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EVALUATION OF ANALGESIC ACTIVITY OF THE METHANOL EXTRACT OF *Solanum aethiopicum* (*Linn*). FRUIT IN MICE

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ABSTRACT
Pain is the first sign of most diseases; pain is a vague unpleasant sensation elicited by external and internal stimuli, which pain is a warning signal primarily protective. Solanum aethiopicum is traditionally used in most African countries for pain treatment without scientific proof; this study was carried out to evaluate the analgesic activities of Solanum aethiopicum fruit methanol extract in mice. The Solanum aethiopicum methanol extract (SAME) was subjected to an acute toxicity test and analgesic studies using an acetic acid-induction and hot plate at 150, 300, and 600mg/kg doses. The superoxide dismutase (SOD) and Glutathione (GSH) levels were also assayed using colourimetric methods. The medial lethal dose of Solanum aethiopicum was greater than 5000mg/kg, revealing that the extract is practically non-toxic after oral administration. The evaluation of the analgesic activity of the extract at all tested doses showed statistically significant antinociceptive activity in both chemicals-induced peripheral and thermal-induced central pain (p≤0.05). The greater analgesic activity was observed by the 150 mg/kg dose of the extract in both the acetic acid-induced writhing test and the hot plate method. The Superoxide dismutase and Glutathione activities were quantified and showed significant (p≤0.05) antioxidant activity, as these enzymes play a crucial role in antioxidant defence. However, the extract had an analgesic activity almost equal to that of Piroxicam (standard drug). The results of this study elucidated that the Solanum aethiopicum methanol extract possessed a significant analgesic activity.
Keywords: Solanum aethiopicum, Analgesic, Pain, Hot Plate, Acetic acid-induced

INTRODUCTION

Pain is the first sign of most diseases, like chest pain, neuropathic, and stomach pain, as well as various organs (Deng, 2011). Pain is described as "an unpleasant sensory and emotional experience associated with actual or potential tissue damages. It's usually initiated by noxious stimuli and transmitted over specialised neural network to the CNS where its interpreted as such, it is a way to protect the body from possible injury" by the International Association for the Study of Pain (IASP) (Merskey, 2017). The currently available standard drugs for pain and inflammation remain the mainstay for managing and treating these disorders (Tamrat *et al*., 2017). However, they are associated with many side effects and toxicities, such as gastric irritation, gastric ulcer. Using NSAIDs increases the risk of cardiovascular adverse events, especially in patients taking COX-2 inhibitors (Geremew *et al.,* 2015). Studies have revealed that whereas NSAIDs typically produce gastrointestinal problems, opiates promote physical dependency, tolerance, and addiction (Hewitt *et al.,* 2009). Opioid analgesics are also associated with many unwanted side effects and toxicities, including disturbing hormonal homeostasis and addiction. Given this, there is a need to intensify research into medicinal plants, which are claimed to be effective in managing pain and inflammation (Schug *et al.,* 2003). *Solanum aethiopicum* is a large group of *Solanaceae* consisting of four cultivars Kumba, gilo, shun and Aculeate. Each cultivar is subdivided into subgroups (Ayodele *et al*.,2018). The girl's subgroup "Gnangnab", whose French name is better eggplant, is a rare cultivar on the local level. *S. aethiopicum* has a high nutritional potential. In addition to this nutritional potential, the vegetable has medicinal properties (Ekweogu *et al*., 2020; Abubakar *et al*., 2021). In Nigeria it is called gauta by Hausa, igbagba by Yoruba and afufa by Igbo tribes (Burkill *et al.,* 2000). However, to the extent of our knowledge, no study has highlighted the effect of *Solanum aethiopicum* on analgesic activity; the present study aimed to evaluate the analgesic activity of *Solanum aethiopicum* fruit methanol extract using acetic acid induce writhing and hot plate test.

MATERIALS AND METHODS

Drugs and chemical

The chemical used for the experiment includes Acetic acids, Methanol, Morphine sulfate, Piroxicam, normal saline and Phosphate Buffer Solution (PBS).

Collection of plant material

The *Solanum aethiopicum* used in this study was purchased from a local market in Kano State, Nigeria. The plant identification and authentication were done in the Department of Plant Biology, Bayero University Kano, with the Voucher number collected as BUKHAN-0501 and kept for future reference. The dried *Solanum aethiopicum* was crushed and pounded into fine powder using a pestle and mortar and pulverised using an electric grinder.

Preparation of plant extract

The methanol extract was prepared by cold extraction technique. About 100g of the dried *S. aethiopicum* powder at room temperature was immersed in 500 ml methanol for 24 h. The mixture was then filtered and repeated using the remaining residue with 300 ml methanol. Both filtrates were combined and concentrated under reduced pressure using a rotary evaporator. The resulting semisolid residue was pounded to dryness under a hot-air dryer to obtain a powdery crude *S. aethiopicum* extract (Sukhdev *et al.*, 2008).

Experimental animals

Wistar mice (20-25g) of both sexes were obtained from the Animal House, Department of Pharmacology and Therapeutics, Bayero University, Kano and were used for the experiment. The animals were house under standard laboratory conditions at room temperature with a relative humidity of 70–80%. They were fed with a standard commercial diet and water at libitum. All the procedure has been used according to the National Institution of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (Albus, 2012).

Acute toxicity study

The acute toxicity study of *Solanum aethiopicum* methanol extract was investigated in mice using the method described by Lorke (1983); this was conducted in two phases. In the first phase, 3 groups of three mice each were used. The 1st, 2nd, and 3rd groups were treated intraperitoneally (*i.p*) with the extract at doses of 10, 100 and 1000mg/kg body weight, respectively. The animals were then observed for 24 hours for signs of toxicity and death. In the second phase, three mice were administered more specific doses, 1600, 2900, and 5000mg/kg of the extract *i.p*. which depended on the result of the first phase and observed for 24 hours for a sign of toxicity and death. Therefore, the medial dose (LD_{50}) was estimated using the relationship $LD_{50}=\sqrt{}$lowest lethal dose ×highest non-lethal dose.

Evaluation of the analgesic activity of the extract

Acetic acid induces the writhing response

The method described by Koster *et al.* (1959), the test was conducted and detected the peripheral analgesic activity of the extract and was performed by randomly dividing overnight fasted mice with free water access. Mice were injected with 10ml/kg *i.p.* acetic acid solution in water (0.6%v/v) thirty minutes after they were given the extract. Analgesic activity of the extract was assessed by counting the number of writhing observed in the mice, which consist of contraction of the abdominal muscle together with stretching of the hind limbs for 30min after a latency period of 5 min. A reduction in the number of writhes as compared to the control group was considered evidence for the analgesic potential of the extract, and it was expressed as per cent inhibition of writhing as follows:

%analgesic activity = (control-treated)*100/Mean writhing count (control)

Hot plate method

The method described by Eddy and Leimbach (1953), the test was conducted to evaluate the central analgesic potential of *Solanum aethiopicum* extract. It was performed by introducing the mice into an open-ended cylindrical space with a floor of a metallic plate heated with a thermos. A plate was heated to a constant temperature of 47° ± 1°C producing the behavioural component that has been measured in terms of their reaction times. The reaction time (in seconds) or latency period was determined as the time the mice took to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 30, 60, 90, and 120 min after the administration of the vehicle (distilled water, standard drug morphine and 150, 300 and 600mg/kg of the extract), the percentage increase in reaction time or pain threshold inhibition was calculated as follows.

%elongation= latency (test)-latency (control)*100/Latency (test)

Determination of oxidative stress markers

Reduced glutathione (GSH) concentration

Glutathione (GSH) was determined by the method of Ellman (1959). About 1 ml of supernatant (0.5 ml Brain tissue homogenate precipitated by 2 ml of 5% TCA) was taken, 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) were added. Absorbance was at a wavelength of 412 nm.

Superoxide dismutase (SOD) activity

Superoxide dismutase was determined by the method described by Fridovich (1989). Brain tissue homogenate of 0.1ml was diluted in 0.9ml of distilled water to make a 1:10 dilution of the microsome. An aliquant mixture of 0.2ml of the diluted microsome was be added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of distilled water. The absorbance was measured over 30 to 150 seconds at 480nm.

Calculations:

Increase in absorbance per minute = (A2 - A1)/2.5

% Inhibition = 100 - {(Incr. in absorbance for sample/Incr. in absorbance of blank) x 100}

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of Adrenaline to adrenochrome in 1 minute.

Statistical Analysis

Data obtained were analysed using one-way analysis of variance (ANOVA) version 23, followed by Bonferroni *post-hoc* test, and values of p<0.05 were considered significant. Results were expressed as Mean ± Standard Error of the Mean (SEM) in tables.

RESULTS AND DISCUSSION

Acute toxicity studies

The result shows that the extract is non-toxic in mice and that the lethal dose was observed to be greater than 5000mg/kg *i.p*. According to Gosselin *et al.* (1984), classification, this extract could be classified as practically non-toxic.

Effect of *Solanum aethiopicum* methanol extract (SAME) on acetic acid-induce writhing

The result showed that the extract possesses analgesic activity at all tested doses (150,300 and 600mg/kg *i.p,*) with a decreased writhing response in treated mice. A significant ($p<0.05$) decrease in the number of writhing responses was observed at all doses when compared to the distilled water (control), and the same with the standard drug (Piroxicam) which shows significantly ($p<0.01$) decreased (Table 1). The extract of *Solanum aethiopicum* decreases the number of writhing, which suggests its possible peripheral analgesic activity. Hence, the maximum reduction was observed at 150mg/kg.

Effect of SAME on the hot plate test

The result of the hot plate test showed that the latency time was significant ($p<0.05$) at tested doses after 90 minutes. This test produced paw-licking and jumping behavioural responses, both of which are considered to be supraspinal responses. The maximum effect was observed after 90 minutes, as shown in Table 2. The most significant ($p<0.01$) increase in latency time was noticed against 150mg/kg of SAME.

Effect of SAME on Glutathione (GSH)

GSH plays a very important role in antioxidant activity, hence the GSH levels were significantly ($p<0.05$) decreased. However, treatment with SAME (150 & 300 mg/kg) significantly ($p<0.05$) increased the GSH levels when compared to the control group. The maximum antioxidant effect was obtained at 150mg/kg, almost equal to the standard drug piroxicam (Table 3).

Effect of SAME on SOD

Treatment with SAME (150-600 mg/kg) revealed a dose-dependent significant ($p<0.05$) increase in the SOD activity when compared to the control group. Likewise, the standard drug piroxicam also significantly ($p<0.05$) increased the SOD activity compared to the control group (Table 4)

Table 1: Effect of *Solanum aethiopicum* extract on acetic acid induce writhing

Treatment (mg/kg)	Mean numbers of writhes
DW 10 ml/kg	31.6±6.337
SAME (600)	15.80±3742*
SAME (300)	16.4±3.778*
SAME (150)	14.2±2.557*
Piroxicam (10)	13.6±3.208**

Values are presented as Mean ± SEM. *P<0.05 **P <0.01 as compared with a control group, one-way analysis of variance ANOVA followed by Bonferroni's *post- hoc* test. n = 6, DW = Distilled water, SAME= *Solanum aethiopicum* methanol extract.

Table 2: Effect of *Solanum aethiopicum* extract on hot plate latency time in mice

Treatment (mg/kg)	**0 Min**	**30 Min**	**60 Min**	**90 Min**	**120 Min**
DW 10 ml/kg	1.08±0.22	0.96±0.15	1.20±0.19	1.41±0.16	1.40±0.16
SAME(600)	1.18±0.15	1.51±0.18*	1.82±0.15*	2.56±0.20*	2.07±0.11*
SAME(300)	1.26±0.18	1.13±0.09	1.39±0.40	2.47±0.35*	1.95±0.13
SAME(150)	1.16±0.12	1.48±0.16*	1.76±0.39*	1.68±0.16*	3.31±0.27**
Morphine (10)	0.94±0.10	1.79±0.03*	2.46±0.35**	2.45±0.19*	3.31±0.27**

Values are presented as Mean ± SEM * p<0.05 **p<0.01 compared to the control group, one-way variance analysis of variance ANOVA followed by Bonferroni's *post-hoc* test. n = 6, Distilled water, SAME= *Solanum aethiopicum* methanol extract.

Table 3: Effect of *Solanum aethiopicum* extract on GSH

Treatment (mg/kg)	GSH(u/mg) protein
DW 10ml//kg	15.75±6.05
SAME (600)	15.62±3.06
SAME (300)	20.51±2.21*
SAME (150)	24.04±1.76*
Piroxicam (10)	24.22±2.38*

Values are presented as Mean ± SEM * p<0.05 as compared to the control group; Analysis was performed with One-way ANOVA followed by Bonferroni's *post-hoc* test. n = 6, DW = distilled water (10mg/kg), SAME= *Solanum aethiopicum* methanol extract

Table 4: Effect of *Solanum aethiopicum* extract on SOD Activity

Treatment (mg//kg)	SOD(u/mg) protein
DW0 10ml/kg	0.75± 0.33
SAME (600)	1.49±0.22*
SAME (300)	1.47±0.21*
SAME (150)	1.29±0.13*
Piroxicam (10)	1.52±0.23*

Values are presented as Mean ± SEM * $p < 0.05$ compared to the control group, one-way analysis of variance ANOVA followed by Bonferroni's *post-hoc* test. n = 6, Distilled water, SAME= *Solanum aethiopicum* methanol extract.

DISCUSSION

The acute toxicity study conducted on *Solanum aethiopicum* showed that, the LD50 in mice was estimated to be >5000mg/kg *i.p.* body weight. The values of LD_{50} obtained showed that, the methanol extract of *Solanum aethiopicum* is considered to be non-toxic when administered intraperitoneally in mice. Acute toxicity evaluates the adverse effects that occur following exposure of the organism to single or multiple doses of a test within 24hrs by a known route (Subramanian *et al.,* 2018). Moreover, the results from acute toxicity serve as a guide in dosage selection for long-term toxicity studies and other studies involving animal use (Loomis and Hayes,1996).

The extract of *S. aethiopicum* significantly reduced the number of acetic acid induced writhes in mice, and revealed analgesic properties similar to the standard drug (Piroxicam) at tested doses (Table 1). the number of abdominal cramps clearly shows that SAME has analgesic activity, and maximum reduction was observed at 150mg/kg. The acetic acid-induced writhing test is a highly recommended model for screening peripheral analgesic potential of test compounds because of its sensitivity and capacity to detect the antinociceptive effect of natural products and test compounds at dose levels that remain inactive for other methods (Tamrat *et al*., 2017). Intraperitoneal injection of acetic acid causes irritation and stimulation of the peritoneal cavity that triggers the synthesis and release of various endogenous inflammatory mediators such as histamine, serotonin, bradykinin substance P, and PGs (Konate *et al*., 2012). These endogenous inflammatory mediators elicited chemical-induced visceral pain, characterised by constriction of abdominal muscles and the extension of the forelimbs and elongation of the body. That is why the acetic acid-induced writhing test is considered a model of visceral pain (Tadiwos *et al.,* 2017). This model has also been associated with increased levels of PGE and PGF2a. Increasing PG levels within the peritoneal cavity enhances inflammatory pain by increasing capillary permeability and activating primary afferent nociceptors (Demise *et al*.,2019).

In hot plate test, the extract showed a significant increase in latency time after 90min (Table 2) and the most significant increased latency time was noticed against 150mg/kg. An increment in the mean response time was utilised in evaluating the central analgesic activity. Narcotic analgesics like morphine have been shown to prolong the mean response time by interacting with the opioid receptors to increase the pain threshold (Gholami *et al*., 2015). In this study, the methanol extract of *Solanum aethiopicum* produced a significant effect by increasing mean response time at all tested doses compared with the control group. The positive group also produced a significant increase in the mean reaction time (Morphine 10mg/kg). This indicates that the extract may possesses central analgesic properties; considering the two experimental results, acetic acid and hotplate tests.

Oxidative stress can activate various transcription factors, which lead to the differential expression of some genes involved in inflammatory pathways. The inflammation triggered by oxidative stress is the cause of many chronic diseases. Polyphenols have been proposed to be useful as adjuvant therapy for their potential anti-inflammatory effect, associated with antioxidant activity, and inhibition of enzymes involved in the production of eicosanoids (Deng *et al*., 2011).

The result of the Oxidative stress biomarkers showed that *Solanum aethiopicum* methanol extract has antioxidant activity. Superoxide dismutase activity constitutes a very important antioxidant defence against oxidative stress in the body. Several studies have been performed that reveal the therapeutic potential and physiological importance of SOD (Deng *et al*., 2011), and the enzymes can serve as an anti-inflammatory agent. Other studies showed that *Solanum aethiopicum* phytochemical screening revealed the presence of Flavonoid and Alkaloids components (Tamrat *et al*., 2017; Abubakar *et al*., 2021). In addition to the flavonoid in *Solanum aethiopicum*, polyphenolic compounds are known to be good natural antioxidants, and this antioxidant activity could be attributed to the presence of polyphenols (Tamrat *et al*., 2017).

Moreover, the flavonoids, which are powerful antioxidants, increase the hydroxylation of the phenolic cycles. It has been demonstrated that the phenolic food compounds of vegetables and other herbal products have bioactive properties beneficial to human health, particularly from free radical scavenging properties, the regulation of enzymatic activity, and the modulation of several cell signalling pathways (Abubakar *et al*., 2021). *Solanum aethiopicum*'s effect on GSH showed that the antioxidant activity of SAME is significantly inhibited oxidative stress at tested doses (150 and 300 mg/kg). Hence, GSH is the most abundant antioxidant in aerobic cells. The results showed that *Solanum aethiopicum* is more active at 150 mg/kg lowest dose than the higher doses in the two tests. The extract had antioxidant activity almost equal to the Piroxicam as a standard drug. However, under these

experimental conditions *Solanum aethiopicum* gave better antioxidant activity compared to vitamin C, and this result could be correlated with *Solanum aethiopicum* as a good source of vitamin C which protects against oxidative stress-induced cellular damage by scavenging protect cells against the effect of free radicals.

CONCLUSION

In conclusion, the LD50 study of the *S. aethiopicum* extract revealed that the plant is safe for folkloric uses. *Solanum aethiopicum* methanol extract possesses interesting analgesic activity in the acetic acid induced writhe in mice, hot plate test and oxidative stress markers. This provides scientific credence for its use in ethnomedicine against inflammatory conditions and pain and the traditional claim of *Solanum aethiopicum* for painful conditions in folk medicines.

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