Mwonjoria J.K. *et al.* / *International Journal of Phytopharmacology.* 2011, 2(1), 22-26.

ISSN 0975-9328
2229- 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

THE ANTINOCICEPTIVE ANTIPYRETIC EFFECTS OF *SOLANUM INCANUM* (Linneaus) IN ANIMAL MODELS

Mwonjoria J. K[1], Kariuki H N,[1] and Waweru FN[1]

[1]Department of Medical Physiology University of Nairobi, Kenya.

ABSTRACT

The root of *Solanum incanum* is used by some Kenyan communities as a folklore remedy for fever, wounds, toothache, and stomach ache. However studies have not been done to validate these claims. The aim of this study was to investigate antinociceptive and antipyretic effects of *Solanum incanum* root extract using animal models. The antinociceptive assays were carried out using tail flick and hot plate tests on CBA mice. The 100 and 200 mg doses of *Solanum incanum* root extract showed significant antinociceptive activity ($p < 0.05$) in both hot plate and tail flick tests. In the antipyretic, assay fever was induced in Sprague Dawley rats using lipopolysacharide (LPS). The 50 mg dose of *Solanum incanum* extract exhibited significant antipyretic effect ($p < 0.05$) at 180 minutes while the 100 mg dose of *S. incanum* exhibited significant antipyretic effect ($p < 0.05$) at 120 and 180 minutes after the lipopolysaccharide pyrogen injection. The results obtained renders support to folklore use of *Solanum incanum* root extract for pain and fever.

Keywords: *Solanum incanum*, Antinociceptive, Analgesic, Antipyretic, Fever.

Introduction

The use of plant parts for therapeutic purposes has been widely practiced in Africa (Sindiga *et al.* 1995). One of the herbs used for fever and analgesia is *Solanum incanum* (bitter or Sodom apple) (Kokwaro, 1993) which belongs to *Solanaceae* family (Schmelzer and Gurib-Fakim, 2008). 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 (Kokwaro, 1993). Elsewhere in Africa *S. incanum* is used for sore-throat, angina, head ache, warts, and benign tumours (Schmelzer, Gurib-Fakim 2008; Dold, Cocks, 2000). Extracts from related herb (*Solanum nigrum*) exhibited antinociceptive and antipyretic effects (Zakaria *et al*., 2006, 2009).
Though the *S. incanum* is extensively used for pain and fever management much of the study has centred on it anti-microbial (Mbaya and Muhammed, 1977; Britto, Senthikumar, 2001) and anti-tumour (Chun-Nan Lin *et al*., 1990; Kupchan *et al*., 1965) effects. 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 (Yun-Lian Lin *et al*., 2000.). Young leaves and stem of *Solanum incanum* have been shown to contain high levels of an alkaloid solasodine (Elsadig and Al-Ansari, 1997). Although *S. incanum* roots extract is used as folklore remedy for pain and fever, studies have not been done to validate these claims. The aim of this study was to investigate these folkloric claims using animal models.

Corresponding Author

Mwonjoria JK
Email; jkmmaina@gmail.com

MATERIALS AND METHODS

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 where a specimen voucher number 2009/JM 3 was deposited.

Preparation of plant extract

The roots were air dried then ground into a powder. 100 grams of the powder was weighed then soaked in dichloromethane methanol mixture at a ratio of 1:1 for 72 hours at room temperature. The mixture was then filtered using Whatman No.1 filter paper. The filtrate was evaporated to dryness at reduced pressure to recover the extract. The weight of the crude extract was 4.2g. The extract was then weighed dissolved in 10% dimethylsulfoxide (DMSO) in Normal saline.

Experimental Animals

All studies were conducted in accordance with guidelines for care and use of laboratory animals. (Wolfensohn and Lloyd, 1998) Experiments involving antinociceptive activity were performed on CBA mice of both sexes weighing 25-30g. The mice were evenly distributed. While antipyretic experiments were performed using male Sprague Dawley rats. The animals were kept in cages in the animal house with a 12 hour light and dark cycle and ambient temperature of 20-25^{o} Celsius. Standard commercial food and tap water was provided *ad libitum*. They were allowed to acclimatize to the laboratory for 7 days before the start of experiments.

Standard drugs

Acetyl salicylic acid (ASA) (Disprin; Reckitt-B) and Morphine (Martindale pharmaceuticals) were used as standard drugs in this study.

Drug administration

The herb extracts, standard drugs, lipopolysaccharide and vehicle (10% DMSO in normal saline) were injected into the peritoneal cavity (i.p).

Sensory motor tests

The mice underwent a sensory motor activity test before the tail flick and hot plate test to rule out cases of sensory motor impairment.

Antinociceptive Asssay

a) Tail flick test

The control group (n = 6) received 2ml / Kg vehicle i.p. while the test groups received 50 mg/Kg, 100 mg/Kg and 200 mg/Kg of *S. incanum* extract, 100 mg/Kg ASA and 5 mg/Kg of morphine in a volume of 2 ml/Kg. The tail flick procedure was carried out using a radiant heat analgesiameter (11 EC, Inc. Mod 33) one hour after the injections with the doses of extract and controls. 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 timer to the nearest second. A 20 second cut-off was imposed to prevent tissue damage from occurring (Eyup and Soner, 2007). Each mouse was tested once.

b) Hot plate test

The test groups of mice (n = 6) received 50 mg/Kg, 100 mg/Kg or 200 mg/Kg doses of the herbal extract, 100 mg/Kg dose of acetyl salicylic acid (ASA) and 5 mg/Kg dose of morphine in a volume of 2ml / Kg intraperitonial while the control group (n = 6) received same volume of 10% DMSO in normal saline (vehicle). One hour after the injections with the doses of extract and controls, the mice were separately placed on the hot plate (model II Inc. mod. 35D) maintained at 50^{o} ±1^{o} Celsius. The assay endpoint (reaction time in seconds) was the first instance of hind paw licking or lifting or jumping. The time between placing the mouse on the hotplate and the end point was measured using a digital timer to the nearest second. A 20 seconds cut off was imposed to prevent tissue damage. Each mouse was tested once.

Antipyretic activity assay

Non-febrile male Sprague Dawley rats weighing between 200-300 grams were used in the study. The test group (n = 7) received 50 mg/Kg and 100 mg/Kg of *Solanum incanum* root extract. The control groups (n = 7) received either the vehicle or 100 mg/Kg acetyl salicylic acid (ASA). The drugs, extracts or the vehicle were administered intraperitonialy (i.p). To induce fever, the rats were injected with 50 micrograms of lipopolysaccharides (LPS pyrogen) from *Esherichia coli* 0111:b4 (Sigma Aldrich) in normal saline intraperitonially (i.p.). The dose was chosen on basis of a previous study (Tavares *et al.* 2004). Rectal (core) temperature was determined using a digital thermometer (DT-01(A)) with the thermister probe inserted in the rectum. The core 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 end point was when the thermometer gave an automatic alarm.

Statistical analyses

The data was expressed as mean ± standard errors of the mean. It was analyzed using one way ANOVA and *Scheffe* post *hoc* test. A value of $p < 0.05$ was taken as the limit of significance.

RESULTS

Tail flick test

The 50 mg dose of *Solanum incanum* extract had no effect. However the 100 and 200 mg doses of *Solanum incanum* extract and 100 mg dose of ASA exhibited a significant antinociceptive effect ($p < 0.05$). Morphine exhibited a highly significant antinociceptive effect ($p < 0.001$) compared to the vehicle. (Fig 1) The mean response time for control (vehicle) was

6.0 ± 0.26 seconds whereas for S*olanum incanum* extract 50 mg/Kg 100 mg/Kg and 200 mg/Kg were 6.83 ± 0.48, 9.33 ± 0.80 and 9.5 ± 0.85 seconds respectively. For ASA and morphine the response time was 8.33 ± 0.21 and 12.5 ± 0.43 seconds respectively.

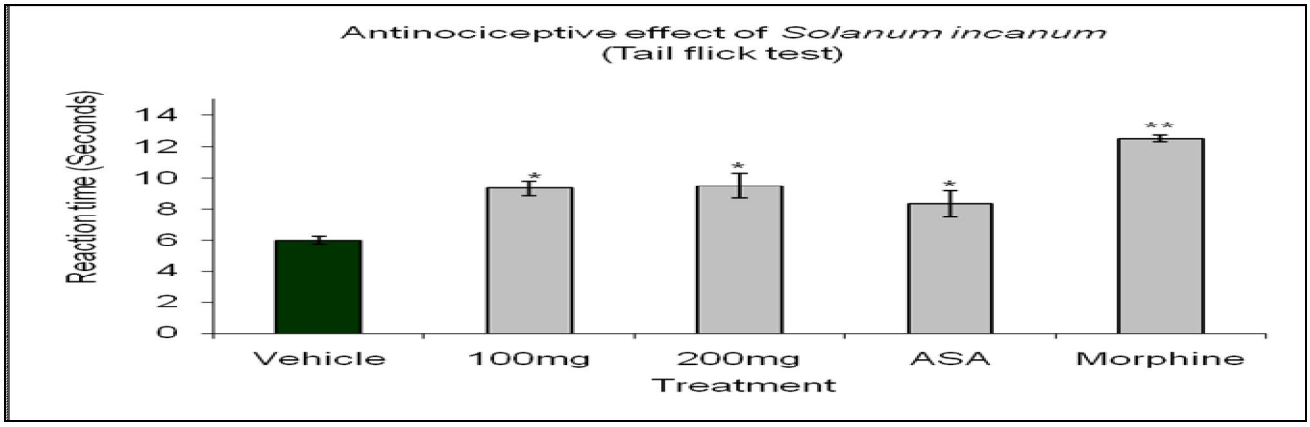
Hotplate test

The 50 mg/Kg dose of *Solanum incanum* extract did not exhibit antinociceptive effect. However, 100 mg/Kg and 200 mg/Kg doses of *Solanum incanum* extract and ASA caused significant antinociceptive effect ($p < 0.05$) when compared with the vehicle, while Morphine showed a highly significant increase in reaction time ($p < 0.001$). (Fig 2) The mean response time for control (vehicle) was 5.0 ± 0.82 seconds whereas that of 50 mg / Kg, 100mg / Kg and 200mg / Kg doses of *Solanum incanum* extract it was 3.5 ± 0.43, 8.5 ± 0.81 and 9.0 ± 0.73 seconds. For ASA and morphine the mean response time was 8.5± 0.50 and 17.67 ± 0.95 respectively.

Antipyretic assay

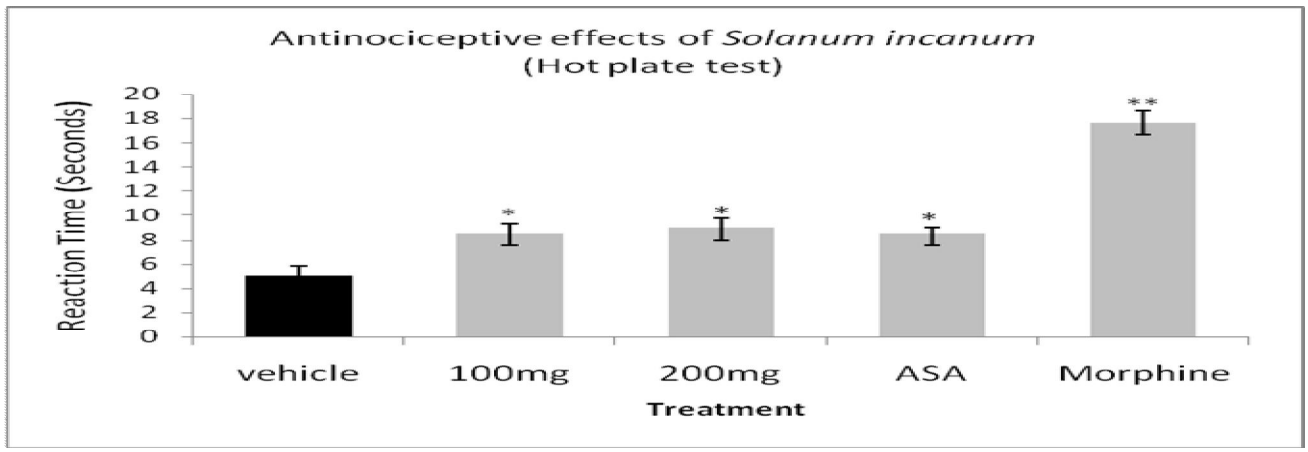
Hyperthermia started developing thirty minutes after injection of LPS pyrogen. There was no significant difference between the means at 30, 60, and 90 minutes following LPS pyrogen injection. The 50 mg/Kg dose *Solanum incanum* extract exhibit significant antipyretic effect ($p < 0.05$) at 180 minutes only while the 100 mg/Kg dose of *Solanum incanum* showed significant antipyretic effect ($p < 0.05$) at 120 and 180 minutes. ASA exhibited significant antipyretic effect ($p < 0.05$) at 120 and 150 minutes and a highly significant antipyretic effect ($p < 0.001$) at 180 minutes. (Fig 3).

Figure 1. Shows antinociceptive effect of *S incanum* in mice (Tail flick test)



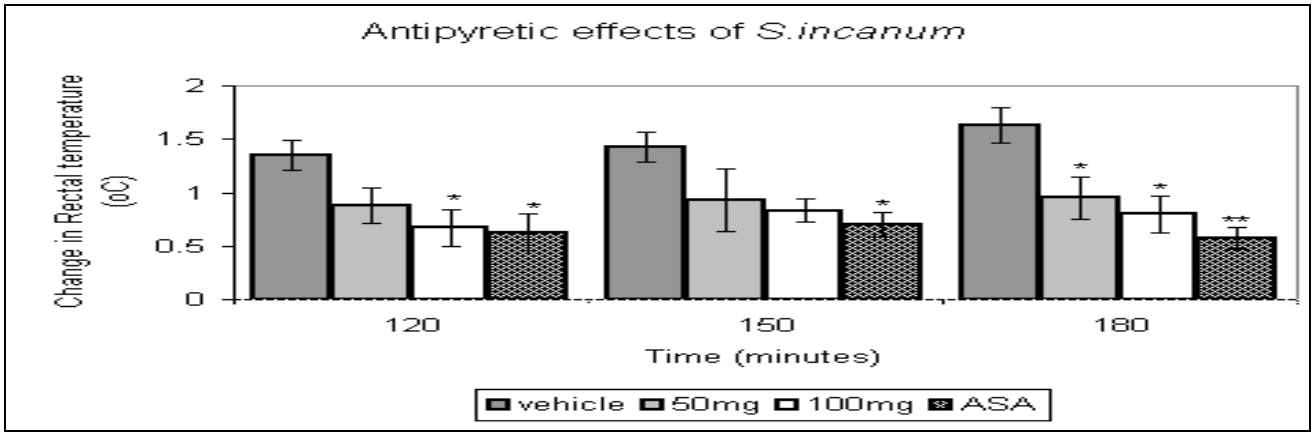
*($p < 0.05$) ** ($p < 0.001$)

Figure 2. The antinociceptive effect of *Solanum incanum* (hot plate test)



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

Figure 3. The effect of *S. incanum* root extract extract on core temperature of rats after LPS pyrogen injection as a function of time.



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

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 (Le Bars *et al.* 2001). 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. The 100 mg/Kg and 200 mg/Kg doses of *Solanum incanum* extract exhibited a significant lengthening of reaction time ($p < 0.05$) ie antinociceptive effect (Le Bars *et al.* 2001) compared to the vehicle. There was no significant difference between the antinociceptive effect of 100 mg/Kg and 200 mg/Kg dose of *Solanum incanum* when compared to 100 mg/Kg ASA. However morphine exhibited a highly significant antinociceptive effect ($p < 0.001$) compared to the vehicle treated animals as well as the 50 mg/Kg, 100 mg/Kg, 200 mg/Kg doses of *Solanum incanum* extract and ASA. The 50 mg/Kg *Solanum incanum* had no significant effect on tail flick test reaction time. The 100 mg/Kg and 200 mg/Kg doses of *Solanum incanum* significantly increased the reaction time ($P < 0.05$) in the tail flick test comparable to that of the ASA dose. Since tail flick is a spinal integrated reflex, it is likely that *Solanum incanum* extract may have acted via the central nervous system by blocking pain pathway at spinal level.

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. In this test, antinociceptive activity of a drug is indicated by increase of reaction time or response latency (Le Bars *et al.*, 2001) In this study, the 50 mg/Kg dose of *Solanum incanum* did not exhibit a significant antinociceptive effect. However, the 100 mg/Kg and 200 mg/Kg doses of *Solanum incanum* root extract significantly increased the reaction time ($p < 0.05$) ie antinociceptive activity, which was comparable to that of 100 mg/Kg of ASA. The morphine dose exhibited a highly significant antinociceptive effect ($p < 0.001$).

Morphine is mainly a centrally acting analgesic (Le Bars *et al.*, 2001) while ASA has both local and central effect (Vane and Botting, 2003). *Solanum incanum* root extract showed comparable antinociceptive effects to the reference drugs used in this study. Since hot plate is supra-spinally mediated response (Le Bars *et al.*, 2001), it is likely that *Solanum incanum* root extract may have exerted antinociceptive effect via a central integrated mechanism. A related herb *Solanum nigrum* which exhibited significant antinociceptive effect (Zakaria *et al.*, 2006, 2009) was shown to have significant neuropharmacological activity with central nervous system depression (Perez *et al.*, 1998). 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 that could be investigated further.

In antipyretic assay, rats injected with LPS pyrogen developed a gradual rise in rectal temperature in all the groups although there was no significant difference between the means up to 90 minutes. However at 120 minutes the 100 mg/Kg dose of *S. incanum* extract and ASA showed significant antipyretic effect ($p < 0.05$) compared to the vehicle.

At 150 minutes only ASA exhibited significant antipyretic effect ($p < 0.05$). Nevertheless at 180 minutes, both the 50mg and 100 mg doses of *S. incanum* extract showed significant antipyretic effect ($p < 0.05$). ASA exhibited a highly significant antipyretic effect ($p < 0.001$). The antipyretic effect observed in this study is similar to observations made by (Zakarial *et al*., 2006) using a related herb *Solanum nigrum*. Chemically, *Solanum incanum* contain ten flavinoids, chlorogenics, adenosine, benzyl-O–beta–D, Xylopyranosyl (1-2)-beta–D, glycopyranoside, Solasodine and three Phenylalkanoic acidics. (Elsadig and Al-Annsari, 1997). It also contains steroid alkaloid solargine. (Kupchan *et al*., 1965) Despite all this, active principles that exert these effects have not been elucidated. However, it is highly probable that *Solanum incanum* root extract contains compound(s) with antipyretic effect that may have crossed the blood brain barrier and inhibited the cyclo-oxygenase activity which acts as the final step in febrogenesis (Tavares *et al*., 2004). Therefore further studies are required to elucidate the nature and mechanism(s) of action of the possible active substance(s) present. This study serves as a first step in determining whether the root extract of the *S. incanum* exerts the claimed antinociceptive and antipyretic effects.

Acknowledgements

We would like to thank the following persons Prof. Patel N.B Chairman of Department of Med. Physiology for his assistance during the studies, Kinyungu Charles., Nzivo Charles., Mugweru, Ng'ang'a Titus, Dr Wambugu and Ndung'u Juliet and for the technical support.

REFERENCES

Britto SJ, Senthinkumar S Antimicrobial activities of *Solanum incanum* leaf extract. *Asian J, Microbio Biotech and Enviro Sci*, 3(1-2), 2001, 65-66.

Chun-Nau L, Chai-Ming L, Ming-Kung C, Kim –Hong G and Sheng-Jeng W The cytotoxic principles of *Solanum incanum*. *J, Nat Prod*, 5(3), 1990, 513-516.

Craft RM, Mogil JS, Aloisi AM, Sex difference in pain and analgesia: the role of gonadal hormones. *Euro J, Pain*, 8, 2004, 397-411.

Le Bars D, Gozariu M, Caddens Animal model of nociception. *Pharmacol Rev*, 53, 2001, 597-652.

Dold AP, and Cocks ML The medical use of some weeds, problem and alien plants in the Grahams town and Peddie district of Eastern Cape S.A. *S.A. J. Sci*, 96, 2000, 467-473.

Elsadig AE, Al-Ansari Roderick JG Changes in steroidal alkaloid Solasodine during development of *Solanum nigrum* & *Solanum incanum*. *Phytochem*, 46 (3), 1997, 489 - 494.

Eyup SA, Soner M, *Eschirichia coli* lipopolysaccharides produce serotype-specific hypothermic response in biometered rats. *Am J, physiol Regul Inter Comp Physiol*, 2007, 1-24.

Kokwaro J, Medicinal plants of East Africa. 2nd Edn East Africa Literature Bureau, Nairobi, 1993: 222-223.

Kupchan SM, Barbouti ST, Knox JK, Laucam CA, *Beta*Solamarine; tumour inhibitor isolated from *Solanum dulcamara*. *Science*, 150(3705), 1965, 827-1828.

Mbaya B, Muhammed S, Antibiotic action of *Solanum incanum* L. *Anti-microb agents and chemother*, 6, 1976, 920-927.

Perez RM., Perez JA., Garcia LMD, Sossa HM, Neural pharmacological activity of *Solanum nigrum* fruit. *J, Ethnopharm*. 62 1998, 45-48.

Schmelzer G.H. and Gurib-Fakim A, Prota Medicines plant 1. Prota Foundation/Backhuys Publishers/CTA Wageningen, Netherlands, 2008, 525-528.

Sindiga I, Nyaigotti-Chacha C, Kanunah MP, Traditional medicine in Africa. East African Education Publishers Ltd Nairobi 1995.

Tavares E, Maria LO Rosaria M, Francisco JM, Neutralization of macrophages inflammatory protein-2 blocks the febrile response induced by Lipopolysaccharide in rats. *J, Thermal Biol*, 29, 2004, 413-42145.

Vane JR, Botting RM, The mechanism of action of aspirin *Thrombosis Research*, 110, 2003, 255-258.

Wolfensohn S. and Lloyd M, Handbook of laboratory animal management and Welfare. 2nd Ed. Blackwell science Ltd, Oxford UK, 1998, 169-216.

Yun-lian L, Wan-yi W, Kuo YH, Non-steroidal constituents from *Solanum incanum* L. *J. Chinese Chem Soc*, 47, 2000,247-251.

Zakaria AZ, Hanan KG, Hairani Z, Nur HMP, Roslan MS, Antinociceptive, anti-inflammatory and antipyretic effects of *Solanum nigrum* chloroform extracts on animal model. *Yakugaku Zasshi*, 126(11), 2006, 1171-1178.

Zakaria ZA, Sulaiman MR, Morsid NA, Aris A, Zainal H, Pojan NH, Kumar GH, Antinociceptive, anti-inflammatory and antipyretic effects of Solanum nigrum aqueous extract in animal models. *Methods Find Exp Clin Pharmacol 2*, 2009, 81-8.