

Bioactivities of *Stixis suaveolens* (Roxb.) Fruit Extract: An Evaluation in Mice Model

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Abstract

The fruits of *Stixis suaveolens* (Roxb.) have been a popular folk medicine among traditional practitioners. However, there are questions about its traditional uses due to lack of scientific evidence. This study was aimed to evaluate the effects of crude methanol extract of fruits of *S. suaveolens* in mice model. The central and peripheral analgesic activity were evaluated using the 'tail flick' and 'writhing' assay respectively. The anti-hyperglycemic potential was assessed by the ability of the crude extract in reducing blood glucose level in mice after oral administration of glucose. Oral administration (400 mg/kg bw) of the extract showed significant ($p < 0.001$) delay in pain sensation and inhibition of acetic acid induced writhing response in mice model. The results were compared with the respective standard morphine (2 mg/kg bw) and diclofenac (50 mg/kg bw). Likewise, in anti-hyperglycemic assay, maximum reduction ($p < 0.001$) of blood glucose level (39.6%) was observed 120 min after oral intake (400 mg/kg bw) of the extract as compared that exhibited by the standard drug, glibenclamide (46.83%). The *in vivo* bioassays confirmed that the crude methanolic extract of fruits of *S. suaveolens* possesses significant central- and peripheral-analgesic as well as anti-hyperglycemic activities. These findings justify its popularity as a traditional medicine and hence demands future study involving isolation and characterization of its bioactive compounds.

Key words: Antidiabetic, antihyperglycemic, analgesic, *Stixis suaveolens*.

Introduction

The Capparaceae (or Cappariaceae), commonly known as the caper family constitutes 650 species of plants dispersed among 30 genera and distributed usually in the tropical regions around the globe (Dhakad *et al.*, 2016). This family is well known for containing numerous species popular among the local populations for their medicinal properties and possess handful of bioactive molecules which could be potential for drug candidates. For example, reports are available about *Crataeva adansonii* Oliv., *Capparis spinosa* L. and *Capparis zeylanica* L. that are being used for the management of rheumatic pains, swellings, backache, indigestion etc.

(Temitopeet *et al.*, 2012; Muthuet *et al.*, 2006; Alzweiriet *et al.*, 2011). Isocodonocarpine, a new spermidine alkaloid, indole derivatives such as capparin, capparilin, capparinin and various other oxygenated heterocyclic constituents have been isolated from various species of this family (Singhet *et al.*, 2011). Species like *Cleome socotrana* has been reported to be rich in bioactive chemicals such as glucosinolates, flavonoids and triterpenoids (Mothanaet *et al.*, 2006).

Stixis suaveolens (Roxb.), a popular medicinal plant belonging to this Capparaceae family and locally known as Hamvuthilota, Modhumaloti etc., is a fruit bearing plant, commonly distributed in the

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forests covering the hill tracts Sylhet, Bangladesh and Tripura, India. In some Asian countries like Vietnam, the root, stem bark and leaves of *S. suaveolens* are being extensively used to treat painful tendons and bones, rheumatism, eye infections etc. (Anh et al., 2019). Fruit parts have been mentioned to be used by the Phom tribe of Nagaland (India) for the management of cough and malaria whilst the indigenous communities in Tripura claim that the whole fruit to possess anti-inflammatory and anti-arthritic properties (Jamir et al., 2017; Zhasa et al., 2015; Biswas et al., 2018). In Bangladesh, the fruits are prescribed by the local traditional practitioners (Kabirajes) for treating chronic ailments such as cardiovascular diseases and asthma (Biswas et al., 2018). Apart from a recent phytochemical study by Anh et al. (2019) and isolation of two new phenolic amides from its leaf by (Ngo et al., 2019), there has been no scientific study conducted with the fruit extract of *S. suaveolens* to confirm its pharmacological activity and evaluating its authenticity as a folk medicine. There has been a popular belief among the general population that due to their natural origin, folk medicines and preparations unlike allopathic drugs, possess no side effects and hence extremely safe for human consumption (Mikhail et al., 2004). However, these general assumptions are not entirely true as traditional or herbal medicines could cause severe side effects, induce adverse reactions or even interfere with other medications, if taken concurrently (Efferth et al., 2011; Ernst et al., 2006). Therefore, authenticating the use of folk medicines by pharmacological and toxicity studies to support the concept of evidence based traditional medicines is a logical obligation for avoiding undesirable health issues and provide better healthcare. As a result, the aim of our study was to evaluate the anti-hyperglycemic- and central- and peripheral-analgesic activities of crude extract of the fruits of *S. suaveolens* in mice model and ultimately impart scientific evidence in order to authenticate its uses as a traditional medicine.

Materials and Methods

Collection of plant material: The fruits of *S. suaveolens* (Roxb.) were collected from the local market, authenticated by a taxonomist, sun dried for several days and then oven dried for 24 hrs at a temperature not exceeding 40°C. The dried fruits were then ground into coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, State University of Bangladesh.

Extraction of plant material: The powdered material (300gm) was taken in a cleaned, amber color reagent bottle (2.5 liters) and soaked in 2.0 litre of methanol for 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a fresh cotton bed and finally with Whatman #1 filter paper. The extract was then concentrated with a rotary evaporator at reduced temperature and pressure.

Drug and chemicals: Glibenclamide and diclofenac sodium were obtained from Square Pharmaceutical Ltd., Bangladesh. Morphine injections were purchased from the retail Pharmacy of Ganashastha Hospital, Dhanmondi. Methanol, Tween-80 and other chemical and reagents were purchased from Merck Specialties, Mumbai. All the chemicals and reagents were of analytical grade.

Experimental animals: Swiss Albino mice (male) weighing between 25-35 g and 4-5 weeks old were obtained from Jahangirnagar University. The mice were kept in the animal house of the State University of Bangladesh and fed with standard rodent feed under strictly maintained environment. Environmental changes were carefully monitored and prior to any experiment, the animals were allowed (4 days) to adjust to the new environmental conditions. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental mice. For each of the in vivo bioassays, the animals were divided into four groups (Group I, II, III and IV), each containing five mice. The first two groups (I and II) served as the negative and positive control, whereas group III

and IV were fed with 200 and 400 mg/kg body weight (p.o.) of crude extract (Kayser *et al.*, 2019).

Assay for central and peripheral analgesic activities: The analgesic activity of *S. suaveolens* crude fruits extract (SSCE) was assessed by the tail flick (central) and writhing (peripheral) method in mice model by following published methods (Sharmin *et al.*, 2012; Owoyele *et al.*, 2012). In the first experiment, the response time to heat was recorded over a period of 90 min, after the oral administration of SSCE and using morphine as the standard. Whereas, the second investigation recorded the inhibition of acetic acid induced 'writhing' response in mice for 4 hours and diclofenac sodium was utilized as the reference standard.

Anti-hyperglycemic activity: The *in vivo* glucose lowering ability of SSCE was analyzed using the popular tail tipping method (Durschl *et al.*, 2012).

After an overnight fasting period, four groups were treated with 200 and 400 mg of SSCE orally, 5 mg glibenclamide, 1% Tween-80 with saline solution, per kg body weight respectively. After 1 hour, 10% glucose solution (2 g/kg bw) was given orally to all the mice and blood glucose level were recorded over a period of 180 min.

Results and Discussion

The central (Table 1) and peripheral analgesic activities of *S. suaveolens* crude extract (Table 2) was evaluated by 'tail flick' and acetic acid induced 'writhing' assay, respectively. These are widely used methods for studying effects on the nociceptive pain in mice model. A significant response to this bioassay indicates the presence of extract/compound(s) capable of inducing analgesia centrally by molecular pathways similar to those followed by morphine

Table 1. Effect of *S. suaveolens* fruit extract in the tail flick assay in Swiss albino mice.

Test group	Mean of tail immersion in min \pm SEM (min)				% Elongation of response (min)			
	0	30	60	90	0	30	60	90
Control (1% Tween-80)	1.79 \pm 0.12	1.99 \pm 0.08	1.99 \pm 0.12	2.04 \pm 0.17	-	-	-	-
Morphine (std.) (2 mg/kg bw)	1.95 \pm 0.12	5.76 \pm 0.15***	8.69 \pm 0.42***	10.3 \pm 0.33***	8.8	189.1	336.0	403.9
SSCE (200 mg/kg bw)	1.99 \pm 0.08	4.22 \pm 0.09***	6.35 \pm 0.31***	6.91 \pm 0.38***	11.2	111.9	219.1	238.1
SSCE (400 mg/kg bw)	2.13 \pm 0.14	4.62 \pm 0.19***	5.61 \pm 0.17***	6.91 \pm 0.31***	18.6	131.7	218.7	238.1

Here, *p < 0.05, **p < 0.01 and ***p < 0.001 when compared with control group; SEM = Standard error of mean; SSCE = *S. suaveolens* fruit extract.

Table 2. Effect of *S. suaveolens* fruit extract in acetic acid induced writhing test in Swiss albino mice.

Test group	Writhing count					Number of writhing Mean \pm SEM	% writhing	% Inhibition of writhing
	M1	M2	M3	M4	M5			
Control (1% Tween-80)	17	17	13	12	16	15.0 \pm 1.05	-	-
Diclofenac sodium (Std.) (50 mg/kg bw)	6	4	4	3	4	4.2 \pm 0.49	28.0	72.0***
SSCE (200 mg/kg bw)	8	8	10	7	7	8.0 \pm 0.55	29.3	46.7***
SSCE (400 mg/kg bw)	4	3	5	5	5	4.4 \pm 0.4	53.3	70.7***

Here, *p < 0.05, **p < 0.01 and ***p < 0.001 when compared with control group; SEM = Standard error of mean; SSCE = *S. suaveolens* fruit extract; M = mouse.

(Sabina *et al.*, 2009). The acetic acid induced 'writhing' response has been associated with the release of prostaglandins in the periphery via COX pathway (Ahmed *et al.*, 2006). Both the doses of SSCE and morphine showed their first sign of action after 30 min and continued for the total 90 min duration of the experiment. The SSCE at dose of 200 mg/kg elongated the response time ($p < 0.001$) to heat by 112% (30 min) and 238% (90 min) in the end.

Whereas the standard drug, morphine inhibited ($p < 0.001$) the response time by 189% (30 min) which increased up to 404% 1.5 hour after administration of the drug. The SSCE also exhibited significant peripheral analgesic activity comparable to the standard, diclofenac sodium. The extract at 400 mg/kg bw inhibited acetic acid induced writhing response by 71% ($p < 0.001$), while 72% reduction ($p < 0.001$) was recorded for the standard. The crude extract of *S. suaveolens* was found to significantly ($p < 0.001$) reduce the writhing response and therefore might contain constituents interfering with the peripheral prostaglandin synthesis pathway (Ferdous *et al.*, 2008). Phytochemicals like flavonoids have been also reported to target prostaglandin synthesis. Likewise, several alkaloids and tannins are being linked with blocking pain perception and antinociceptive activity (Ramesh *et al.*, 1998; Uche *et al.*, 2011; Ramprasath *et al.*, 2006). The promising

central and peripheral analgesic activities of the plant could be the reason for its use in treating pain, cough, malaria etc. by the folk practitioners. Elevated body temperature and muscle ache during malaria and common colds are common symptoms and prostaglandins are one of the major mediators for such manifestations (Dorsey *et al.*, 2000; Milton *et al.*, 1998).

During assay for anti-hyperglycemic activity, the crude extract of *S. suaveolens* displayed marked anti-hyperglycemic effect in the mice, as shown in Table 3. Here, the plant extract, at the dose of 200- and 400-mg/kg bw, started to show significant ($p < 0.05$) effect after 60 minutes of oral administration, while the standard drug, glibenclamide initiated its effect ($p < 0.05$) 30 min. prior to the crude extract and continued for the whole 3 hours. The SSCE at 400 mg/kg resulted in a maximum of 39.6% reduction ($p < 0.001$) of blood glucose 120 min after the oral intake while glibenclamide (5 mg/kg) reduced the blood glucose by 46.83% ($p < 0.001$) at the same time. Thus, SSCE showed significant lowering of blood glucose after glucose-induced hyperglycemia in Swiss albino mice. Increased synthesis of insulin by the β -cells in the pancreas and increased uptake of glucose by liver and muscle tissues are the two common mechanisms that could reduce sugar concentration in the blood (Michael *et al.*, 2010).

Table 3. Effect of *S. suaveolens* fruit extract on the glucose-induced hyperglycemia in mice model.

Test groups	Average blood glucose level (mmol/l)					% Inhibition (min)	
	Before treatment	After treatment (min)				120	180
	0 min	30	60	120	180		
Control (1% Tween-80)	4.84±0.61	10.34±0.31	9.12±0.44	7.32±0.69	5.7±0.70	-	-
Glibenclamide (std.) (5 mg/kg bw)	4.74±0.57	6.84±2.05*	6.66±0.77*	3.89±0.20***	3.80±0.18*	46.83	33.4
SSCE (200 mg/kg bw)	5.16±0.18	10.06±0.44	6.32±0.29*	4.76±0.26***	4.42±0.32	34.9	18.2
SSCE (400 mg/kg bw)	5.7±0.14	7.22±0.09	6.26±0.22*	4.42±0.39***	4.66±0.34	39.6	22.5

Here, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared with the control group; SEM = Standard error of mean; SSCE = *S. suaveolens* fruit extract.

Phyto-constituents such as alkaloids are inherently hypoglycemic in nature whereas flavonoids have been found to enhance peripheral glucose uptake and cellular glycolysis (Zheng *et al.*, 2012; Brahmachari *et al.*, 2011). Similarly, saponins have been found to stimulate pancreatic β -cells thereby increasing insulin concentration in the blood (Hu *et al.*, 2014). Various heterocyclic compounds like indole derivatives, alkaloids (isocodonocarpine), flavonoids and triterpenoids have been reported to be abundant in various species belonging to the Capparaceae family. Therefore, the hypoglycemic activity of the SSCE could be attributed due the presence of such phytochemicals.

Conclusion

The present *in vivo* biological studies, indicate for the first time, that the crude extract of fruits of *S. suaveolens* contains phytoconstituents capable of inducing significant analgesic and anti-hyperglycemic properties. These bioactivities could be the basis of its reported traditional uses. For example, the analgesic property of SSCE could be the reason for its use against malaria, pain and arthritis. The crude methanolic extract of its fruit have been found to be safe and induced no untoward effects at a maximum dose of 400 mg/kg bw during the experimental period in Swiss albino mice. Our current study warrants future phytochemical research with *S. suaveolens* in order to isolate and characterize the compound(s) responsible for its anti-hyperglycemic and analgesic activities.

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