ± SEM
Formalin only	3.00 ± 0.21
Diclofenac	1.50 ± 0.22*
25 mg	1.67 ± 0.17**
12.5mg	2.17 ± 0.21
6.5 mg	2.17 ± 0.21
Vehicle	2.83 ± 0.21

*$p<0.05$, **$p<0.001$ against the vehicle



***p<0.05, ****p<0.001 against the vehicle

Figure 2: Effect of flavonoids rich fraction of *S. incanum* on formalin induced edema in mice

Inflammation is a normal biological process that occurs in response to the presence of damaging stimuli, with ultimate goal of elimination of the etiology of tissue injury and initiating healing process. It involves migration of immune cells, recruitment of inflammatory cells and release of mediators at the site of damage [24]. Intake of flavonoids has been found to have a significant effect on the function of the immune system including development of pro-inflammatory cells by inhibiting critical enzyme systems involved. Such enzymes include phospholipases A_2, protein kinase C, tyrosine kinases [25]. The anti-inflammatory activity of flavonoids involves alteration of the function of enzyme systems involved in the inflammatory process which involve binding of the flavonoids with ATP catalysts on the enzyme sites competitively. These biological catalysts, enhance signal transduction and immune cells activation such as tyrosine and serine-threonine protein kinases [26]. Flavonoids have been found to deactivate enzymes that play a role in activation of cells e.g. phosphodiesterases [27]. Alternative mechanisms of action of the anti-inflammatory activity of flavonoids include inhibition of biosynthesis of mediators of inflammation that act as powerful signaling molecules influencing migration of leucocytes. Flavonoids also inhibit expression of inducible nitric oxide synthase isoforms, lipooxygenase and cyclooxygenase (COX) which are impotant in production of large number of mediators such as prostanoids, nitric oxide (NO), leukotrienes (slow reacting substance of anaphylaxix) [27]. Several flavonoids have been reported to significantly inhibit platelet function i.e. adhesion, aggregation, and secretion presumably through inhibition of inhibitors of cyclic AMP phosphodiesterase and arachidonic acid metabolism [28].

In the study the 6.5mg and 12.5mg doses significantly suppressed ($p< 0.05$) the anti-inflammatory effects while the 25mg dose highly significantly reversed the formalin induced inflammatory changes compared to the vehicle. Consequently, the flavonoids rich fraction of *S. incanum* may have suppressed inflammation by alteration of the function of enzyme systems involved in the inflammatory process, inhibition of phosphodiesterases involved in cell activation or may have blocked the biosynthesis mediators of inflammation that act as powerful signaling molecules influencing migration of leucocytes and hence limit the chemotaxis. Migration of leukocytes is an important phenomenon in induction of edema. Accumulation of the leucocytes in in the region of injury results in their degranuration and release of pro-inflammatory mediators [6]. The extract could as well have suppressed the expression of the enzymes that play a key role in edema formation such as the inducible nitric oxide synthase isoforms, COXas well as lipooxygenase, which are responsible for the production of a great amount of NO, prostanoids, slow reacting substance of anaphylaxis. These substances cause relaxation of the arteriole smooth muscles, opening of pores and copious exudates of intravascular fluid [29]. These and perhaps many more mechanisms may be hypothesized as the possible modes of action of the flavonoids rich fraction of *Solanum incanum.*

CONCLUSION

From the study, the flavonoids rich fraction of *S. incanum* roots showed significant antinociceptive and anti-inflammatory effects which lenders support to the findings of other studies as well as the folklore claims that the plant root posses' substances with both analgesic and anti-inflammatory effects.

Conflicts of Interest

No conflict of interest among authors.

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