ANTINOCICEPTIVE, ANTIPYRETIC AND ANTI-INFLAMMATORY EFFECTS OF *SOLANUM INCANUM* LINNAEUS IN ANIMAL MODELS

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Declaration

This thesis is my own original work and has not been presented for a degree program in any other university.

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DEDICATION

I dedicate this work to my dear father Samwel Mwonjoria, my wife Mary, son Sam, and daughters Bridget and Jacqueline for your support and prayers.

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ABSTRACT

The root of *Solanum incanum* is used by some Kenyan communities as a folklore remedy for fever, wound healing, fever and toothache. However scientific studies have not been done to validate these claims. The aim of this study was to investigate antinociceptive, antipyretic and anti-inflammatory effects of *Solanum incanum* root extract using animal models.

The antinociceptive assay was carried out using hot plate and tail flick on white CBA mice. In the antipyretic assay fever was induced in Sprague Dawley rats using lipopolysaccharide (LPS) while carrageenan induced paw edema was used as the model for acute inflammation.

The 100 and 200 mg/Kg dose of *Solanum incanum* root extract showed significant ($p < 0.05$) antinociceptive activity to both hot plate and tail flick tests The 50 mg/kg dose of *Solanum incanum* extract exhibited significant ($p < 0.05$) antipyretic effect at 180 minutes while the 100 mg/Kg dose of *S. incanum* exhibited significant antipyretic effect at 120 and 180 minutes after lipopolysaccharides pyrogen injection. . The 50, 100 and 200mg / Kg doses of *Solanum incanum* failed to show significant anti-inflammatory effects ($p > 0.05$)

Therefore from these observations, it's highly probable that *Solanum incanum* root extract contains compounds with both antinociceptive and antipyretic but no anti-inflammatory effects. Hence this may render support to folklore use of *Solanum incanum* root extract for pain and fever.

CHAPTER 1: LITRATURE REVIEW

1.1 Introduction

Plant extracts have been used as remedy for various ailments. The Assyrians and the Egyptians were aware of the analgesic effects of a decoction of myrtle or willow leaves for joint pain while Hippocrates recommended chewing willow leaves for analgesia in childbirth (1). The use of herbs for therapeutic purposes has been widely practiced in Africa (2). Among the plants used for fever and inflammatory treatment includes *Solanum incanum* which belongs to *Solanaceae* family (3). Other members of this family includes vegetables such as potatoes (*Solanum tuberosum*), tomato *(Solanum lycopersicum)*, black nightshade (*Solanum nigrum*), and brinjal *(Solanum melongena)*. In addition *Datura spp.* and *Atropa belladona* also belongs to this family. The herb *Solanum incanum* is a perennial bushy herb. It is widely distributed in Kenya where it is considered a weed. However the herbs is used especially by the Maasai, Kikuyu and Samburu peoples of Kenya as soup flavour where the root is boiled and the concoction mixed with the soup especially for nursing mothers.

The root extract of *Solanum incanum* is used as a remedy by various East African communities for several medical problems e.g. fever, stomach ache, throat and chest infection (2). The juice from ripe fruits is applied topically as a remedy for wounds (5). The fact that the herb is also used as a folklore remedy for wound could mean that it may have anti-inflammatory effects as well.

The aim of this study was to establish whether the root extract of *Solanum incanum* has antinociceptive, antipyretic and anti-inflammatory effect.

1.1.1 *Solanum incanum*

The use of plant parts for therapeutic purposes has been widely practiced in Africa (5). One of the herbs used for fever and analgesia is *Solanum incanum* (bitter or Sodom apple) (2) which belongs to *Solanaceae* family (3). It is a perennial bushy herb which is widely distributed in Kenya where it is considered a weed. The herb is used by several East African communities as a remedy for tooth-ache, stomach-ache, fever, and chest pains, snake bite and ear ache (2). Elsewhere in Africa *S. incanum* is used for sore-throat, angina, head ache, warts, and benign tumors (3, 4). Fruit extract were shown to exhibit antimicrobial effects (6) while the extract from the leaves exhibited similar effect (7). The extract from the herb was found to have anti-tumor effect (9). Chloroform extract from a related herb *Solanum nigrum* (widely used as a vegetable in Kenya) showed a significant antipyretic, antinociceptive and anti-inflammatory effect in mice (10, 11), while *Solanum lycocarpum* has anti-inflammatory effects (12). *Datura spp. & Atropa belladonna* are sources of atropine and hyoscine which have parasympatholytic activity. Several alkaloids have been isolated from this family which includes;-

steroid alkaloids; Solasosine and Solargine isolated from freshly ripe fruits of *Solanum incanum* and beta Solamarine from *Solanum dulcamara* which was found to inhibit sarcoma in mice (13). The glycosidal alkaloid solanocapsine from seeds of *Solanum seudocapsicum* showed antimicrobial effects, while solanin had an antifungal effect.

Six compounds were isolated from the aerial parts of *Solanum incanum* that includes ten flavinoids, chlorogenics, adenosine, benzyl-O-beta-D xylopyranosyl (1-2)-beta-D-glucopyranoside and three phenylalkanoic acids. One of the compounds kaempferol 3 -O- (6'''-O- 25-dihydroxycinnamoyl)-beta-D-glucopyranosyl (1-2) beta-D-glucopyranoside was identified as a new compound (14). Young leaves and stem of both *Solanum incanum* and *nigrum* have been shown to contain high levels of an alkaloid solasodine (15).

1.1.2 Inflammation

The term refers to a generalized non-specific beneficial response of tissue to injury. It involves a complex cascade of events both local and systemic. The local events involve phagocytic cells recruitment, removal of both endogenous and exogenous debris (16), while systemic responses involve haemostatic changes (17). Cellular mechanisms of inflammation involve relaxation of vascular smooth muscle cells causing vasodilation, alteration of vascular permeability due to contraction of cytoskeleton in epithelial cells, migration of phagocytes to inflamed area and phagocytosis (18). Inflammatory process is involved in pathogenesis of various diseases and has both acute and chronic phase.

The acute phase is characterized by fever, pain and edema while in chronic phase there is cellular proliferation, activation of complement, fibrinolytic system and hyaluronidase activity.

The acute inflammatory model can be induced by carrageenan (19), formalin (20), serotonin, histamine, bradykinnin and prostaglandins.

1.1.3 Pain

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. In muscles, visceral, and cutaneous tissues, transduction of high threshold input involving chemical, thermal and mechanical stimuli to electrophysiological activity occurs at the specialised free nerve endings of nociceptive primary afferent. The transductive mechanism involves activation of cationic channels on free nerve endings directly by biophysical properties of the high threshold noxious stimuli, and directly by changing the composition of micro- and macro- environments produced by such stimuli or trauma. (21).

Thermal nociceptive transduction is mediated via vanilloid type receptor cation channels (TRPV). TRPV1 responds to noxious heat greater than 45 degrees centigrade and are capsaicin

sensitive. TRPV2 is not capsaicin sensitive and has a thermal threshold of 52 degrees centigrade. Cold and menthol – receptor CNR – 1/T8 responds to noxious cold i.e. 8–25°C and menthol. Both cold and hot responsive channels respond to thermal changes by increasing $Na,^{+}$ K^{+} or Ca^{++} ion channels flux causing membrane depolarization and transduction (21.22. 23). High threshold mechanical stimuli affect non-specific channels by distorting collagenous bridging elements between membranes of free nerve endings and surrounding tissue matrix. This causes changes in channel configuration producing inward Na^{+}, K^{+} and Ca^{++} currents (21. 23).

1.1.3.1 Pain receptors

Nociceptors exhibit graded membrane potential which is intensity and temporal dependent. Activation of voltage gated Na^{+}channels causes propagation of depolarization along the nociceptor membrane while both Na^{+} and Ca^{++} influx cause release of Ca^{++} from intracellular stores, a signaling molecule which affects the nociceptive threshold. Long term changes in Ca^{++} dependent signaling mechanism alters early and late phase of gene expression to modify transcription. The resulting protein synthesis alters neuronal microstructure. All these changes results in sensitization of peripheral afferents High threshold stimuli may release arachdonic acid from the membranes which is acted upon by cyclo-oxygenase 2 (COX-2) to form prostaglandin E-2 (PGE-2). The latter acting via protein kinases signaling enhances responsiveness of the affected primary afferents to both noxious stimuli (Hyperpathia) and non-noxious stimuli; (Allodynia) (24).

1.1.3.2 Transmission of pain sensation

From nociceptors pain sensation is transmitted to spinal cord via A-delta and C-fibers. A-delta fibers are small (1-5um) diameter myelinated nerve fibers that conduct fast pain and have small receptive fields. Type II thermosponsive A-delta fibers are capsaicin sensitive and respond to noxious heat at 40 – 45°C while type I are not capsaicin sensitive and respond at 52 – 56°C. Slow pain is conducted via small unmyelinated C-fibers which are 0.25-1.5mm in diameter. They are stimulated by products of cell disruption, inflammation cascade and immune mediators and have a large receptive area.

A-delta cold afferents have optional responses at about 8°C. The C- fibers constitute majority of cutaneous nociceptive innervation and also innervate visceral organs. Polymodal A-fibers are abundant in testes and structures surrounding the heart (21).

1.1.3.2.1 Transmission in dorsal horn of spinal cord

Majority of nociceptive primary afferent fibers that project to the dorsal horn A- delta fibers terminate in laminae I, II and IIa while C- fibers terminate in laminae II, IIa and V. Glutamate is an excitatory transmitter between primary efferent nociceptors and dorsal horn cells. High intensity or prolonged C-fiber activity causes the release of the substance P. They also produce calcitonin gene related peptide (CGRP) that enhance (NO) nitric oxide level and increase peripheral vasodilation (21).

1.1.3.2.1 Dorsal Horn Afferents

Majority of A-delta and C-fibers synapse with neurons in the dorsal horn that transform afferent input. Majority of second-order neurons decussate and ascend in the anterolateral quadrant as the

spinothalamic tract while a few pass ipsilaterally. Neospinothalamic tract has fibers from laminae I that project to parabrachial nucleus and fibers from laminae I and II that terminate in Ventroposterior lateral nucleus of thalamus. Paleospinathalamic tract is mainly comprised of axons from laminae IIa and V that project to parabrachial nucleus and rostral ventral medulla, caudal Pons and midbrain. Spinothalamic pathways project into the raphe nulei, magnocellullar nuclei. The spinotectal pathway project into the periaqueductal and periventricular grey of the midbrain. These areas are linked by ascending projections to the cortex and limbic system (21).

1.1.3.3 Antinociceptive systems

Pain can be modulated at different levels including the spinal cord. Inhibitory interneurons from laminae I and V receives collaterals from A–delta and C-fibers. These interneurons synapse on primary afferent and secondary order neurons within the horizontal spinal cord segment. The transmitter substances released by these neurons include GABA, glycine, dynorphin and endogenous cannabinoids. Other sites where pain modulation takes place are dorsal column by activation of A-delta mechano-stimulation, brain stem level and mid brain by the sensory cortex (21, 25).

Several hormones that have been shown to modulate pain include corticotrophin releasing hormone (21) and sex hormones (26).

1.1.3.4 Management of Pain

Several methods are used in management of both acute and chronic pain. They include use non-steroid anti-inflammatory drugs (NSAIDs), opioids, acupuncture, nerve blockade etc.

The NSAIDs are widely used and are available over the counter. Examples include acetylsalicylic acid, para-cetamol etc. These groups of drugs have antipyretic and analgesic as

well as anti-inflammatory effects. Most NSAIDs act peripherally by inhibiting cyclo-oxygenase mediated synthesis of prostaglandins (27). Prostaglandins sensitize peripheral nerve endings hence facilitating pain behavior (28). Diring *et al.* showed that the central analgesic effect of NSAIDs is due to the inhibition of spinal cyclo-oxygenase activity (29).

Opioid Analgesics

Opioids analgesics are very potent examples include Morphine, Pethidine, Codeine, Heroin etc. The analgesic effect of opiods is mediated via mu delta or kappa opioid receptors. They cause analgesia in the central nervous system by reducing neuronal excitability (30).

1.1.3.5 Antinociceptive Tests

Several tests have been developed to assess behavioral antinociception in experimental animals. These include writhing / abdominal constriction, formalin test, tail flick, hot plate and paw pressure tests.

1.1.3.5.1 Acetic acid induced abdominal constriction (Writhing Test)

The acetic acid test is mainly used to study visceral pain. It involves injection of the irritant e.g. acetic acid in to the peritoneum of an animal. The irritant induces behavioral response in the experimental animal. In mice the response consists of a wave of constriction and elongation that passes caudally along the abdominal wall and twisting of the trunk. This is followed by extension of the hind limbs (31). Various substance can induce abdominal constriction e.g. acetylcholine, hypertonic saline, phenylbenzoquinone etc. However acetic acid and phenylbenzoquinone are the more frequently used substances for writhing test due to their longer time latency. Abdominal

writhing test is commonly used as a screening test due to its simplicity. It is a sensitive method for investigating the antinociceptive effects of drugs (32).

1.1.3.5.2 Formalin Test

In this test low concentration s of formalin is injected into the dorsal surface of the paw of the experimental animal. The behaviour observed after formalin administration includes licking, biting, elevation or shaking the injected paw. Time spent in these behavioral states is noted as the latency of nociception. Two phases of nociception are observed which represent acute and chronic pain. The two phases are thought to involve two distinctly different stimuli. The early phase is observed between 0-5 minutes and is thought to be due to direct stimulation of nociceptors by the chemical (33).

The late phases start 15-30 minutes after formalin injection and it is due to inflammatory pain process (34).

1.1.3.5.3 Tail Flick Test

The two variants of tail flick are radiant heat and heat emersion methods. The application of thermal radiation on the tail of the animal provokes the withdrawal of the tail by a brief but vigorous movement. It is the reaction time of this movement that is recorded and often referred to as tail flick latency. The lengthening of the reaction time is interpreted as an analgesic action. The flick of the tail is a spinal reflex. Advantages of this method are its simplicity and the small inter-animal variations in reaction time measurements under a given set of controlled conditions (35).

1.1.3.5.4 Hot Plate Method.

This test involves placing a rat or mouse on a metallic plate heated by a thermode or boiling water. A plate heated to a constant temperature produces the behavioral components in the test animals that can be measured in terms of their reaction times namely paw–licking and jumping. Both responses are supra-spinally integrated. The paw licking behaviour is only affected by opioids while jumping reaction time is increased by analgesics such as aspirin, para-cetamol etc especially when temperature is 50 °C or less (35).

1.4 Fever

Fever is perhaps the oldest and the best manifestation of infectious diseases (36, 37), the rise in body temperature observed during fever is as a result of change in set-point of thermostat in the hypothalamus (38). The causes of fever include microbial infections, injury, immunological reactions, neoplasm and inflammations (39, 40, 41, 42). The initial step in Febrogenesis is the release by leukocytes of pro-inflammatory cytokines e.g. Interleukin-1 beta, Interleukin-6 also known as endogenous pyrogen or leucocytic pyrogen after stimulation by the exogenous pyrogen (43, 44, 45).

Noxious stimuli cause the tissues to liberate arachdonic acid from the cell membranes, which is converted to prostaglandin by the leukocytes. Synthesis of prostaglandin E-2 in the pre-optic area of the anterior hypothalamus is evoked by exogenous and endogenous pyrogen is the final step in the development of fever. Macrophage inflammatory protein-2 (MIP-2), a powerful chemotaxic cytokine for neutrophils is an important mediator in lipopolysaccharide initiated febrogenesis via the prostaglandin dependent pathway (46). Prostaglandin E 2 acts through four Prostaglandin E

series of receptors namely EP1, EP2, EP3 and EP4. The EP3 receptor subtype appears to play the most important role in fever and hyperalgesic effects of prostaglandin E2 (47).

Khalid Benarmar *et al.*; showed that Macrophage inflammatory protein -1 beta produces fever via a parallel pathway independent of the classical prostaglandin dependent pathway adduced in the development of fever for most endogenous pyrogen such as Interleukin-1 beta and Interleukin-6 (39).

Tumour necrosis factor alpha (TNF) acts as an endogenous pyrogen but under certain conditions behave as an antipyretic by stimulating the production of Interleukin-10 (Cytokine synthesis inhibiting factor) (46).

1.5 JUSTIFICATION

Solanum incanum root is used by some Kenyan communities as folklore remedy for fever, ulcers and toothache (2). However scientific studies have not been done to confirm these effects. This study investigated these folkloric claims and provided scientific data that may be used to develop a cheaper alternative method for management of pain and fever.

1.6 HYPOTHESES

1.6.1 **Null Hypothesis** (HO)

Solanum incanum root extract has no antinociceptive, antipyretic, and anti-inflammatory effects in animal models

1.6.1 **Alternative Hypothesis** (HA)

Solanum incanum root extract has antinociceptive, antipyretic and anti-inflammatory effects in animal models.

1.7 OBJECTIVES

1.7.1General objective

To determine the antinociceptive, antipyretic and anti-inflammatory effects of *Solanum incanum* root extract using animal models.

1.7.1 Specific objectives

To determine the;

(a) Antinociceptive effect of *Solanum incanum* using mice

(b) Antipyretic effect of *Solanum incanum* using rats

(c) Anti-inflammatory effect of *Solanum incanum* using mice

CHAPTER 2: MATERIALS AND METHODS

2.1 Collection of Plant materials

Fresh roots of *Solanum incanum* were collected in Kasarani area of Nairobi (Kenya) in March 2009. Botanical identification was done in the University of Nairobi Herbarium at the School of Biological Sciences against sample collected by Kanyingi and Mathenge voucher specimen number 32 / 81.

2.2 Preparation of plant extract

The roots were air dried then ground into a powder. Approximately 100 grams of the powder was weighed then soaked in dichloromethane /methanol mixture at a ratio of 1:1 for two hours at room temperature. The mixture was then decanted and more DCM methanol added to the powder. The mixture was allowed to stand overnight before being decanted. The latter procedure was repeated twice. The solution obtained was then filtered using Whatman No.1 filter paper. The filtrate was evaporated to dryness at reduced pressure to obtain the extract. Then 0.05grams of the extract was weighed dissolved in 20 ml of 10% dimethylsulfoxide (DMSO) in normal saline to make a stock solution.

2.3 Experimental Animals

The institution had no animal care and ethics committee. However permission was obtained from the department of Medical physiology and all the studies were conducted in accordance with guidelines for care and use of laboratory animals (48).

Experiments involving antinociceptive and anti-inflammatory activity were performed on CBA mice (*Mus musculus*) weighing 25-30g while antipyretic experiments were performed using Sprague Dawley rats (*Rattus norvegicus*). The animals were kept in animal house with a 12 hour light and dark cycle and ambient temperature of 20-25° Celsius. Standard commercial food and tap water was provided *ad libitum*. They were allowed to acclimatize to the laboratory conditions for 7 days before the start of experiments.

2.3 Standard drugs

The following were the standard drugs used in this study; Acetyl salicylic acid (ASA) (Disprin; reckitt-B), Diclofenac sodium (voltaren) were bought in a retail Pharmacy in Nairobi. Morphine (Martindale pharmaceuticals) was provided by the department of Medical Physiology, University of Nairobi, while Carrageenan (Sigma), Lipopolysaccharide (LPS) (Sigma) was bought at Kobian Store Kijabe street Nairobi.

2.3.1 Drug administration

The *Solanum* root extract, standard drugs, LPS and normal saline were injected into the peritonial cavity (i.p) of the experimental animals at volume of 2ml. The 50, 100 and 200 mg/kg doses of the *S. incanum* extract and standard drugs were injected one hour before the subsequent treatment in order to give them time for distribution. Carrageenan was injected into the sub-plantar surface of the right hind paws of mice.

2.4.0 Bioassays

The antinociceptive and ant-inflammatory activity of *Solanum incanum* root extract was assessed using CBA strain of mice divided into groups (n = 6) while the antipyretic activity assay were carried out using Sprague Dawley rats divided into groups (n = 7)

2.4.1Sensory motor test

In the sensory motor test the mice were placed on observation stands with vertical placed cylindrical bars and their motility observed. Lack or reduced activity of the animals was interpreted as sensory motor impairment.

2.4.2 The Hot plate test

The test groups of mice (n = 6) received 50, 100 or 200 mg/kg of the herb extract, 100 mg/Kg of acetyl salicylic acid (ASA) and 5 mg/kg of morphine in a volume of 2ml / kg intraperitonial (i.p.), while the negative control group (n = 6) received same volume of 10% DMSO in normal saline in normal saline (vehicle). One hour after injection with the herb, vehicle or the drugs, the mice underwent sensory motor activity test. Time was measured using a digital watch. The mice were then separately placed in the hot plate (model II Inc. mod. 35D) maintained at $50^{o} \pm 1^{o}$ Celsius. The assay endpoint (reaction time in seconds) was the first instance of hind paw licking or lifting or jumping out. The time between placing the mouse on the hotplate and the end point was measured using a digital timer to one decimal place and to the nearest second. A 20 seconds cut off was imposed to prevent tissue damage. Each mouse was tested once (35)

2.4.3 Tail flick test

One hour after injections of the test drugs, the mice were separately tested for sensory motor activity. The negative control group (n = 6) received 2ml / kg vehicle i.p. while the test groups (n =6) each received 50, 100 and 200 mg/kg of herb extract, 100 mg/Kg ASA and 5 mg/Kg of morphine in a volume of 2 ml/kg. Tail flick procedure was carried out using a radiant heat analgesiameter (11 EC, Inc. Mod 33). Radiant heat was directed 5mm from the tip of the tail of the mice. The endpoint was indicated by the flick of the tail away from the radiant heat. The reaction time was measured using a stop timer (KADIO KD-1069) to the nearest second. A 20 second cut-off was imposed to prevent tissue damage from occurring (35). Each mouse was tested once.

2.4.4 Anti-inflammatory activity assay

Anti-inflammatory activity assay was carried out using the procedure as described in Omosire *et al.* (49). Hind paw edema in mice was used as a model of acute inflammation. A volume 0.1ml of 1% carrageenan in normal saline was injected into the sub-plantar surface of the right hind paws of mice. The control group (n = 6) received 2 ml/Kg of 10% DMSO in normal saline (vehicle). The test groups received 50, 100 , 200 mg/Kg of *Solanum incanum* root extract or 15 mg/Kg diclofenac sodium(i.p.) one hour before carrageenan injection. Time was measured using a digital watch. Two hours after carrageenan injection, the mice were anaesthetized by dropping them in a jar containing cotton wool dipped in chloroform. Both right and left hind limbs were cut identically at the ankle joint and weighed. The difference in weight between the right and left gave the degree of edema developed in the right hind limbs (49). The paw weight measurement in this study was preferred to volume displacement method due to unavailability of digital vernier calipers and plesthysmometer while analytical balances were readily available.

2.4.5 Anti-pyretic activity assay

Non-febrile male Sprague Dawley rats weighing between 200-300 grams were used in the study. The test group (n = 7) received either 50 or 100 mg/kg of *Solanum incanum* root extract. The negative control group (n = 7) received the vehicle (10% DMSO in normal saline) while the positive control group received 100 mg/kg acetyl salicylic acid (ASA).

The drugs, extracts or the vehicle were administered intraperitonialy (i.p). The animals were housed in cages with an ambient room temperature of 20-25° Celsius and a 12:12 hour light and dark cycle. Food and water was provided *ad. libitum.*

To induce fever, the rats were injected intraperitonially (i.p.) with 50 micrograms of Lipopolysaccharide (LPS pyrogen) from *Escherichia coli* 0111:b4 (Sigma Aldrich) in normal saline. The dose was chosen on basis of a previous study (46). Rectal temperature temperature was determined using a digital thermometer (DT-01(A)) with thermister probe inserted in the rectum. The rectal temperature was taken before the injection with the drugs and vehicle, then every 30 minutes after LPS pyrogen injection for the next 3 hours. The temperature reading after the thermometer gave an automatic alarm was taken as the assay end point. Calibration was done before the start of experiments using mercury thermometer.

2.6 Statistical analyses

The data obtained in the study was tabulated then expressed as mean ± standard errors of the mean. It was analyzed using one way ANOVA and *Scheffe's* post *hoc* test.

Windows KwikStat (WINKS) SDA 6.08 and Excel statistical packages were used to compute the data obtained in the results. A value of $p < 0.05$ was taken as the limit of significance while $p < 0.001$ was taken to be highly (very) significant

CHAPTER 3: RESULTS

3.1 Sensory motor tests

The drugs, extracts and vehicle treated mice showed normal exploratory behavior, grooming and climbing down the wooden observation stand. Some climbed up and down the stand. Hence they did not show any sign of sensory motor impairment as a result of various treatments. (Table 1)

Table 1

The Sensory motor tests result

Mice used for Tail flick test (36)		Mice used for hot plate test (36)	
impaired	Not impaired	Impaired	Not impaired
0	36	0	36

3.2 Tail flick test result

The effects of 50, 100, and 200 mg/kg doses of the root extract on the tail flick reaction time were investigated. The baseline reaction time was determined by administering the vehicle (10% DMSO in normal saline) intraperitonially. The 50 mg/kg *Solanum incanum* extract did not show significant ($p > 0.05$) change in mean reaction time. The 100 and 200 mg/kg doses of *Solanum incanum* extract and 100 mg/kg dose of ASA exhibited a significant ($p < 0.05$) increase in mean reaction time (antinociceptive effect). Morphine induced a very significant ($p < 0.001$) antinociceptive effect (Figure 1).

The mean response time for control (vehicle) was 6.0 ± 0.26 seconds whereas for S*olanum incanum* extract 50, 100 and 200 mg/kg were 6.837 ± 0.48, 9.33 ± 0.80 and 9.5 ± 0.85 seconds respectively. For ASA and morphine the mean response times was 8.33 ± 0.21 and 12.5 ± 0.43 seconds respectively.

Table 2

The antinociceptive effects of *S. incanum* in mice using tail flick test

Treatment / Dose (n = 6)	Tail flick reaction time (seconds)
Vehicle	6.0 ± 0.26
50 mg/kg *S. incanum* extract	6.83 ± 0.48
100 mg/kg *S. incanum* extract	9.33 ± 0.80 *
200 mg/kg *S. incanum* extract	9.5 ± 0.85 *
100 mg/kg ASA	8.33 ± 0.21 *
5 mg/kg Morphine	12.5 ± 0.43 **

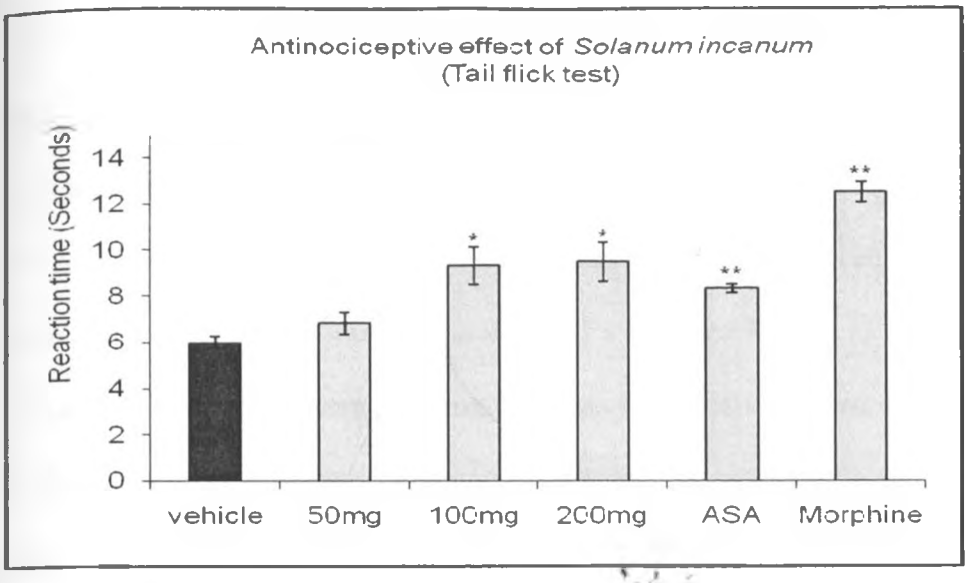
* ($p < 0.05$) **($p < 0.001$)

Values in (Table 2) represent reaction time (seconds) ± S.E.M.

The 100 and 200 mg / kg doses of the extract showed significant ($p < 0.05$) antinociceptive effects that was comparable to ASA while morphine induced a very significant ($p < 0.001$) antinociceptive effect.

Figure 1

The antinociceptive effect of *Solanum incanum* in mice(Tail flick test)



(n = 6) *($p < 0.05$) **($p < 0.001$)

The 100 and 200 mg/kg doses of extract and ASA exhibited significant ($p < 0.05$) antinociceptive effects while morphine induced a highly significant ($p < 0.001$) effect.

3.3 Hotplate test results

The 50 mg/kg dose of *Solanum incanum* extract did not exhibit significant increase in reaction time. However, 100 and 200 mg/kg doses of *Solanum incanum* extract and ASA caused significant ($p < 0.05$) increase in reaction time when compared with the vehicle. Morphine induced a highly significant ($p < 0.001$) increase in reaction time. (Figure 2) The mean response time for control (vehicle) was 5.0 ± 0.82 seconds whereas for 50, 100, and 200 mg/kg doses of *Solanum incanum* extract it was 3.5 ± 0.43, 8.5 ± 0.81 and 9.0 ± 0.73 seconds respectively. For ASA and morphine the mean response time was 8.5± 0.50 and 17.67 ± 0.95 respectively. (Table 3) There was no significant difference between the means of vehicle and 50 mg/kg *S. incanum* extract treated animals.

Table 3

The antinociceptive effects of *S. incanum* in mice using hot plate test

Treatment / Dose (n = 6)	Hot plate; reaction time (seconds)
Vehicle	5.0 ± 0.82
50 mg/Kg *S.incanum* extract	3.5 ± 0.43
100 mg/Kg *S. incanum* extract	8.5 ± 0.81 *
200 mg/Kg *S. incanum* extract	9.0 ± 0.73 *
100 mg/Kg ASA	8.5 ± 0.50 *
5 mg/Kg Morphine	17.67 ± 0.95**

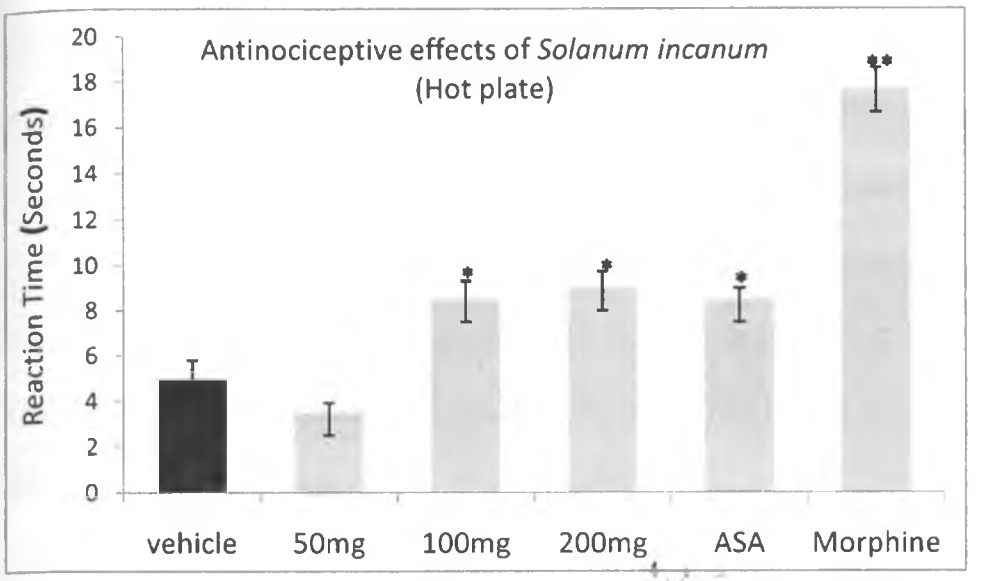
* ($p < 0.05$) ** ($p < 0.001$)

Values in (Table 3) represent reaction time in seconds ± S.E.M.

The 100 and 200 mg/kg doses of *S. incanum* showed significant ($p < 0.05$) antinociceptive effects.

Figure 2;

The antinociceptive effect of *Solanum incanum* (hot plate test)



(n = 6) *($p < 0.05$) ** ($p < 0.001$)

The 100 mg/kg dose of extract and ASA exhibited significant ($p < 0.05$) antinociceptive effects while morphine induced a highly significant ($p < 0.001$) effect.

3.4 The Antipyretic effect assay

Hyperthermia started developing thirty minutes after injection of LPS pyrogen. There was no significant difference ($p < 0.05$) between the means at 30, 60, and 90 minutes following LPS pyrogen injection (Table 4 and Figures 3a & 3b). However,120 minutes after pyrogen injection both the 100 mg/kg dose of *Solanum incanum* extract and ASA showed significant ($p < 0.05$) antipyretic effects while the 50 mg/kg dose of *Solanum incanum* dose had no apparent antipyretic effect. (Figure 3a) One hundred and fifty minutes after LPS pyrogen injection only ASA exhibited significant antipyretic effect

($p < 0.05$). (Figure 3b) Nevertheless 180 minutes following LPS pyrogen injection, the 50 and100 mg/kg dose of *Solanum incanum* extract exhibited significant ($p < 0.05$), antipyretic effects while ASA induced a very significant($p < 0.01$) antipyretic effect (Figure 3c).

Table 4

The antipyretic effects of *S. incanum* extract on lipopolysaccharide pyrogen induced fever in rats

	Time in minutes after LPS pyrogen injection					
Treatment/Doses	30min	60min	90min	120min	150min	180min
Vehicle	0.23 ± 0.06	0.55 ± 0.14	0.48 ± 0.13	1.35 ±0.14	1.43 ±0.14	1.63± 0.17
50mg/kg extract	0.6 ±0.30	0.65 ±0.23	0.78 ±0.17	0.88 ±0.17	0.93 0.29	0.95 0.20*
100mg/kg extract	0.23 ±0.15	0.42 ± 0.16	1.03 ±0.19	0.67± 0.17*	0.83± 0.11	0.8 ±0.17*
100mg/kg ASA	0.23± 0.15	0.43 ±0.12	0.57 ±0.13	0.63 ±0.17*	0.7 ±0.12*	0.57 0.10*

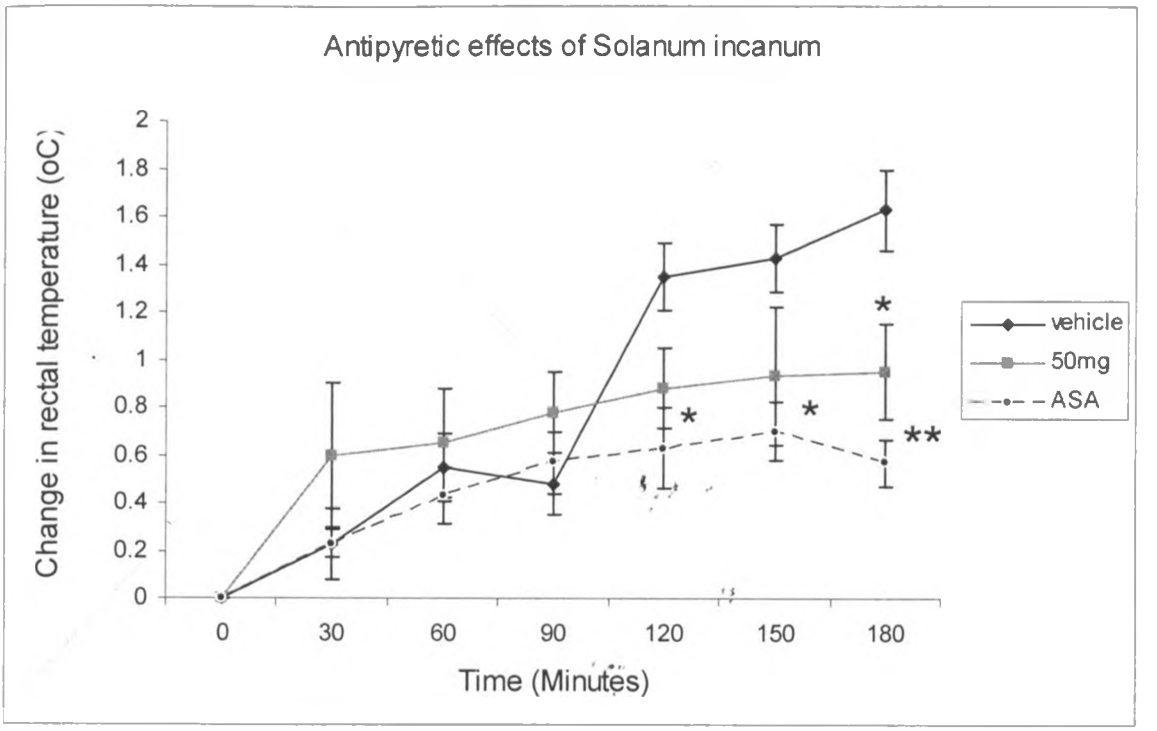
(n = 7) * ($p < 0.05$) ** ($p < 0.01$)

The values in (Table 4) represent changes in rectal temperature in ^{o} Celsius ± S.E.M.

The 50 mg/kg dose of the extract exhibited significant ($p < 0.05$) antipyretic effect 180 minutes only while the 100 mg/kg dose of the extract exhibited significant($p < 0.05$) antipyretic effects at 120 and 180 minutes after LPS pyrogen injection.

Figure 3a

The effect of 50 mg/kg dose of *Solanum incanum* extract on mean rectal temperature of rats after LPS pyrogen injection as a function of time

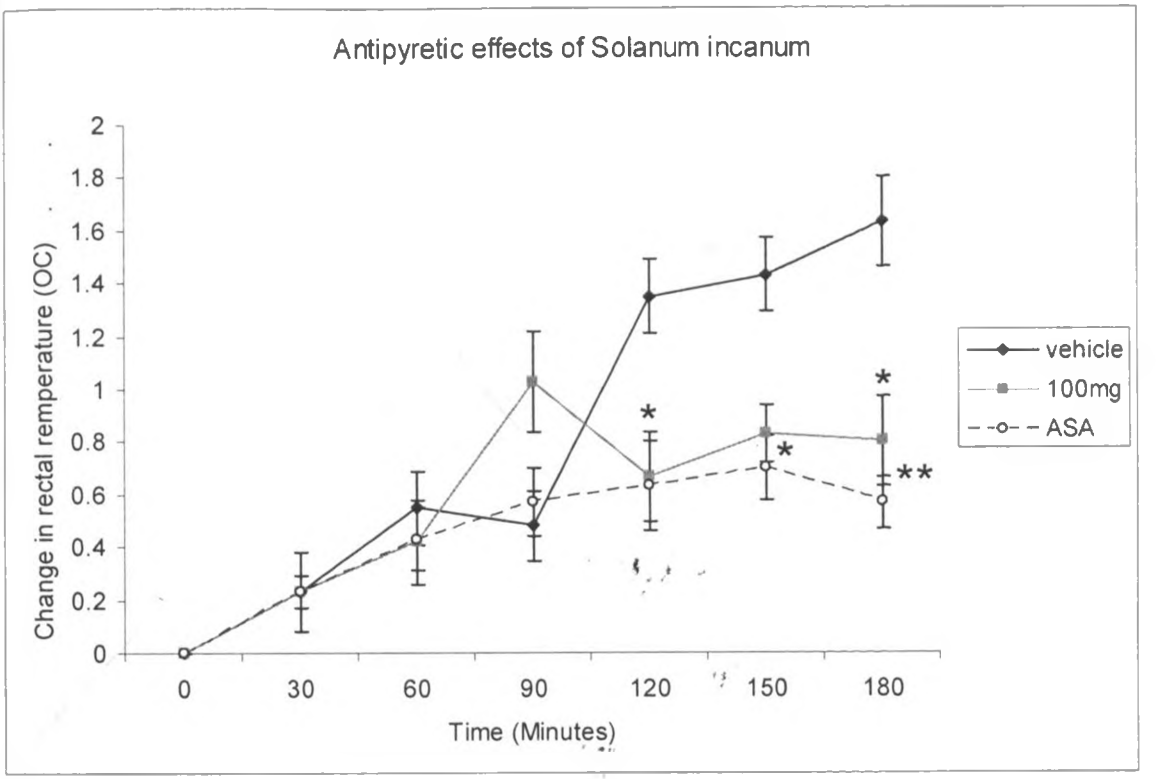


(n = 7) *($p < 0.05$) ** ($p < 0.001$)

There was a gradual rise in temperature in all the cases. However there was no significant difference observed between the various treatments and the vehicle at 30, 60 and 90 minutes after LPS pyrogen injection. The 50 mg/kg dose of *Solanum incanum* extract exhibited a significant antipyretic effect ($p < 0.05$) at 180 minutes after LPS pyrogen injection while ASA induced a significant ($p < 0.05$), antipyretic effects at 120 and 150 minutes and a highly significant($p < 0.001$) effect at 180 minutes after LPS pyrogen injection.

Figure 3b

The effect of 100 mg/kg dose *Solanum incanum* extract on mean rectal temperature of rats after LPS pyrogen injection as a function of time

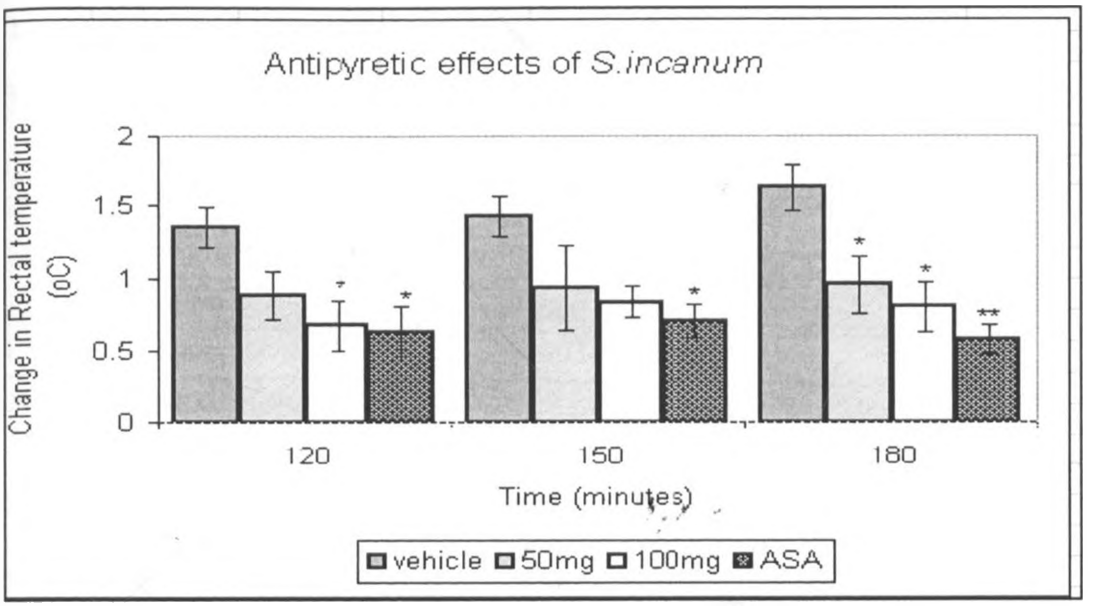


(n = 7) *($p < 0.05$) ** ($p < 0.001$)

Rise in rectal temperature occurred gradually throughout the three hours. Nevertheless both the 100 mg/kg dose of *Solanum incanum* and ASA did not exhibit significant ($p > 0.05$) antipyretic effects for the first 90 minutes following the LPS pyrogen injection. The 100 mg/kg dose of *Solanum incanum* extract showed significant antipyretic effects ($p < 0.05$) at 120 and 180 minutes after LPS pyrogen injection. ASA exhibited significant antipyretic effect ($p < 0.05$) at 120 and 150 minutes and a highly significant effect ($p < 0.001$) at 180 minutes after LPS pyrogen injection.

Figure 3c

The effect of the *Solanum incanum* extract on rectal temperature of rats at 120, 150 and 180 minutes after LPS pyrogen injection as a function of time



(n = 7) * ($p < 0.05$) ** ($p < 0.001$)

The 50 mg/kg dose of *S. incanum* exhibited significant antipyretic ($p < 0.05$) effect at 180 minutes only while the 100 mg/kg dose showed significant ($p < 0.05$) antipyretic effect at 120 and 180 minutes. ASA induced significant ($p < 0.05$) antipyretic effect at 120 and 150 minutes and a highly significant antipyretic ($p < 0.001$) effect at 180 minutes after LPS pyrogen injection.

3.5 Anti-inflammatory activity assay

(Table 5) shows the effect of various doses of *S. incanum extract* on carrageenan induced acute paw edema in mice. The 50, 100, and 200 mg/kg doses of the *S. incanum* extract did not exhibit significant ($p < 0.05$) effect in acute paw edema reduction when compared to the control. This

was in contrast to diclofenac sodium which induced a significant ($p < 0.05$) reduction in acute paw edema (anti-inflammatory effect).

Table 5

The anti-inflammatory effects of *S. incanum* extract

Treatment/ Dose (n = 6)	Paw edema in grams
Vehicle	0.023 ± 0.002
50 mg/kg herb	0.013 ± 0.004
100 mg/kg herb	0.020 ± 0.007
200 mg/kg herb	0.006 ± 0.003
15 mg/kg Diclofenac	0.003 ± 0.002*

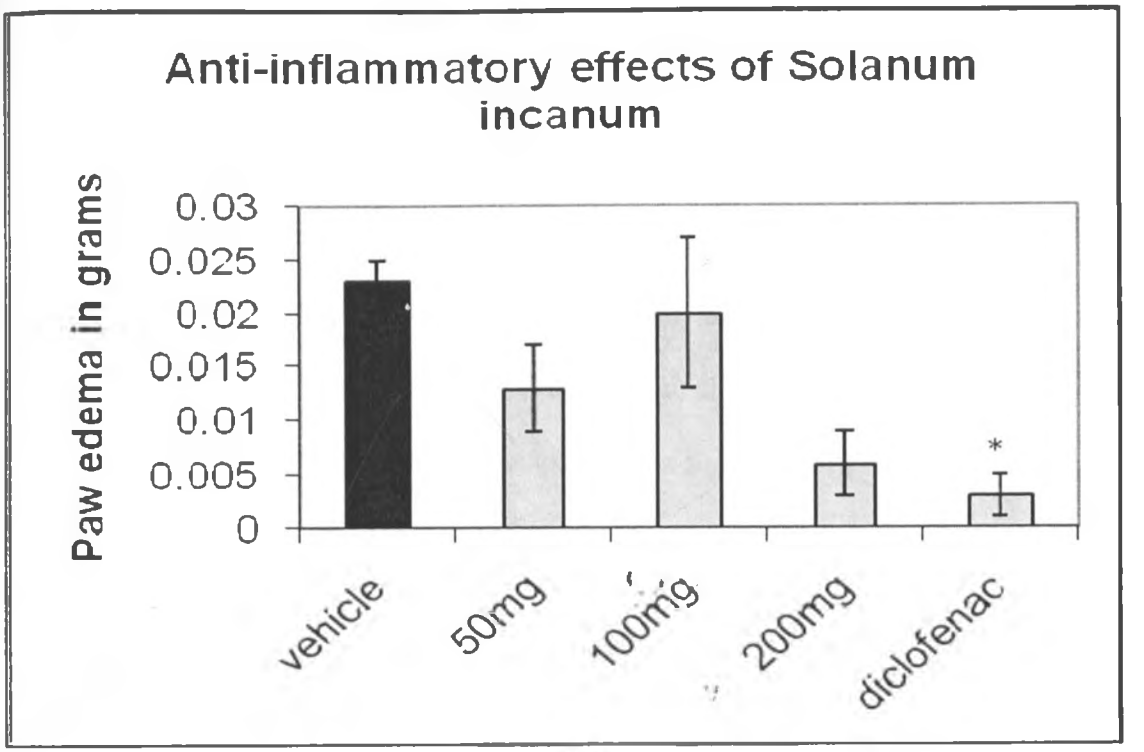
*($p < 0.05$)

The values in the (Table 5) above represent paw edema in grams ± S.E.M.

None of the three doses of the *S. incanum* extract (namely 50, 100, and 200 mg/kg) exhibited significant anti-inflammatory effects on carrageenan induced paw edema in mice.

Figure 4

Effect of *Solanum incanum* extract on carrageenan induced paw edema in mice



(n = 6) * ($p < 0.05$)

The 50, 100, and 200 mg/kg doses of *S. incanum* extract did not show significant ($p > 0.05$) decrease in edema paw weight. Hence the extract may not have significant anti-inflammatory effect.

CHAPTER 4 : DISCUSSION

The test animals that underwent sensory motor test did not show signs of sensory motor impairment and hence were used for subsequent experiments.

The tail flick test is a spinally mediated nociceptive test that is commonly used to study pain mechanisms. Mice show quantifiable behaviour when tested using this method (35).

In this study the dichloromethane: methanol extract of *Solanum incanum* was found to prolong the reaction time after radiant heat was directed to the tail of the test mice. This reaction time was comparable to ASA but much more less than that as result of morphine. The 100 and 200 mg/kg doses of *Solanum incanum* extract exhibited a significant ($p < 0.05$) lengthening of reaction time (antinociceptive) effect (35) compared to the vehicle. There was no significant difference between the antinociceptive effect of 100 and 200 mg/kg doses of *Solanum incanum* when compared to ASA. However morphine exhibited a highly significant ($p < 0.001$) antinociceptive effect compared to the vehicle treated animals, as well as with the 50, 100, 200 mg/kg doses of *Solanum incanum extract* and ASA. Though the 50 mg/kg *Solanum incanum* extract had no significant effect on tail flick test reaction time, the 100, 200 mg/kg doses of the herbal extract significantly ($P < 0.05$) lengthened the reaction time in the tail flick test that was comparable to ASA. Tail flick response is a spinally integrated reflex, hence it is likely that *Solanum incanum* extract may have acted via the central nervous system.

The hot plate test is commonly used to study nociception. The test is a supra-spinally integrated response and involves paw licking behavior and jumping by the animals. Paw licking is only affected by opioids while jumping is also affected by non steroidal anti-inflammatory drugs. Mice show clear and quantifiable behavior when tested on hot plate at temperature between 42

°C to 50 °C. In this test, antinociceptive activity of a drug is indicated by increase of reaction time or response latency (35).

In this study, 100 and 200 mg/kg doses of *Solanum incanum* root extract significantly ($p < 0.05$) increased the reaction time (antinociceptive activity). The 100 mg/kg dose of *Solanum incanum* extract showed a significant antinociceptive activity ($p < 0.05$) that was comparable to that of 100 mg/kg dose of ASA. However, morphine dose induced a very significant ($p < 0.001$) antinociceptive effect compared to ASA and *Solanum incanum* root extract.

The 50 mg/kg dose of *Solanum incanum* did not exhibit a significant ($p > 0.05$) antinociceptive effect. The 100 and 200 mg/kg doses of *Solanum incanum* and ASA exhibited significant ($p < 0.05$) antinociceptive activity, while morphine dose induced a highly significant ($p < 0.001$) antinociceptive effect when compared to 50 mg/kg dose of *Solanum incanum* root extract and the vehicle. In both tail flick and hot plate tests, the 50 mg/kg dose of *S. incanum* failed to exhibit significant antinociceptive effect. However the 100 and 200 mg/kg doses of the *S. incanum* did so, though there was no significant difference between the effects of the two doses of the extract, hence the effect of the herb may not be dose dependent. Morphine is a centrally acting analgesic (35) while ASA has both local and central effect (27) *Solanum incanum* root extract showed comparable antinociceptive effects to the reference drugs used in this study. The hot plate is a supra spinally mediated response (35) hence it is likely that *Solanum incanum* root extract may have centrally acting substance(s) with antinociceptive effect.

In this study male Sprague Dawley rats injected with LPS developed transient hyperthermia within the first 30 minutes. There was a gradual rise in the mean change of rectal temperature in all the groups but there was no significant difference between the means. At both 60 and 90 minutes following LPS pyrogen injection, substantial development of hyperthermia was recorded

in the vehicle, 50 and 100 mg/kg doses of *Solanum incanum* and ASA but none of the means from the four treatments was significantly different from the other. However at 120 minutes following LPS pyrogen injection the 100 mg/kg dose of *Solanum incanum* extract and ASA showed significant ($p < 0.05$) difference in rectal temperature change compared to the vehicle (antipyretic effect).

At 150 minutes following LPS pyrogen injection significant ($p < 0.05$) antipyretic effect was observed in ASA treated rats only. Yet at 180 minutes after LPS pyrogen injection the 50 and 100 mg/kg doses of *Solanum incanum* extract showed significant ($p < 0.05$) antipyretic effect while ASA induce a highly significant ($p < 0.001$) antipyretic effect. The 50 mg/kg dose of extract showed significant ($p < 0.05$) antipyretic effect at 180 minutes only while the first significant ($p < 0.05$) antipyretic effect observed with the 100 mg/kg dose of extract occurred at 120 minutes after LPS pyrogen injection. This difference in antipyretic onset time may indicate possibility of doses response relationship. The antipyretic effect observed in this study is similar to observations made by Zakaria *et al using* a closely related herb *Solanum nigrum* (11, 12). Several chemicals isolated from *Solanum incanum* includes ten flavonoids, chlorogenics, adenosine, benzyl-o–beta –D, xylopyranosyl (1-2)-beta –D, glycopyranoside, solasodine and three phenylalkanoic acids (14). It also contains steroidae alkaloids solargine(15). Despite all this, the active principle that exerts this effect has not been elucidated. The present study serves as a first step in determining whether the root extract of the *Solanum incanum* roots exerts the claimed antipyretic effects. In the study, 25% and 30% of rats treated with 50 and 100 mg/kg doses of *Solanum incanum* extract respectively developed hypothermia after LPS pyrogen injection. Eyup and Soner showed that administration of high doses of LPS pyrogen provoked a hypothermic response in rats probably due to development of shock (50). However in this study

hypothermia developed in response to injection of low dose of LPS pyrogen and only in *Solanum incanum* extracts treated animals. Therefore it appears that *Solanum incanum* extract potentates the development of the condition after injection of LPS pyrogen.

In the anti-inflammatory activity assay, there was an apparent but statistically insignificant ($p > 0.05$) reduction in carrageenan induced paw edema in the *S. incanum* treated mice especially with the 200 mg/kg dose. (Figure 4)

Closely related herbs *Solanum nigrum* (10, 11) and *Solanum lycocarpum* (12) were reported to exhibit significant anti-inflammatory effects. Therefore it likely that *S. incanum* root extract may contain low concentrations of substances with anti-inflammatory effects. Probably higher doses of the extract such as 400, 800 mg/kg and above may exhibit this effect.

CHAPTER 5: CONCUSION AND RECOMMENDATIONS

5.1 CONCLUSIONS

In this study the 100 and 200 mg/Kg doses of *Solanum incanum* root extract were observed to significantly ($p < 0.05$) increase the reaction time in the tail flick test. ASA induced a similar effect while morphine induced a very significant ($p < 0.001$) antinociceptive effect. Tail flick is a spinally mediated reflex (35) therefore it can be speculated that the 100 and 200 mg/kg doses of *Solanum incanum* may have exhibited antinociceptive activity via blockage of pain pathway at the spinal level. The 100 and 200 mg/kg doses of Solanum *incanum* root extract and ASA dose significantly ($p < 0.001$) increased the reaction time ($p < 0.05$) in the hot plate test while morphine induced a very significant ($p < 0.001$) antinociceptive effect in the same test. The fact that response to hot plate test is supra spinally integrated (35) may indicate that *Solanum incanum* root extract probably exerted antinociceptive effect via a central integrated mechanism. A related plant (herb) *Solanum nigrum* which exhibited significant antinociceptive effect (10, 11) was shown to have an additional significant neuropharmacological activity which included depression of central nervous system (51). Such an effect may be associated with a central antinociceptive activity. In summary *Solanum incanum* root extract may contain substances with both spinal and supra spinal antinociceptive activity.

In the current study the 100 mg/kg dose of S*olanum incanum* extract exhibited significant ($p < 0.05$) antipyretic effect at 120 and 180 minutes after LPS pyrogen injection. The 50 mg/kg S. *incanum* extract exhibited significant ($p < 0.05$) antipyretic effect at 180 minutes after LPS pyrogen injection while ASA induced significant ($p < 0.05$) antipyretic effect at 120, 150 and

180 minutes. More or less similar effects were observed by (*Zakaria et al.)* using a related herb *Solanum nigrum* (10, 11). From the results it is highly probable that *Solanum incanum* root extract contains compound(s) with antipyretic effect.

In this study the 50, 100 and 200 mg/kg doses of *Solanum incanum* failed to exhibit significant anti-inflammatory effect. This may rule out existence of high concentration of anti-inflammatory compounds in *Solanum incanum* root extract. However related herbs S*olanum nigrum* (10, 11) and S*olanum lycocarpum* (12) are reported to have significant anti-inflammatory of activity.

5.2 RECOMEDATIONS

The *Solanum. Incanum* extract exhibited significant antinociceptive effect with both tail flick and hot plate test. This implies that it may contain substances with central antinociceptive effects. These effects needs to be investigated further.

Solanum incanum root extract exhibited significant antipyretic effect meaning that further studies are warranted to elucidate the nature and mechanism(s) of action of the possible active substances present.

The *Solanum incanum* root extract doses of 50 ,100,and 200 mg/Kg doses did not exhibit significant anti-inflammatory effect. This may indicate probable lack of anti-inflammatory substances in the extract. Higher doses of the herb could be used to conclusevly asses the presence or otherwise of its anti-inflammatory activity.

During the study it was noted that *S. incanum* appeared to potentate development of lipopolysacharide fever. This toxicological effect(s) should be investigated.

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APPENDIX

ANTINOCICEPTIVE EFFECT ASSAY

Tail flick test values represent reaction time (seconds)

vehicle	50mg	100mg	200mg	ASA	morphine
6	5	10	9	8	12
6	7	7	8	9	12
6	7	10	9	9	14
7	6	12	12	8	11
5	8	7	7	8	13
6	8	10	12	8	13

Hot plate test reaction time (seconds)

vehicle	50mg	100mg	200mg	ASA	morphine
5	5	9	8	9	15
4	2	12	9	10	17
6	4	7	12	9	17
5	3	9	7	9	17
5	3	7	10	7	18
2	4	7	8	7	22

ANTI-INFLAMMATORY EFFECT ASSAY

mice No.	vehicle	50mg	100mg	200mg	Diclofenac
1	0.03	0.01	0	0	0
2	0.03	0.01	0.01	0	0
3	0.02	0	0.05	0	0
4	0.02	0.01	0.02	0.01	0
5	0.02	0.02	0.03	0.02	0.01
6	0.02	0.03	0.01	0.01	0.01

Values represent paw edema weight in grams

ANTIPYRETIC EFFECT ASSAY

(Values represent change in rectal temperature in °C.

Vehicle

Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	0	0.4	0.4	1.1	1.8	1.9	1.9
2	0	0	0.8	0.8	1.4	1.1	1.7
3	0	0.2	0.2	0.3	1.4	1.6	1.9
4	0	0.4	0.9	1.2	1.6	1.8	2.1
5	0	0.1	0.9	0.8	1.2	1.2	1.3
6	0	0.3	0.1	0.5	0.7	1.0	0.9
7	0	1.1	0.9	1.0	1.1	1.1	1.6

50mg dose (herb)

Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	0	1.1	1.1	1.8	1.6	1.5	1.4
2	0	0	0.2	0.6	0.5	0.3	0.4
3	0	1.6	1.4	1.1	0.5	0.4	0.9
4	0	-0.4	-0.1	0.7	0.9	1.1	1.0
5	0	0.4	1.0	0.9	0.7	0.3	0.4
6	0	0.9	0.3	1.1	1.1	1.8	1.6

100mg dose (herb)

Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	0	-0.1	0.9	0.6	0.3	0.6	0.3
2	0	0.4	0.0	0.1	1.4	1.2	0.2
3	0	0	-0.1	0.0	0.1	0.4	1.2
4	0	-0.21	0.3	0.1	0.6	1.0	1.0
5	0	0.0	0.7	0.9	0.8	1.0	1.2
6	0	0.0	0.7	1.2	0.8	0.8	0.9
7	0	0.9	0.9	0.6	0.7	0.5	0.3

ASA

Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	0	0.6	0.8	0.6	0.3	0.6	0.3
2	0	1.0	0.8	0.6	1.1	0.9	0.6
3	0	0.3	0.0	0.3	0.3	0.4	0.9
4	0	-0.2	0.4	1.1	1.2	1.2	0.7
5	0	0.2	0.2	0.1	0.2	0.4	0.2
6	0	0.4	0.4	0.7	0.7	0.7	0.4
7	0	0.7	0.7	0.3	0.3	0.4	0.4

Serial numbers = Rat No.

RAW DATA SHEET; ANTIPYRETIC EFFECT ASSAY

	Vehicle						
Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	37.1	37.5	37.5	38.2	38.9	39.0	39.0
2	37.8	37.8	38.6	38.6	39.2	38.9	39.5
3	37.5	37.7	37.7	37.8	38.9	39.1	39.4
4	37.0	37.4	37.9	38.2	38.6	38.8	39.1
5	37.1	37.2	38.0	37.9	38.0	38.3	38.4
6	37.5	37.8	37.6	38.0	38.2	38.5	38.4
7	37.7	37.8	38.6	38.7	38.8	38.8	39.3
	50mg dose (herb)						
Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	37.2	38.3	38.3	39.0	38.8	38.7	38.6
2	37.0	37.0	37.2	37.6	37.5	37.3	37.4
3	37.6	39.2	39.0	38.7	38.1	38.0	38.5
4	37.6	37.2	37.5	38.3	38.5	38.7	38.5
5	37.1	37.5	38.1	38.0	37.8	37.4	37.5
6	37.0	37.9	37.3	38.1	38.1	38.8	38.6
7	37.6	35.6	35.4	35.4	36.1	36.3	36.4
8	37.3	34.7	35.1	35.1	36.3	36.7	36.8
	100mg dose (herb)						
Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	37.1	38.2	38.0	37.7	37.4	37.7	37.4
2	37.6	38.0	37.6	37.7	39.0	38.8	37.8
3	37.2	37.2	37.1	37.2	37.3	37.6	38.8
4	37.8	35.7	38.1	37.9	38.4	38.8	38.8
5	37.0	37.0	37.7	37.9	37.8	38.0	38.2
6	37.8	37.8	38.5	39.0	38.6	38.6	38.7
7	37.5	38.4	38.4	38.1	38.2	38.0	37.8
8	37.6	35.8	35.9	35.6	35.6	35.4	35.6
9	37.3	34.7	35.4	36.9	36.2	36.1	37.3
10	37.6	36.9	36.7	36.4	37.0	37.3	37.7
	ASA						
Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**
1	37.0	37.6	37.8	37.6	37.3	37.6	37.3
2	37.5	38.5	38.3	38.6	38.6	38.4	38.1
3	37.0	37.3	37.0	37.3	37.3	37.4	37.9
4	37.4	37.2	37.8	38.5	38.6	38.6	38.1
5	37.4	37.6	37.6	37.5	37.6	37.8	37.6
6	37.6	38.0	38.0	38.3	38.3	38.3	38.0
7	37.3	38.0	38.0	38.6	37.6	37.7	37.7

(Values represent rectal temperature in °C). Serial numbers = Rat No.