Phytochemical analysis and antioxidant, analgesic and thrombolytic activity investigation of methanol extract of *Pisum sativum* seed

Amer Khorshed Alam and Chand Sultana Khatun

Abstract

The aim of the present study was to investigate the presence of major phytochemicals and the analgesic, antioxidant and thrombolytic effects of methanol extract of the seeds of a plant *Pisum sativum*. Phytochemical analysis of the extract of *Pisum sativum* seeds indicated the presence of Glycoside, Alkaloid, Flavonoids, Saponin and Tannin types of compounds. Significant analgesic effect was monitored (p<0.05) as the oral extract dose of 500 mg/kg showed 42.75% inhibition of writhing movements compared to standard drug Diclofenac which inhibited 74.20% writhing movement. Also in present study the methanol extract of *Pisum sativum* seeds have mild antioxidant activity. The IC$_{50}$ of the extraction was 489.25 µg/ml, whereas IC$_{50}$ of reference anti-oxidative agent Ascorbic acid was 16.28 µg/ml. *Pisum sativum* extracts showed thrombolytic activity of (18.25 ± 0.04%) comparing with standard Streptokinase (66.98 ± 0.11%).

Keywords: *Pisum sativum*, antioxidant, thrombolytic, analgesic

1. Introduction

Plants and plant-derived sources not only provide us foodstuff, shelter but also they provide remedies for many years. Different chemical constituents contained in plant exhibit different activities for alleviating abnormal health of human or animals. Therefore, traditional medicine practitioners appreciate to use different parts of plant having several chemical constituents. Considerable portion of current diseases are caused due to the ‘oxidative stress’ which results in enormous amount of free radicals, causing tumor, atherosclerosis and cardiovascular illnesses [1]. Cells of the human body ensure themselves against harm caused by free radicals by catalysts such as ascorbic acid, tocopherol and glutathione [2]. However, frequently these defensive systems are becoming upset by different neurotic techniques, and cell reinforcement supplements are imperative to battle oxidative harm. This is because much consideration has been controlled towards the improvement of medicine with solid cell reinforcement properties. Thrombus formed in the circulatory system due to the loss of homeostasis leads to complications such as vascular-blockage, atherosclerosis, myocardial or cerebral localized necrosis and in many cases death. Current anticoagulants have many limitations such as high risk of bleeding due to intracranial hemorrhage, severe anaphylactic reaction and lack of specificity to gastrointestinal bleeding or hypertension that is why tremendous efforts have been directed towards the discovery and development of natural products such as the oral anticoagulants [3].

Drugs which are currently used for pain management and inflammatory conditions in most cases are either steroidal like corticosteroids or non-steroidal like NSAIDs. All of these drugs possess more or less adverse effects such as renal failure, allergic reactions, hearing loss or affecting platelet function. On the contrary many plant derived medicines had been used from centuries ago without any serious adverse effects. Thus, large scale researches should be conducted to develop new pain management medicines with plant based origin [4, 5].

*Pisum sativum* is an annual plant, with a life cycle of one year. It is a cool season crop grown in many parts of the world; planting can take place from winter to early summer depending on location. Seeds provide nutrients that are important for maintaining bone health. They are an excellent source of vitamin K1, which activates osteocalcin, the major non-collagen protein in bone. Osteocalcin anchors calcium molecules inside of the bone. Therefore, without enough vitamin K1, osteocalcin levels are inadequate and bone mineralization is impaired [6]. It also serves as a very good source of vitamin B6 and folic acid. These two nutrients help to reduce the buildup of a metabolic byproduct called homocysteine, a dangerous molecule can obstruct collagen cross-linking, resulting in poor bone matrix and osteoporosis.
One study showed that postmenopausal women who were not considered deficient in folic acid lowered their homocysteine levels simply by supplementing with folic acid by itself. In addition to affecting bone health, homocysteine contributes to atherosclerosis through its ability to damage the blood vessels, keeping them in a constant state of injury. Therefore the folic acid and vitamin B6 in green peas are supportive of cardiovascular health as well. In fact, folic acid is so important for cardiovascular function that a major 1995 study concluded that 400 micrograms per day of folic acid could prevent 28,000 cardiovascular deaths per year in the United States [1].

This research investigated the phytochemical presence, antioxidative potential, thrombolytic effect and analgesic effect of *Pisum sativum* seed’s methanol extract.

2. Materials and Methods

2.1 Plant Materials

*Pisum sativum* seeds were collected from Mohammadpur, district Dhaka and the plant authentically were confirmed form the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

2.2 Preparation of Plant Extract

The collected plant seeds were dried at room temperature (30 ± 3 °C), for 30 days to ensure the active constituents free from decomposition. The dried seeds were powdered in an electrical grinder after overnight drying in an oven below 50 °C. The powder was extracted with methanol at room temperature. The bottle were kept at room temperature and allowed to stand for 11 days with occasional shaking. When the solvent become concentrated, the liquid alcohol contents were filtered, the remaining liquid was evaporated. The dried mass obtained was placed at room temperature for 28 days to ensure the active constituents free from decomposition. The dried seeds were powdered in an electrical grinder after overnight drying in an oven below 50 ± 3 °C, for 30 days to ensure the active constituents free from decomposition. The dried seeds were powdered in an electrical grinder after overnight drying in an oven below 50 °C. The powder was extracted with methanol at room temperature. The bottle were kept at room temperature and allowed to stand for 11 days with occasional shaking. When the solvent become concentrated, the liquid alcohol contents were filtered, the remaining liquid was evaporated. The dried mass obtained was placed at room temperature for 28 days to ensure the active constituents free from decomposition.

2.3 Phytochemical Screening Methods

2.3.1 Test for Glycosides

2 ml solution of the extract was taken into a test tube. 1 ml mixture of Fehling solution was added into the test tube. The tube was placed in a water-bath at 60 °C. If a brick red color form that shows the presence of glycosides.

2.3.2 Test for Alkaloids

In testing for Alkaloids, about 0.5 g of each extract will be stirred with 5 ml of 1 percent aqueous hydrochloric acid on a water bath; 1 ml of the filtrate is to be treated with a few drops of mayer's reagent and a second 1 ml portion is to be treated the same way with Draganoff's reagent. Presence of orange-red color indicates the presence of alkaloid.

2.3.3 Test for Flavonoids

A small quantity of test residue was dissolved in 5 ml of ethanol (95% v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. If the pink, crimson or magenta color is developed within a minute or two that mean flavonoids are present.

2.3.4 Test for Tannins

About 5 g of each portion of plant extract will be stirred with 10 ml distilled water, filtered, and ferric chloride reagent will then be added to the filtrate. If dark green or deep blue color is obtained, tannins are present.

2.3.5 Test for Saponins

A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth is obtained, saponins are present [8].

2.4 Analgesic property test

2.4.1 Drugs and Chemicals

Acetic acid was collected from laboratory of the University. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh.

2.4.2 Experimental Animals

Eight week-old Swiss albino mice were (50-52 g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25 °C, humidity 60-70%, 12 hours light: 12 hours dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

2.4.3 Experimental Protocols

For Analgesic test 16 mice were divided into three groups-

- Negative Control (NC Group, Vehicle 0.5% MC, n = 3)
- Standard Group (ST, Diclofenac sodium 10 mg/kg, n = 3)
- Extract Group (ML Group, 500 mg/kg, n = 3)

For analgesic test all mice were divided into three groups (Control Group, Standard Group and Extract Group). Each group comprises 3 mice. Control group (received 0.5% Methyl cellulose), Standard Group (received Diclofenac sodium 10 mg/kg), and Extract Group (received 300 mg/kg extract).

2.4.4 Acetic acid-induced writhing test for Analgesic activity

The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Acetic acid was administered intraperitoneally to NC group. Test samples and vehicle was administered orally 30 min before administration of 1% acetic acid to extract group. Diclofenac-Na was administered 30 min before administration of 1% acetic acid to standard group. The mice were observed for specific contraction of body referred to as “writhing” for the next 30 min [9,10].

2.4.5 Statistical Analysis

All values were expressed as mean ± Standard Deviation (SD). Statistical comparison were performed by One-way analysis of variance (ANOVA), followed by using Dunnet test. Results were considered as significant when p values less than 0.05 (p<0.05).

2.5 Antioxidant Property Test

2.5.1 Reagents

Methanol, DPPH (1, 1 - diphenyl - 2 – picrylhydrazyl - hydrate)

2.5.2 Procedure

Stock solution of the plant extract was prepared in methanol from which a serial dilution was carried out to obtain concentration of 1 μg/ml, 5 μg/ml, 10 μg/ml, 50 μg/ml, 100 μg/ml, 500 μg/ml and 1000 μg/ml. Diluted solutions (2 ml)
Values were expressed in mean value. Each group comprised 3 animals (n=3); p<0.05 Dunet test as compared to Control Group. Control Group animal received vehicle (1% Tween 80 in water), Standard Group received Diclofenac 10 mg/kg body weight, Extract Group was treated with 500 mg/kg crude extract of *Pisum sativum*.

### 3.3 Result of Anti-oxidant Test

Table 3 shows antioxidant activities of standard antioxidant ascorbic acid.

### Table 4: Antioxidant activity of Extract Solution

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance (nm)</th>
<th>% of DPPH remaining</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.802</td>
<td>98.53</td>
<td>1.47</td>
</tr>
<tr>
<td>10</td>
<td>0.701</td>
<td>86.11</td>
<td>13.89</td>
</tr>
<tr>
<td>50</td>
<td>0.614</td>
<td>75.42</td>
<td>24.58</td>
</tr>
<tr>
<td>100</td>
<td>0.478</td>
<td>58.73</td>
<td>41.27</td>
</tr>
<tr>
<td>500</td>
<td>0.314</td>
<td>38.58</td>
<td>61.42</td>
</tr>
<tr>
<td>1000</td>
<td>0.197</td>
<td>24.21</td>
<td>75.79</td>
</tr>
</tbody>
</table>

Table 4 shows antioxidant activities of *Pisum sativum* extract.
The antioxidant activity of the methanol extract of *Pisum sativum* is evaluated using DPPH free radical scavenging activity method. The methanol extract of *Pisum sativum* has minor antioxidant activity. The IC$_{50}$ of the extraction is 489.25 µg/ ml, whereas IC$_{50}$ of Ascorbic Acid is 16.28 µg/ ml.

### 3.4 Thrombolytic activity test

**Table 5:** Thrombolytic activity (in terms of % clot lysis) of *Pisum sativum*

<table>
<thead>
<tr>
<th>Sample</th>
<th>% of clot lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>3.14 ± 0.31</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>66.98 ± 0.11</td>
</tr>
<tr>
<td>Extract</td>
<td>18.25 ± 0.04</td>
</tr>
</tbody>
</table>

SK = Streptokinase (positive control), ME= Methanol extract, Blank= Water as negative control.

Addition of SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C, showed 66.98±0.11% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 3.14±0.31%. The mean difference of in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study *Pisum sativum* displayed mild thrombolytic activity (18.25 ± 0.04 %).

### 3.5 Discussion

Phytochemical screening of *Pisum sativum* plant extract displayed that it contains Carbohydrate, Alkaloids, Glycoside, Flavonoid, Saponin and Tannins.

The analgesic tests displayed that it has good analgesic properties because the standard analgesic drug Diclofenac- Na inhibited 74.20% acetic acid induced writhing while the oral dose prepared by plant extract inhibited 42.75% acetic acid induced writhing.

The antioxidant test showed that the plant extract has mild antioxidant activity because the standard antioxidant agent ascorbic acid was found to have IC$_{50}$ of 16.28 µg/ ml while the IC$_{50}$ of the plant extract is 489.25 µg/ ml.

The thrombolytic test displayed that it has mild thrombolytic activity because the standard thrombolytic agent Streptokinase was found to have caused 66.98±0.11% lysis of the clot and the plant extract caused 18.25 ± 0.04 %.

### 4. Conclusion

The results stated above showed that the methanol extract of *Pisum sativum* possessed good analgesic effects. Among other activities it showed mild antioxidant and thrombolytic activities. It is evident that *Pisum sativum* has potential for further research due to its numerous beneficial properties.

### 5. Acknowledgement

Authors are grateful to Square pharmaceuticals for providing Diclofenac-Na, and Beacon pharmaceuticals for providing Streptokinase for this research.

### 6. Conflict of interest

The authors declare that there is no conflict of interest about this article with any institution.

### 7. References


