Arch. Pharm. Res. **15**(1), 95-98 (1992)

Presence of Acetylcholine-like Substance(s) in *Sesamum indicum*

***Anwar-ul Hassan Gilani and Khalid Aftab**

**Department of Pharmacology, Medical College, The Aga Khan University, Karachi-74800, Pakistan and HEJ Research Institute, of Chemistry, University of Karachi, Karachi, Pakistan*

(Received September 3, 1991)

Abstract □ Alcoholic extract of seeds of *Sesamum indicum* (SI, 1-30 mg/kg) caused hypotensive action in anesthetized rats. Heart rate was also decreased at slightly higher doses (10-30 mg/kg). Pretreatment with atropine (2 mg/kg) abolished these cardiovascular responses. In isolated spontaneously beating atria from guinea-pigs, SI caused decrease in force and rate of atrial contractions. In isolated guinea-pig ileum and rat uterus, SI (100-1000 ug/ml) produced contractile responses. All these actions of SI were abolished in the presence of atropine (1 μM). These results indicate that alcoholic extract of seeds of *Sesamum indicum* contains acetylcholine-like constituent(s) which explains some of the folkloric uses of plant.

Keywords □ *Sesamum indicum*, seed extract, hypotensive, cholinergic.

Sesamum seeds locally known as 'TIL' have multiple medicinal uses and are available in three different varieties (black, white and red or brown)[1]. The plant *Sesamum indicum* belongs to the family Pedaliaceae[2] and is extensively cultivated in the warmer regions. White variety seeds is richer in oil. The seeds and its oil have been used traditionally, since olden times as an emmenagogue and abortifacient[3]. The sesamum seeds yields 47 to 57 per cent of oil which is used in canning fish and as an emollient. This is also reported to have a diuretic, laxative and lactagogue properties[4]. Plant has a folkloric reputation as an antihypertensive in South Asia region[5] and sesamum seed oil is traditionally used in Philippines as purgative as well as antirheumatic agent[6]. While several phytochemical studies have been carried out on *Sesamum indicum*, the plant has not been widely studies scientifically for its pharmacological activities. Two lignins, sesamin, and sesamolin have been isolated from seed oil of *S. indicum*[7,8] while sesamin along with sesangolin was also found in another specie, *S. angolense*[9]. These lignins have been reported to have potentiating effect on the insecticidal activity of pyrethrum[7,9]. Presence of saponins and alkaloids has also been found in seeds of *S. indicum*[10,11]. In the present study, we describe some pharmacological actions of alcoholic extract of white seeds of *Sesamum indicum* in an attempt to rationalize some of the folkloric uses of this indigenous medicinal plant.

*Correspondence to : Dr. Anwar Gilani

EXPERIMENTAL METHODS

Extraction

White seeds of *Sesamum indicum* were procured from local market and plant material was identified with the help of a Botanist at the University of Karachi, Karachi. The seeds of *Sesamum indicum* were ground in a grinder, soaked in 70% methanol and kept at room temperature for two weeks. This procedure was repeated twice and combined methanolic extract was evaporated under reduced pressure resulting in brown material.

Cardiovascular effects in anesthetized rats

Wistar rats of either sex (200-250 g) were anesthetized with pentothal sodium (80 mg/kg, i.p.). The trachea was exposed and cannulated to facilitate

spontaneous respiration. The arterial blood pressure was recorded from the carotid artery *via* the arterial cannula connected to a pressure transducer (model statham P_{23} AS strain gauge transducer) and heart rate was recorded on Grass model 79 polygraph. Drugs and extracts were slowly injected via the cannula inserted into external jugular vein followed by saline flush. The animal was allowed to equilibrate for at least 30 min before administration of any drug.

Mean blood pressure (MBP) was calculated by the following formula: diastolic BP plus one-third pulse width. Changes in blood pressure and heart rate (HR) were expressed as percent of control values obtained immediately before the administration of test substance.

Isolated tissue experiments

These experiments were carried out by the method previously used in our laboratory[12,13].

Guinea-pig atria : Guinea-pigs of either sex (400-600 g) were killed by cervical dislocation. Paired atria were removed carefully and mounted into a 20 m*l* tissue bath filled with Krebs-Hensilet solution maintained at 32℃ and aerated with 5% CO_2 in O_2. The composition of physiological salt solution was (mM): NaCl, 118.2; KCl, 4.7; $MgSO_4$, 1.2; D-glucose, 11.7; $NaHCO_3$, 25.0 and $CaCl_2$, 2.5. The spontaneous atrial contractions were recorded *via* force displacement transducer (FT-03) on a Grass model 79 poly-graph. The preparation was allowed to equilibrate under 1 g resting tension for at least 30 min before administration of any drug.

Guinea-pig ileum : Segments of ileum about 2 cm long obtained from guinea-pigs were suspended in a 10 m*l* tissue bath filled with Tyrode's solution maintained at 37℃ and aerated with 5% CO_2 in O_2. The composition of the Tyrode's solution was (mM): NaCl, 136.9; KCl, 2.7; $MgCl_2$, 1.1; NaH_2PO_4, 0.4; $NaHCO_3$, 11.9; D-glucose, 5.6; $CaCl_2$, 1.8 (pH 7.4).

An initial loading of 0.7 g was applied to the tissue and isotonic contractions to acetylcholine (ACh) were recorded with a Bioscience transducer (T_3) coupled with Bioscience (PR 200) chart recorder. The tissue was exposed for up to 20 s to a constant concentration of ACh which produced a sub-maximal response, then washed by over flow and the cycle repeated at 3 min intervals until constant responses were recorded (usually 10-15 contractions).

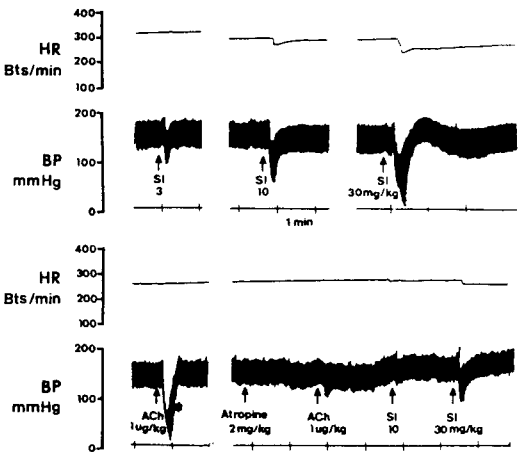

Fig. 1. Tracing from a typical experiments showing dose-dependent hypotensive and bradycardiac effects of plant extract (SI) in the absence (upper panel) and presence of atropine (lower panel) in anesthetized rat.

Rat uterus : Young female rats (170-200 g) pretreated with stilbesterol (0.1 mg/kg, s.c., 24 h) were killed by a blow on the head. The middle 2 cm of the uterine horns were cut longitudinally and each strip was mounted in a 10 m*l* tissue bath containing De Jalon's solution at 32℃ and gassed with a mixture of 95% of O_2 and 5% of CO_2. The composition of the De Jalon's solution was (mM): NaCl 154.0, KCl 5.6, D-glucose 2.8, $NaHCO_3$ 6.0, $CaCl_2$ 0.5 (pH 7.4). The tissue was allowed to equilibrate under 0.5 g basal tension for 20 min before isotonic contractions were recorded as described for ileum.

Drug Sources

The following reference materials were obtained from the sources specified : Acetylcholine chloride, atropine sulphate, norepinephrine and stilbesterol (Sigma) and pentothal sodium (Abbott Laboratories, Karachi, Pakistan).

RESULTS

Cardiovascular effects in anesthetized rats

In anesthetized rats, plant extract (1-30 mg/kg) caused fall in systolic as well as diasystolic blood pressure in a dose-dependent manner. Fig. 1 shows

Table I. Effect of seed extracts of *sesamum indicum* on mean blood pressure (MBP) and heart rate (HR) in anesthetized Rats

Dose (mg/kg)	No. of observation	% Fall*	
		MBP (mm Hg)	HR (beats/min)
1	5	28.9± 2.5	0
3	6	35.5± 9.4	2.8± 0.9
10	6	54.9± 8.7	9.5± 2.4
30	6	62.3± 6.9	37.8± 5.6

*Values shown represent mean± standard error of the mean.

original tracing of hypotensive effect of seed extract in a typical experiment, whereas combined effect of different experiments is shown in Table 1. The hypotensive effect was very brief returning to normal within one minute. At the low dose (upto 3 mg/kg) plant extract produced negligible change in heart rate, however bradycardia was observed at doses 10-30 mg/kg and was dose-related (Table 1). Pretreatment with plant extract did not alter the reflex vasoconstrictor response to bilateral carotid occlusion and pressor response to norepinephrine also remained unchanged (data not shown). Pretreatment with atropine (1 mg/kg) abolished the hypotensive response of acetylcholine (1 ug/kg) almost completely. Similarly, atropine completely blocked the hypotensive effect of plant extract at doses upto 10 mg/kg, whereas hypotensive effect of larger dose (30 mg/kg) was partially blocked (Fig. 1).

Guinea-pig atria

In spontaneously beating paired atria, the seed extract of *Sesamum indicum* at the concentration range of 100-1000 µg/ml caused a progressive decrease in force as well as rate of atrial contractions and the results are shown in Fig. 2. These responses were persistence and the tissue recovered only after the drug was washed out. Pretreatment of tissue with atropine (1 µM) abolished the inhibitory responses of the plant extract both on force and rate of atrial contractions, similar to that of acetylcholine (1 µM).

Guinea-pig ileum and rat uterus

In ileum, methanolic extract of *Sesamum indicum* caused concentration-dependent contractile responses. At the concentration of 1 mg/ml, contractile response of the plant extract was comparable to submaximal concentrations of acetylcholine (0.1 µM) and histamine (0.2 µM) as shown in Fig. 3. Pretreatment of tissue with atropine (1 µM) abolished the contractile responses of plant extracts similar to that of acetylcholine whereas contractions induced by histamine remained unaltered. In rat uterus, plant extract also produced contractile responses and blocked by atropine as in ileum (data not shown).

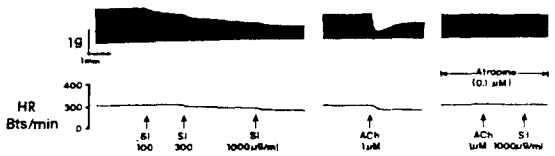

Fig. 2. Tracing showing effect of atropine on negative inotropic and chronotropic responses of plant extract (SI) and acetylcholine (Ach) in spontaneously beating paired atria from guinea-pig.

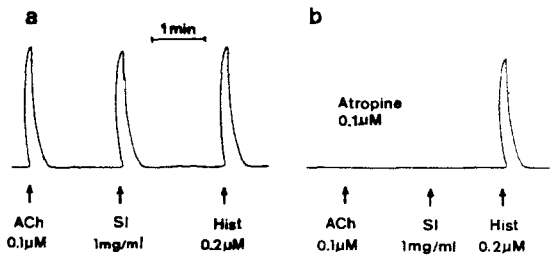

Fig. 3. Effect of atropine on ileal contractions induced by plant extract (SI), acetylcholine (Ach) and histamine (Hist.) in isolated guinea-pig ileum.

DISCUSSION

Seed extract of *Sesamum indicum* (SI) produced fall in blood pressure and heart rate in anesthetized normotensive rats. Pretreatment of atropine abolished the cardiovascular responses of SI as well as of acetylcholine although hypotensive effect of higher dose of SI was partially blocked. Acetylcholine is known to cause fall in blood pressure by activation of muscarinic receptors located on the epithelium of blood vessel[14]. Similarly, activation of muscarinic receptors in the heart results in decrease in heart rate[15]. Atropine is a competitive antagonist of acetylcholine at muscarinic receptors[16] and bloc-

kade of SI effect by atropine suggests that hypotensive and bradycardiac effects of plant extract are mediated through mechanism(s) similar to that of acetylcholine.

The fact that the high dose of SI produced partial response in atropinized animal, indicates clearly that SI displaced some of atropine from receptors as a result of competition between agonist and antagonist. It is a characteristic of pharmacological agonists (receptor activating drugs) that increasing dose can revive the agonist response by displacing competitive antagonists from receptors[16].

Cholinergic activity of SI was further confirmed when the extract was tested *in vitro* experiments using cardiac preparation. In spontaneously beating atria of guinea-pig, SI produced the responses similar to that of acetylcholine which was blocked by atropine.

Thus it is clear from these data that seed extract of *Sesamum indicum* contains constituent(s) which lower blood pressure and decrease heart rate by activation of acetylcholine muscarinic receptor in blood vessels and heart, respectively.

Acetylcholine muscarinic receptors are also present in different smooth muscles. When tested on smooth muscle preparations of guinea-pig ileum and rat uterus, SI produced contractile responses in these tissues. Atropine blocked contractile responses of both SI and acetylcholine without affecting histamine response suggesting that agonist effect of SI on ileum is atropine-sensitive similar to that of acetylcholine and different from that of histamine. Acetylcholine promotes peristaltic movements of the gut resulting in laxative effect[15] and similar cholinergic action of SI explains the laxative effect reported by traditional healers[4]. Similarily uterine stimulant effect found in this study is probably responsible for the plant's folkloric reputation as abortifacient[3]. Thus results of this study indicate that alcoholic extract of seeds of *Sesamum indicum* contains acetylcholine-like substance(s) which rationalize some of the traditional uses of this indigenous medicinal plant. Bioassay-directed isolation of pure compound(s) is in progress.

LITERATURE CITED

1. Nadkarni, A. K.: *Indian Materia Medica*, Popular Book, Bombay (1976).
2. Nasir, E. and Ali, S. I.: *Flora of Pakistan*, Fakhri Printing Press, Karachi, (1972).
3. Baquar, S. R.: *Medicinal and Poisonous Plants of Pakistan*, Printas Karachi, p. 408 (1989).
4. Chopra, R. N., Nayar, S. L. and Chopra, I. C.: *Glossary of Indian Medicinal Plants*, Council of Scientific and industrial research, New Dehli (1956).
5. Perry, L. M.: *Medicinal plants of East and Southeast Asia.*, The MIT Press, Cambridge, (1980).
6. Watt, J. M., Gardina, M. and Brandwigk, B.: *The Medicinal and Poisonous Plants of Southern and Eastern Africa.*, 2nd Edn. E&S Livingston, Edinburgh, p. 833 (1962).
7. Beroza, M.: *J. Am. Oil Chemists So.*, **31**, 302 (1954) cited by Jones *et al.*, 1962 (ref. 9).
8. Bedigian, D. Seigler, D. S. and Harlan, J. R.: *Biochem. Syst. Ecol.* **13**, 133 (1955) cited by Potterat *et al.*, 1987 (ref. 11).
9. Jones, W.A., Beroza, M. and Becker, E.D.: Isolation and structure of sesangolin, a constituent of *Sesamum angolenses*. *J. Org. Chem.*, **31**, 3232 (1962).
10. Fenwick, D. E. and Oakenfull, D. J.: *J. Sci. Food Agric.*, **34**, 186 (1983) cited by Potterat. *et al.*, 1987 (ref. 11).
11. Potterate, O., Evans, H. S., Msonthi, J. D. and Hostettman, K.: Two antifungal nephthoxirene derivatives and their glycosides from *Sesamum angolense*. *Helvetica Chemica Acta*, **70**, 1551 (1987).
12. Gilani, S. A. H. and Cobbin, L. B.: Cardioselectivity of himbacine: a muscarine receptor antagonist. *Naunyn-Schiedeberg's Arch. Pharmcol.*, **332**, 16 (1986).
13. Gilani, S. A. H.: Comparison of anticholinergic actions of gallamine and himbacine. *Revista Pharmacol. Clin. Expt.* **6**, 23 (1988).
14. Furchgott, R. F. and Zawadzki, J. V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373 (1990).
15. Gilman, A. G., Rall, T. W., Nies, A. and Taylor, P.: *The Pharmacological Basis of Therapeutics*, 8th edn. Pergamon Press, New York, p. 122 (1990).
16. Arunlakhshana, O. and Schild, H. O.: Some quantitative uses of drug antagonists. *Br. J. Pharmacol.*, **14**, 48 (1959).