African Journal of Pharmacology and Therapeutics Vol. 9 No. 1 Pages 22-26, 2020
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Research Article

Analgesic activity of ethanolic leaf extract of *Solanum anomalum*

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Background: *Solanum anomalum* Thonn. ex Schumach. (family *Solanaceae*) is a shrub with edible fruit consumed locally for nutritional and medicinal purposes.

Objective: To evaluate the leaf extract of *Solanum anomalum* for analgesic properties in mice.

Method: The ethanol leaf extract of *Solanum anomalum* (70-210 mg/kg) was evaluated for analgesic activity against nociception in mice using acetic acid-induced writhing, formalin-induced hind paw licking and thermally-induced pain models.

Results: The leaf extract significantly inhibited nociception in all the models tested in a dose-dependent fashion.

Conclusion: The leaf extract possesses analgesic activity which confirms its use in traditional medicine in the treatment of pains.

Keywords: *Solanum anomalum*, analgesic, medicinal plant

Published: May, 2020

1. Introduction

Solanum anomalum Thonn. ex Schumach. (family *Solanaceae*) is a shrub growing up to 2 metres tall. The stem, branches and midribs of the leaves have prickles up to 5 mm long. The edible fruits are gathered from the wild and consumed locally. Both the fruits and the leaves are used medicinally. The plant is sometimes cultivated or semi-cultivated for its fruits. It is found in West tropical Africa - Sierra Leone to southern Nigeria, Cameroon and DR Congo. It is known as 'childrens' tomatoes'. They are more commonly used as a condiment in soups and sauces and the fruits are eaten raw or cooked (Burkill, 2000). The sap from the leaves and fruits is drunk, or taken by enema 1 - 2 times daily, as a treatment for leprosy and gonorrhoea (Burkill, 2000). The fruits are used as a laxative and digestive (Burkill, 2000). They are also served ground up in soups and sauces as an appetizer for sick persons, sometimes mixed with fruits of Parkia (Burkill, 2000). The crushed fruits are applied topically to promote maturation of inflamed parts of the fingers and toes (Burkill, 2000). The fruit juice is applied to sores on the ears to alleviate pain (Bukenya and Hall, 1988). Offor and Ubengama (2015) reported the antidiabetic activity of the fruit of this plant. The leaf extract has been reported to possess anti-inflammatory (Okokon et al, 2017), anticonvulsant and depressant (Okokon et al, 2019) activities. Although *Solanum anomalum* is used widely in traditional medicine for the treatment of various diseases, there is paucity of information on the biological activity of the leaves of this plant. We report in this study the analgesic activity of the leaf extract of the plant.

2. Methods

2.1 Plants collection and extraction

The plant material *Solanum anomalum* (leaves) were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in August, 2015. The plant was identified and authenticated by a taxonomist in the Department of

Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium (UUH: **No 75 (a)**).

The plant parts (leaves) were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using a mortar and pestle. The powdered material was macerated in 50% ethanol. The liquid filtrate was concentrated and evaporated to dryness *in vacuo* at 40˚C using rotary evaporator and stored in a refrigerator at - 4˚C.

2.2 Phytochemical Screening

Phytochemical screening of the crude extract was done employing standard procedures and tests (Trease and Evans, 1989; Sofowora, 1993). These were carried out to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

2.3 Animal Husbandry

Albino Swiss mice (19 – 28g) of either sex were obtained from the University of Uyo animal house. They were housed in standard cages with saw dust beddings and maintained on standard animal pellets (Guinea Feeds, Nigeria) and water *ad libitum*. The animals were maintained under standard conditions (12 hours light/ 12 hours dark cycle). Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo (CHS/AE/015/18). All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of laboratory Animals (NIH Publications No. 80-23) revised in 2002.

2.4 Determination of median lethal dose (LD_{50})

The median lethal dose (LD_{50}) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke (1983). This involved intraperitoneal administration of different doses of the extract (100 -1000 mg/kg) to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD_{50} was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

2.5 Evaluation of analgesic potential of the extract

Acetic acid induced writhing in mice

Writhings (abdominal constrictions consisting of the contraction of abdominal muscles together with the stretching of hind limbs) resulting from intraperitoneal (i.p) injection of 2% acetic acid (Sigma, USA, Analar grade), was induced according to the procedure described by Santos et al, (1994), Correa et al, (1996) and Nwaforet al, (2010). The animals were divided into 5 groups of 6 mice per group. Group 1 served as negative control and received 10 mL/kg of normal saline, while groups 2, 3 and 4 were pre-treated with 70, 140, and 210 mg/kg doses of *Solanum anomalum* leaf extract intraperitoneally, and group 5 received100 mg/kg of acetyl salicylic acid (Sigma, USA). After 30 minutes, 0.2ml of 2% acetic acid was administered intraperitoneally (i.p). The number of writhing movements was counted for 30 minutes. Anti-nociception (analgesia) was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with extracts.

Formalin – induced hind paw licking in mice

The procedure adopted was similar to that described by Hunskaar and Hole (1987), Correa and Calixto (1993), Gorki et al, (1993) and Okokon and Nwafor, (2010). The animals were injected with 20 µL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffer, 10 mM) under the surface of the right hind paw. The amount of time spent licking the injected paw was timed and considered as the indication of pain. Adult albino mice (20 – 25 g) of either sex randomized into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 hours before being used but allowed access to water. The animals in group 1 (negative control) received 10 mL/kg of normal saline, groups 2 - 4 received 70, 140, and 210 mg/kg doses of the extract, while group 5 received 100 mg/kg of acetyl salicylic acid (ASA) 30 minutes before being challenged with buffered formalin. The responses were measured for 30 mins after formalin injection.

Thermally induced pain in mice

The effect of the extract on hot plate induced pain was investigated in adult mice. The hot plate was used to measure the response latencies according to the method of Vaz et al, (1996) and Okokon and Nwafor, (2010). Adult albino mice (20 – 25 g) of either sex randomized into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 hours before being used but allowed access to water. The animals in group 1 (negative control) received 10 mL/kg of normal saline, groups 2 - 4 received 70, 140, and 210 mg/kg doses of the extract, while group 5 received 100 mg/kg of acetyl salicylic acid (ASA) 30 minutes before the experiment.

In these experiments, the hot plate was maintained at 45□±1˚C, each animal was placed into a glass beaker of 50 cm diameter on the heated surface, and the time(s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30-second cut off was used to prevent tissue damage.

The animals were randomly divided into 5 groups of 6 mice each and fasted for 24 hours but allowed access to water. Group 1animal served as negative control and received 10 mL/kg of normal saline. Groups 2, 3 and 4 were pretreated intraperitoneally with 70, 140, and 210 mg/kg doses of *Solanum anomalum* leaf extract respectively, while group 5 animals received 100 mg/kg of acetyl salicylic acid intraperitoneally, 30 minutes prior to the placement on the hot plate.

2.6 Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One- way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between the means were considered significant at 1% and 5% level of significance i.e. $p < 0.05$. Graphpad Instat 3 (USA) was used for the analyses.

3. Results

The phytochemical screening of the ethanol extract of the leaf of *Solanum anomalum* revealed the presence of alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids.

The median lethal dose (LD_{50}) was calculated to be 724.5 mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

The administration of *Solanum anomalum* extract (70-210 mg/kg) demonstrated a dose-dependent reduction in acetic acid-induced writhing in mice. The reductions were statistically significant ($p<0.05$) relative to control and not comparable to that of the standard drug, ASA (**Table 1**).

The extract exhibited a dose- dependent analgesic effect on formalin-induced hind paw licking in mice. The extract prominently inhibited the two phases of formalin-induced paw licking with a more considerable inhibition of the second phase. These inhibitions were significant when compared to the control ($p< 0.05$) and comparable to that of the standard drug, ASA (**Table 2**).

Table 1: Effect of *Solanum anomalum* leaf extract on acetic acid induced writhing in mice.

Dose (mg/kg)	Time Intervals (hr)						
	5	10	15	20	25	30	TOTAL
Control	6.66±0.88	11.66±1.20	24.66± 1.85	17.0± 0.57	13.00±1.15	11.0± 1.00	83.98±6.65
Extract							
70	4.33± 0.33	5.33±1.45	13.0±2.00 ^{b}	12.66±0.88 ^{b}	11.66±0.66	7.66± 0.33	54.64±5.65^{a}
140	3.33± 0.33	5.33± 2.96	9.00±1.52^{c}	9.66 ± 0.88^{c}	8.33±1.76	7.0±1.00^{a}	42.65±8.45^{c}
…..210	0.33± 0.33^{c}	3.00±0.57 ^{b}	4.33±1.20^{c}	4.00± 0.57^{c}	5.66±0.88^{b}	4.00± 0.57^{c}	21.32±4.12^{c}
ASA 100	4.00±0.57^{a}	6.00±0.57	8.33± 0.88^{c}	8.66± 0.33^{c}	7.66±0.66^{c}	4.00 ± 0.57^{c}	38.65±3.58^{c}

Data expressed as mean ± SEM. significant at $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$ when compared to control n = 6. ASA: acetylsalicylic acid

Table 2: Effect of *Solanum anomalum* leaf extract on formalin- induced hind paw licking in mice.

Dose (mg/kg)	Time Intervals (hr)						
	5	10	15	20	25	30	TOTAL
Control	24.33±0.33	22.37±0.66	20.66±0.44	14.33±0.23	13.03± 0.14	10.16±0.16	104.88±1.96
Extract							
70	4.33± 0.33	5.33±1.45	13.0±2.00 ^{B}	12.66±0.88 ^{B}	11.66±0.66	7.66± 0.33	54.64±5.65^{a}
140	3.33± 0.33	5.33± 2.96	9.00±1.52^{c}	9.66 ± 0.88^{c}	8.33±1.76	7.0±1.00^{a}	42.65±8.45^{c}
…..210	0.33± 0.33^{c}	3.00±0.57 ^{B}	4.33±1.20^{c}	4.00± 0.57^{c}	5.66±0.88^{b}	4.00± 0.57^{c}	21.32±4.12^{c}
ASA 100	4.00±0.57^{a}	6.00±0.57	8.33± 0.88^{c}	8.66± 0.33^{c}	7.66±0.66^{c}	4.00 ± 0.57^{c}	38.65±3.58^{c}

Data expressed as mean ± SEM. significant at $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$ when compared to control n = 6. ASA: acetylsalicylic acid

Table 3: Effect of *Solanum anomalum* leaf extract on hot plate test

Group	Dose (mg/kg)	Reaction time (sec) (mean ± SEM)	% inhibition
Control	-	4.92± 0.23	
S. anomalum	70	5.97± 0.22	21.3
	140	13.25 ± 0.50 ^{a}	169.3
	210	20.51± 0.74^{b}	316.8
ASA	100	29.53±3.48^{b}	500.2

Data expressed as mean ± SEM. Significant at $^{a}p< 0.05$, $^{b}p< 0.001$ when compared to control. n = 6. ASA: acetylsalicylic acid

The extract (70-210 mg/kg) exhibited a dose-dependent effect on thermally-induced pain in mice. This inhibition was only statistically significant ($p<0.001$) when compared to the control at the highest dose of the extract**.** The effect of the extract was not comparable to that of the standard drug, ASA (**Table 3**).

4.0 Discussion

The extract significantly reduced acetic acid-induced writhing, formalin-induced hind paw licking and also delayed the reaction time of animals (mice) to thermally induced pain. Acetic acid causes inflammatory pain by increasing capillary permeability (Amico-Roxas et al*,* 1984; Nwafor et al*,* 2007), and in part through local peritoneal receptors from peritoneal fluid concentration of PGE_2 and $PGF_{2\alpha}$ (Deraedt et al*,*1980; Bentley et al*,*1983). The acetic acid-induced abdominal writhing is a visceral pain model in which the processor releases arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Franzotti et al*,* 2002). It is used to distinguish between central and peripheral pain. These results suggest that the extract may be exerting its action partly through the lipoxygenase and/or cyclooxygenase system.

The inhibition of acetic acid-induced writhing by the extract at all doses employed suggests an antinociceptive effect which might have resulted from decreased synthesis of arachidonic acid metabolites.

Formalin- induced pains involve two different types of pains which are in phases; neurogenic and inflammatory pains (Vaz et al*,* 1996, 1997).This model measures both centrally and peripherally mediated activities that are characteristic of biphasic pain responses. The first phase (0 to 5 min), named neurogenic phase provoked the release of bradykinin and substance P while the second and late phase initiated after 15 to 30 min of formalin injection resulted in the release of inflammatory mediators such as histamine and prostaglandin (Wibool et al*,* 2008; Yi-Yu et al*,* 2008). The first phase of formalin-induced hind paw licking is selective for centrally acting analgesics such as morphine (Berkenet al*,* 1991), while the late phase of formalin-induced hind paw licking is peripherally mediated. The extract ability to inhibit both phases of formalin-induced paw licking suggests its central and peripheral activities as well as its ability to inhibit bradykinins, substance P, histamine and prostaglandins which are mediators in these pains.

It was observed that the extract significantly delayed the reaction time of the thermally- induced (hot plate) test. This model is selective for centrally acting analgesics and indicates narcotic involvement (Turner, 1995) with opioid receptors.

Phytochemical screening of the leaf extract shows that the leaf extract contains alkaloids, flavonoids, cardiac glycosides, tannins, saponins, and terpenes (Okokon et al*,* 2017). Some of these phytoconstituents found to be present in the leaf extract of plant in this study may be responsible for the observed reported activity. Sesquiterpenes like β-caryophyllene and (*E*,*E*)-α-farnesene have been reported to possess analgesic potentials (Ahmed et al*,* 1997;Chavanet al*,* 2012) and may also contribute to the observed analgesic activity. Flavonoids are known to act through inhibition of the cyclooxygenase and lipoxygenase pathways (Liang et al*,* 1999; Carlo et al*,* 1999), phospholipase A_2 and phospholipase C (Middleton et al*,* 2000). Some flavonoids exert their antinociception via opioid receptor activation activity (Suh et al*,* 1996; Rajendranet al*,* 2000; Otuki et al*,* 2005). The extract has been reported to exhibit analgesic activity. The presence of these compounds (polyphenolics, flavonoids, monoterpenes, sesquiterpenes and triterpenes) in this plant might account for the activity and may in part explain the mechanisms of its actions in this study.

5.0 Conclusion

The results of this study demonstrated that *Solanum anomalum* possesses analgesic properties which supports its use in traditional medicine for treatment of pain.

Conflict of Interest declaration

The authors declare no conflict of interest.

Acknowledgement

The authors are grateful to Ms. Sussan Attah of Department of Pharmacology and Toxicology, University of Uyo for her technical assistance.

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