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A pharmacological study on *Stixis suaveolens* (Roxb.) using experimental animal model

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ABSTRACT

The présent study was aimed to screen crude methanol fruit extract of *S. suaveolens* for biological activity in mice model. The central analgesic activity was assessed using the 'tail flick' method. Whereas the peripheral analgesic activity was determined by acetyl salicylic acid induced writhing method. The anti-hyperglycemic potential was evaluated by the ability of the crude extract in reducing blood glucose level after oral glucose administration. The CNS stimulating activity was evaluated by the well-known phenobarbitone induced sleep bioassay. In 'tail flick' bioassay, the oral dose of crude extract resulted in 238% delay (P<0.001) in response time, comparable to the effect of standard morphine but its effect lasted till 90 min after administration, whereas morphine had a greater duration of action. The crude extract at 400 mg/kg showed significant (P<0.001) inhibition of writhing similar to the standard diclofenac (50 mg/kg). Maximum reduction of glucose level (39.6%) was observed 120 min after oral intake of the extract while the result was lower compared to the 46.83% reduction by the standard glibenclamide. Significant (p<0.01) reduction in time for onset of sleeping as well as the total sleeping time was recorded in mice receiving the crude extract. The extract resulted in delayed sleeping intervals by 156.4 min (91.6 min in control group) and the duration of sleep was reduced to 83.6 min while 148.4 min was recorded in the control. The *in vivo* bioassays confirms that the extract from the fruit of *S. suaveolens* possess significant analgesic (acting both centrally and peripherally), glucose lowering and CNS stimulating ability which has been the reason behind its popularity as a traditional medicine.

Keywords: Antidiabetic, antihyperglycemic, analgesic, antidepressant, *Stixis suaveolens*

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INTRODUCTION

Stixis suaveolens (Roxb.), belonging family Capparaceae, locally known as hamvuthilota, modhumaloti etc., is a fruit bearing plant, commonly distributed in the forests covering the hill tracts of both Sylhet (Bangladesh) and Tripura (India) region. In some Asian countries like Vietnam, the root, stem bark and leaves of this plant are being extensively used to treat painful tendons and bones, rheumatism, eye diseases etc.[1] Locally, besides consumption, the fruit is being prescribed by traditional practitioners (kabirajes) for chronic diseases such as cardiac diseases and asthma.[2] 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 confirming any pharmacological activity and evaluating its authenticity as a folk medicine.[1,3] There has been a popular belief among the general population that due to their natural origin, folk medicines or preparations unlike allopathic drugs possess no side effects and hence extremely safe for human consumption.[4] 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.[5,6] Therefore, authenticating the use of folk medicines by pharmacological and toxicity studies (evidence based traditional medicine) is a logical obligation for avoiding undesirable health issues and provide better healthcare. As a result, the aim of our study was to conduct intense *in vivo* biological assays for the evaluation of the medicinal properties of crude *S. suaveolens* fruit extract in mice model; assess its safety and efficacy and ultimately impart scientific evidence in order to authenticate its use as a traditional medicine.

MATERIAL AND METHODS

Collection of plant material and preparation of crude extract

The fruits of *S. suaveolens* (Roxb.) were collected from the local market, sun dried for several days

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and then oven dried for 24 hours at considerably low temperature (not more than 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. The powdered material (300gm) was taken in a cleaned, amber color reagent bottle (2.5 liters) and soaked in 2.0 L of methanol. The container with its content was sealed by bottle cap and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixtures were then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The volume of the filtrate was then allowed to evaporate at ambient temperature until approximately 70% solvent was evaporated.[7]

Chemicals used

Glibenclamide, phenobarbitone, acetyl salicylic acid and diclofenac sodium were the products Square Pharmaceutical Ltd., Bangladesh. Morphine was purchased from the pharmacy of Ganashastha hospital, Dhanmondi. Methanol, Tween-80 and other chemical reagents were the products of Merck specialities, Mumbai. All the reagents were ensured to be of analytical grade.

Experimental animals

Swiss Albino mice (male) weighing between 25-35 g and 4-5 weeks old were obtained from the animal house at the 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 the *in vivo* bioassays the animals were divided into four groups (Group I, II, III and IV) of 5 individuals. 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 per kilogram body weight (p.o) of crude extract.[8]

Data analysis

All the results were recorded in triplicates and results expressed as the mean ± standard error mean (SEM). In order to determine the significance in variables between groups, One way ANOVA and Post Hoc Dunnett's test were performed using SPSS version 25.0 and p value less than 0.05 was considered to be statistically significant.

Determination of central and peripheral analgesic activity

The analgesic activity was assessed using the tail immersion (central) and writhing (peripheral) method proposed by Pizziketti, *et al.,* 1985 and Kaushik, *et al.,* 2012 respectively.[9,10] In the first experiment 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 salicylic acid induced 'writhing' response in mice for a duration of 4 hr and diclofenac sodium was considered as the reference standard.

Determination of antihyperglycemic activity

The *in vivo* glucose lowering ability of SSCE were analyzed using the popular tail tipping method.[11] After an overnight fasting period, four groups of (n = 5) were treated with 200 and 400 mg of SSCE orally, 5 mg glibenclamide, 1% Tween-80 with saline solution, per kg body weight respectively. 1 hr. later 10% glucose solution (2 g/kg b.wt.) were given orally to all the mice and blood glucose level were recorded over a period of 180 min.

Determination of CNS stimulating activity (Antidepressant activity)

The antidepressant potential of the crude fractions were determined using the phenobarbitone induced sleeping time test according to the works of Williamson *et al.,* 1996.[12] The SSCE at two different doses were fed to the experimental groups followed by intraperitoneal injection of phenobarbitone sodium. All the animals were then monitored to record the time of onset of sleep and total sleeping time for each individual.

RESULTS

Central and peripheral analgesic activity of S. suaveolens crude extract

Both the SSCE doses and morphine showed their first sign of action on the 30 min mark and continuing for the total 90 min hence the duration of the experiment. The SSCE at dose of 200 mg/kg (p.o.) elongated the response time ($P<0.001$) to heat by112% (30 min) and 238% (90 min) in the end. Whereas the morphine inhibited ($P<0.001$) the response time by 189% (30 min) which increased up to 404% 1.5 hr after administration. The SSCE also exhibited significant peripheral analgesia comparable to the standard diclofenac sodium. The extract at 400 mg per kg inhibited salicylic acid induced writhing response by 71% ($P<0.001$) while 72% reduction ($p<0.001$) was recorded for the standard.

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

	Mean of tail immersion ± SEM				% Elongation of response			
Test groups	**0 min**	**30 min**	**60 min**	**90 min**	**0 min**	**30 min**	**60 min**	**90 min**
Control (1% tween-80)	1.79±0.12	1.99±0.08	1.99±0.12	2.04±0.17	-	-	-	-
Morphine (std.) (2 mg/kg)	1.95±0.12	5.76±0.15***	8.69±0.42***	10.3±0.33***	8.8	189.1	336.0	403.9
SSCE (200 mg/kg)	1.99±0.08	4.22±0.09***	6.35±0.31***	6.91±0.38***	11.2	111.9	219.1	238.1
SSCE (400 mg/kg)	2.13±0.14	4.62±0.19***	5.61±0.17***	6.91±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* crude fruit extract, n = Sample size

Table 2 ***Effect of S. suaveolens crude fruit extract in the ASA induced writhing test in Swiss albino mice***

	Writhing count					Number of writhing Mean ± SEM	% writhing	% Inhibition of writhing
Test groups	**M1**	**M2**	**M3**	**M4**	**M5**			
Control (1% tween-80)	17	17	13	12	16	15.0±1.05	-	-
Diclofenac sodium (Std.) (50 mg/kg)	6	4	4	3	4	4.2±0.49	28.0	72.0***
SSCE (200 mg/kg)	8	8	10	7	7	8.0±0.55	29.3	46.7***
SSCE (400 mg/kg)	4	3	5	5	5	4.4±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* crude fruit extract, M = mouse

Table 3 ***Effect of S. suaveolens crude fruit extract on the glucose induced hyperglycemia***

		Average blood glucose level (mmol/L)						
		`Before treatment	After treatment				% Inhibition	
Test groups	**n**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**	**120 min**	**180 min**
Control (1% tween-80)	5	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)	5	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)	5	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)	5	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 control group. SEM = Standard error of mean, SSCE = *S. suaveolens* crude fruit extract, n = Sample size

Antihyperglycemic activity of S. suaveolens crude extract

The SSCE at 200 and 400 mg/kg.b.wt., started to show significant (p<0.05) effect 60 min. after oral administration while the standard glibenclamide initiated its effect (p<0.05) 30 min. prior to the crude extract and continued for the whole 3 hr. 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 blood glucose by 46.83% (p<0.001) at the same time.

Effect of S. suaveolens crude extract on the CNS

The oral administration of SSCE resulted in marked delay in the onset of sleep as well as reduction of duration of sleeping period among the test animals after inducing sedation by phenobarbitone sodium. The extract at 200 and 400 mg/kg caused dose

Table 4 ***Effect of S. suaveolens crude fruit extract on the central nervous system of Swiss albino mice***

Groups	n	Time of onset of sleep (minutes)	Total sleeping time (minutes)
Control (1% tween-80)	5	91.6±6.22	148.4±6.22
SSCE (200 mg/kg)	5	138.2±11.60**	101.8±11.62**
SSCE (400 mg/kg)	5	156.4±14.59**	83.6±14.58**

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

dependent elevation of sleep onset time (p<0.01) to 138.2 and 156.4 min respectively while 91.6 min were recorded in case of control. Similarly, both doses of extract decreased the total sleeping time significantly (p<0.01) of 101.8 and 83.6 min respectively whereas the animals in the control group had a sleeping time of 148.4 min.

DISCUSSION

The 'tail flick' method is a common method widely used for studying effects on the nociceptive pain in mice model. A significant response to this bioassay indicates presence of agents capable inducing analgesia centrally by molecular pathways similar to those followed bymorphine.[13] The acetic acid induced 'writhing' response have been associated with the release of prostaglandins in the periphery via COX pathway.[14] The crude extract of *S. suaveolens* were found to significantly (p<0.001) reduce the writhing response and therefore might contain phytochemicals interfering with the peripheral prostaglandin synthesis pathway.[15] Phytochemicals like flavonoids have been reported to target prostaglandin synthesis. Likewise, several alkaloids and tannins are being linked with blocking pain perception and antinociceptive activity.[16,17,18]

The 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 pancrease and increased uptake of glucose by liver and muscle tissues are the two common mechanisms that could reduce sugar concentration in the blood.[19] Phytoconstituents such as alkaloids are inherently hypoglycemic in nature whereas flavonoids have been found to enhance peripheral glucose uptake and cellular glycolysis.[20,21] Similarly saponins were found to stimulate pancreatic β-cells thereby increasing insulin concentration in the blood.[22]

CNS stimulants have been suggested to be a viable option for prevention and treatment of depression.[23] The SSCE has shown to possess significant amount of CNS activating potential by decreasing both sleeping time interval and sleeping time. Barbiturates like phenobarbitone exhibit their sedative activity by acting on the GABAergic receptors, increasing the influx of chloride ions and inhibition the generation of action potential.[24] *S. suaveolens* crude extract might possess phytochemicals which could inhibit the action of barbiturates. There have been records of molecules derived from plants i.e.,bicuculline and picrotoxin which are strong GABA antagonists capable of reversing the effect of phenobarbitone.[25]

As numerous drugs could be linked back to plant origin, similarly the current available CNS-active agents i.e., stimulants are mostly derived from plants.[26,27] Therefore there is a huge possibility of finding new phytochemicals with CNS stimulant or anti-depressant activity with better efficacy. The SSCE has shown to possess significant amount of CNS activating potential.

CONCLUSION

The present *in vivo* biological studies, for the first time reports that the crude fruit extract of *S. suaveolens* possess phytoconstituents capable of inducing significant analgesic, antihyperglycemic and CNS stimulant activity *in vivo*. These biological properties could be the basisof its reported traditional use. The crude methanolic extract of its fruit have been found to be safe and induced no side effects at a maximum dose of 400 mg/kg.b.wt in Swiss albino mice. This study will evoke future phytochemical research in order to isolate and characterize the bioactive molecules responsible for the antihyperglycemic, analgesic and CNS stimulant activity. Hence, further extensive studies on isolation of the phytoconstituents and investigation on clinical level could lead to the discovery and development of new therapeutic entities.

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