

Investigation of the Analgesic and Antioxidant Activity from an Ethanol Extract of Seeds of *Sesamum indicum*

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Abstract: The present study was intended to investigate the analgesic and antioxidant activity of ethanol extract of seeds of *Sesamum indicum* (Linn.) (family: Pedaliaceae). It was observed that the extract showed a significant inhibition on the writhing response produced by induction of acetic acid. The intensity of writhing inhibition of the extract was increased with the increase of dose. The extract produced about 48.19 and 75.46% writhing inhibition at the doses of 250 and 500 mg kg⁻¹, respectively, which was comparable to the standard drug ibuprofen where the inhibition was about 71.82% at the dose of 25 mg kg⁻¹. This study also intended to investigate the antioxidant property of the ethanolic extract of this sample. The ethanol extract showed 92.00% inhibition and 56.00% reduction ability in hydrogen donation and reducing power assays, respectively at maximum concentration of the extract tested. The antioxidant activity of the extract in all these *in vitro* assays was compared with standard antioxidant (ascorbic acid).

Key words: *Sesamum indicum*, analgesic effect, free radical, hydrogen donation

INTRODUCTION

The *Sesamum indicum* (Linn.) (family: Pedaliaceae) is a flowering plant in the genus *Sesamum*. The precise natural origin of the species is unknown, although numerous wild relatives occur in Africa and a smaller number in India. It is a pharmaceutically important plant specially its seeds, which accumulates a variety of secondary metabolites including phenolic compounds, terpenes, limonoids and steroids for which it is used traditionally as herbal medication for many years both for the benefits of whole body and also in cosmetic preparations as free-radical scavenger (Ghani, 2003). Now a days, the findings of natural antioxidants has been increased greatly especially from natural plants (Jayaprakasha *et al.*, 2000). The antioxidant activities of brown pigment, extract of n-hexane and extract of supercritical carbon dioxide extraction of black sesame seeds has been investigated by Hu *et al.* (2004).

The objectives of present study was to investigate the scientific basis of the traditional use of this plant and to explore the richest sources of natural antioxidants also the novel sources with analgesic activity. The present study was focused to determine the antioxidant potential of ethanol extract of *Sesamum indicum* employing hydrogen donation and reducing power assays also the inhibition on the writhing response produced by induction of acetic acid.

MATERIALS AND METHODS

Preparation of extract: For this present investigation, *Sesamum indicum* was collected from Batiaghata, Khulna, Bangladesh in December 2007 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 32578) and a voucher specimen was also deposited there. The collected plant parts (seeds) were separated from undesirable materials or plants or plant parts. They were air dried for 3 weeks. The plant parts were grinded into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. The powdered material was successively extracted using ethanol (80%) for 72 h in Soxhlet apparatus. The extract was evaporated under reduced pressure to obtain a solid mass. It rendered an oily concentrate of brownish color. The gummy concentrate was designated as crude extract of ethanol.

Chemicals: 0.7% acetic acid solution, ibuprofen 2,2'-diphenyl-1-picryl hydrazyl (DPPH) and ascorbic acid.

Analgesic activity

Study design: The acetic acid induced writhing method is an analgesic behavioral observation assessment method

that demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution intraperitoneally (Ira Thabrew *et al.*, 2003) and then observing the animal for specific contraction of body referred as writhing. A comparison of writhing was made between positive control (ibuprofen), control and test sample given orally 30 min prior to acetic acid injection.

Young Swiss-albino mice aged 4-5 weeks; average weight ranges from 20-28 g were used for the experiment. The mice were purchased from the animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR). Experimental animals were randomly selected and divided into four groups denoted as group I, II, III and IV consisting of 10 mice in each group. Each group received a particular treatment i.e., control, positive control (ibuprofen) and two doses of the extract.

Preparation of sample suspension: To prepare suspension of the test samples at the doses of 250 and 500 mg kg⁻¹ b.wt., 250 and 500 mg of samples were measured. The extract was triturated in unidirectional manner by the addition of small amount of Tween-80. After proper mixing of extract and Tween-80, the distilled water was slowly added. The final volume of the suspension was made 5 mL. To stabilize the suspension, it was shaken well by vortex mixture.

For the preparation of ibuprofen at the dose of 25 mg kg⁻¹ b.wt., 6.25 mg of ibuprofen was taken and a suspension of 2.5 mL was made.

For preparation of 0.7% acetic acid solution, 0.7 mL glacial acetic acid was mixed with 99.3 mL of distilled water.

Methodology: Distilled water (for control) was given orally by means of a feeding needle in test group I, samples were given orally by means of a feeding needle in test group III and IV and ibuprofen (positive control) was given intraperitoneally in standard or positive control group II. Then after 30 min, acetic acid solution (0.7%) was administered intraperitoneally in all groups. A 15 min interval was given to ensure proper absorption of the administered substances. Then the number of squirms (writhings) was counted for 15 min (Table 1).

Antioxidant activity: The preliminary investigations of antioxidant activity of the extract were made by employing the following assays.

Hydrogen donation assay: The hydrogen-donating ability of lyophilized extract was determined using DPPH, stable nitrogen centered radical by employing the method of

Table 1: Experiment profile to assess the effect of ethanolic extract of *Sesamum indicum* seeds on acetic acid induced writhing of mice

Animal groups	Treatments	No. of animals	Doses (kg b.wt.)	Routes of administration
I (Control)	1% Tween-80 solution in water	10	30 mL	Oral
II (Positive control)	Ibuprofen	10	25 mg	Intraperitoneal
III (Test group)	Ethanolic extract of <i>Sesamum indicum</i>	10	250 mg	Oral
IV (Test group)	Ethanolic extract of <i>Sesamum indicum</i>	10	500 mg	Oral

Blois (1958). DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic anti-oxidants (Cao *et al.*, 1997). In this assay, a known volume of extract solution (0~200 µg mL⁻¹) was added to 0.1 mM ethanolic DPPH in a cuvette. The decrease in DPPH absorption at 517 nm as per time variation was correlated with the scavenging action of the test compound. The radical scavenging activity was expressed as the inhibition percentage and monitored as per the equation:

$$\text{DPPH radical scavenging (\%)} = (1 - \text{AS}/\text{AC}) \times 100$$

where, is AC is absorbance of control and AS is Absorbance of sample solution. The DPPH solution without sample solution was used as control. IC₅₀ value (the concentration of sample mg mL⁻¹ required to scavenge 50% DPPH free radical) was calculated from the inhibition curve. Ascorbic acid was used as positive control.

Reducing power assay: The reducing power of extracts was determined by the method of Oyaizu (1986). In this assay, the absorbance was measured spectrophotometrically at 700 nm. The increase in absorbance of the reaction mixture was interpreted as increase in reducing activity of the extract and the results were compared with ascorbic acid (positive control). The percentage reduction of the sample as compare with ascorbic acid was calculated by using the formula:

$$\text{Reduction (\%)} = [1 - (\text{As}/\text{AC})] \times 100$$

where, AC is absorbance of standard at maximum concentration tested and AS is absorbance of sample.

RESULTS AND DISCUSSION

The analgesic effect on each mouse of all groups was observed carefully to count the number of writhing

Table 2: Statistical evaluation of the results shown in table

Groups	Mean±SEM (writhing %)	Inhibition (%)	t-test	p-value
I	11.0±1.27(100)	0.00		
II	3.1±0.721(28.18)	71.82	4.290	0.001
III	5.7±2.39(51.81)	48.19	2.385	0.050
IV	2.7±0.966(24.54)	75.46	2.793	0.010

SEM: Standard error of mean

that they had made in 15 min. Table 2 shows the effect of the ethanolic extract of seeds of *Sesamum indicum* on acetic acid induced writhing in mice. It was found that the extracts cause a significant ($p < 0.001$) inhibition on the writhing response. The intensity of writhing inhibition of the extract was increased with the increase of dose. The extract produced about 48.19 and 75.46% writhing inhibition at the doses of 250 and 500 mg kg⁻¹, respectively, which was comparable to the standard drug ibuprofen where the inhibition was about 71.82% at the dose of 25 mg kg⁻¹.

The results of antioxidant effect of 80% ethanol extracts in different *in vitro* assays are described below and shown in Fig. 1 and 2.

Hydrogen donation assay: The extract showed the hydrogen donation ability in a dose dependent manner with maximum inhibition of 92.00% at the concentration 120 µg mL⁻¹. The IC₅₀ value of extract was found to be 60.0 µg mL⁻¹. Kinetic studies depicted a sharp decrease in absorbance from the initial reaction time up to 9 min with concentration 20–120 µg mL⁻¹; afterwards a steady state was obtained (Fig. 1).

Reducing power assay: The reducing capacity of extract serve as a significant indicator of the potential antioxidant activity (Kanatt *et al.*, 2005). The IC₅₀ value of extract was found to be 110 µg mL⁻¹. It was noticed that the extract showed lower reducing power potential than that of natural antioxidant i.e., ascorbic acid (Fig. 2).

Analgesic activity of *Sesamum indicum* was tested by acetic acid-induced writhing model in mice. Acetic acid, which is used to induce writhing in mice, causes analgesia by liberation of endogenous substances, which then excite the pain nerve endings (Trongsakul *et al.*, 2003). The extract produced a significant writhing inhibition at the doses of 500 mg kg⁻¹, which is comparable to the standard drug Ibuprofen at the dose of 25 mg kg⁻¹. Based on this, it could be concluded that the ethanolic extract of *Sesamum indicum* possess analgesic activity and the mode of action might involve a peripheral mechanism.

It has also been demonstrated in various *in vitro* assays that the compounds, extracted from plant materials, have good antioxidative properties due to which these compounds are finding increasing use as additional dietary supplements (Moure *et al.*, 2001;

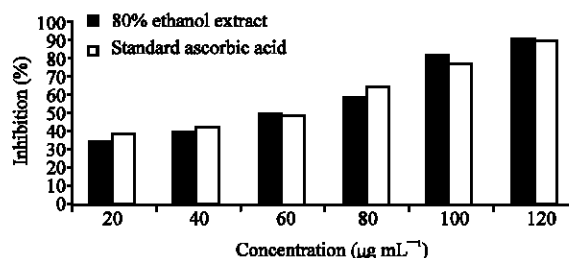


Fig. 1: Antioxidant effects of methanol extract employing hydrogen donation assay

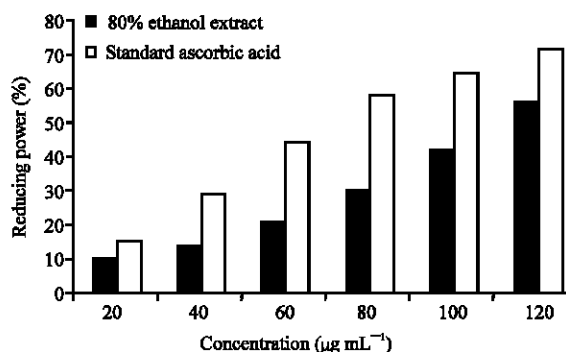


Fig. 2: Antioxidant effects of methanol extract employing reducing power assay

Andersen *et al.*, 2003). The hydrogen donating potential is known to be one of the various mechanisms for measuring antioxidant activity. In DPPH assay, the radical scavenging ability of the extract was determined by the DPPH which itself is a stable nitrogen-centered free radical. The color of ethanolic DPPH solution changes from purple to yellow, due to the formation of diphenylpicrylhydrazine (a stable diamagnetic molecule), upon reduction by either the process of hydrogen radical or electron-donation (Oktay *et al.*, 2003). It can be concluded from the results obtained in the present investigation that the 80% ethanol extract can prove to be a powerful inhibitor of hydroxyl radicals, in addition to being a good reducing agent and further studies are needed to be done before reaching to any concrete conclusion. Efforts are being in progress to evaluate this extract in number of other assays and to identify the active principles, responsible for their bioactivity by different spectroscopic method.

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