Investigating the Protective Effect of *Solanum melongena*

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Abstract - The aim of this study is to investigate the protective effect of *Solanum melongena* (*S. Melongena*). Different solvent were used to extract the fractions. Antiplatelet activity was monitored using dual channel Lumi aggregometer, antioxidant enzymes were measured using kits purchased from RANDOX, UK while calcium channel blocking activity was screened on guinea pig ileum using isolated organ bath assembly. Aqueous fraction, Ethyl acetate fraction and Chloroform fraction potently inhibited platelet aggregation, antioxidant and calcium channel...
blocking activity respectively. These results indicated that all fractions of *S. Melongena* possesses antioxidant properties but aqueous fraction posses both AA and PAF antiplatelet activity and chloroform fraction acts as calcium channel blocker. We hypothesized that fractionation process may be responsible for the modification of the active compounds present in the extracts of *S. Melongena* and hence for their respective properties. Moreover, PAF aggregation cycle molecules which are involved in blocking may not be soluble either in ethyl acetate or chloroform.

**Keywords** - solanum melongena, platelet aggregation, antioxidant, calcium channel blockers, traditional medicine

**INTRODUCTION**

**Traditional Use**

The use of medicinal plants in therapeutics or as dietary supplements goes back beyond recorded history, but has increased substantially in the last decades (Woods 1999, Khan et al. 2001, WHO 2002). Plants having medicinal values are generally designated as medicinal plants (Iwu 1986). *S. Melongena* (Brinjal Solanaceae), a culinary vegetable that is also considered as medicinal plant, has been used in the Indian system of medicine for centuries. Various parts of the plant are useful in the treatment of inflammatory conditions, cardiac debility, neuralgia, ulcers of nose, cholera, bronchitis and asthma (Warrier et al 1996). Its antioxidant (Sudheesh et al 1999, Noda et al 1998; 2000), CNS depressant (Vohera et al 1984), analgesic (Mutalik et al 2003) and hypolipidemic (Sudheesh et al 1997) activities have already been reported.

**Known Pharmacological Activity**

Although Sudhesh et al. (1999) reported antioxidant activities of *S. Melongena*; however, it is likely that the plant possesses additional antioxidant enzymes. Over the past two decade or so, considerable evidence has been gathered in support of the hypothesis that free-radical–mediated oxidative
stress processes and specific products arising from them play a key role in the pathogenesis of a number of diseases. Low levels of total antioxidant status (TAS) has been reported in liver damage, myocardial infarction and angina pectoris (Mitrevski et al. 1996), cancer (Salgo et al. 1997), diabetes (Maxwell et al. 1997), rheumatoid arthritis (Salgo et al. 1997) and in male infertility (Gavella et al. 1996). Low levels of glutathione peroxidase (GPx) may increase the incidence of cardiovascular diseases, such as Keshan disease and atherosclerosis (Baines et al. 1997). Super oxide dismutase (SOD) low level has been reported in patients suffering from angina pectoris and myocardial infarction (Sushil et al. 1995; Vukelic et al. 1997). These diseases are the major cause of morbidity and mortality in mankind and no longer exclusive to the industrialized nations but also affect developing countries such as Pakistan.

**Rationale of the study**

In developing countries, remedies prepared by a traditional healer from plants of the local flora are the only drugs available for a large number of people. In Pakistan, for example, there are at least 45,000 traditional healers of whom about three-quarters are practicing in rural areas. This figure has not changed significantly over the years. Over 70% of the country’s population relies upon herbal remedies. However, these traditional remedies have not been widely investigated scientifically, mainly because of the lack of proper communication between traditional and modern health professionals, absence of modern technology, and a shortage of qualified scientists in the field of Natural Products Pharmacology.

More recently we have seen a revival of interest at a global level in the natural products for the health care. World Health Organization has emphasized the importance of scientific evaluation into the indigenous herbal remedies. In many countries of the world, native medicinal plants are looked upon as a possible addition to WHO list of “Essential Drugs” once their value has been clinically proven. Pakistan is rich in herbal wealth and a large number of medicinal plants grow abundantly particularly in the Northern areas (Nasir and Ali 1972). It is likely that scientific studies for the exploration of new drugs from indigenous sources may yield fruitful results. This study aimed at exhibiting the effects of *S. Melongena* on oxidative stress induced pathogenesis. For that purpose, we investigated antiplatelet, antioxidant and calcium channel blocking potential of *S. Melongena* in different fractions.
MATERIALS AND METHODS

Plant Material

*S. Melongena* was procured from the market. Fruit part collected weighed approximately 5 Kg. Identification was carried out by Dr. Humaira Gul (Department of Botany, Faculty of Sciences, University of Karachi, Pakistan). A specimen has been kept in our laboratory for future reference. Fruit pulp at room temperature was mashed and stored in a tightly closed container for future use.

Preparation of plant extracts

Mashed fruit was soaked in 5 lit 70% aqueous methanol for 3 days with occasional shaking. It was then filtered through a muslin cloth and then through a Whatman qualitative Grade 4 (20–25 μm) filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure to a thick, semi-solid mass of dark brown color, the crude extract. Fractionation of the crude extract was done by standard phytochemical procedures using different organic solvents (Williamson et al., 1998). A known quantity of the crude extract (50 g) was dissolved in 50ml distilled water. This was then introduced in a separating funnel. Ethyl acetate (50 ml) was then added into the same separating funnel. This mixture was shaken vigorously, regularly allowing the air to escape out. It was kept for about 30 min to let the two layers separate. The upper layer of ethyl acetate was acquired and the same procedure was repeated twice and all the ethyl acetate layers were collected and concentrated in a rotary evaporator to obtain the ethyl acetate fraction (EtAc). Chloroform (50 ml) was then added to the remaining layer and the same process was repeated as with ethyl acetate, finally obtaining the chloroform fraction (Chlm). The yield of both fractions was 20% and 25%, respectively, while the remaining layer was the aqueous layer (Aq).

Chemicals and Reagents

Verapamil, aspirin, vitamine C, arachidonic acid(AA), platelet activating factor(PPF), hydrogen peroxide \( \text{H}_2\text{O}_2 \) and sodium citrate were purchased
from Sigma Chemical Company, St Louis, MO, USA. Kits for antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS)) were purchased from Randox, UK.

All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh in normal saline on the day of the experiment.

Solution

Tyrod’s solution in mM [KCl 2.7, NaCl 136.9, MgCl$_2$ 1.1, NaHCO$_3$ 11.9, NaH$_2$PO$_4$ 0.4, glucose 5.6 and CaCl$_2$ 1.8 (pH 7.4)]. For making solution of PAF, we used 2mg/vial, which is 3.83 mM, dissolved and diluted (1:100) in saline to make 38.3 µM. 5-10 µL of this is added to PRP to cause aggregation.

For making solution of AA, we used 20 µL of ethanol, 730 µL of 0.2% sodium carbonate (W/V in water) was added to a vial of AA (10 mg,) which gave 40.6 mM stock solution. A maximum of 20 µL was used to induce aggregation.

Animals

Experiments were performed according to animal ethic rules of Aga Khan University. Local Guinea pigs (1.5–2 kg) of either sex were used in the study, and were bred and housed in the animal house of The Aga Khan University under controlled environment (23–25 °C). Animals were exposed to regular dark and light cycles. Animals were given tap water ad libitum and a standard diet consisting of (g/kg): flour, 310; choker, 310; molasses, 12; salt, 5.8; nutrivet L, 2.5; potassium metabisulphate, 1.2; vegetable oil, 38; fish meal, 170; and powdered milk, 150.

BIOLOGICAL ACTIVITY EXAMINED

Preparation of platelets

Blood was taken after getting human ethic approval. Samples were collected from human blood via venepuncture from normal volunteers reported to be free of medication for 7 days. Blood samples were mixed with 3.8 % (w/v) sodium citrate solution (9:1) and centrifuged at 260g for 15min at 20°C to obtain platelet rich plasma (PRP). The remaining blood samples were
centrifuged at 1200g for 10 min to obtain platelet poor plasma (PPP). Platelet count was determined by phase contrast microscopy and all aggregation studies were carried out at 37°C with PRP having platelet counts between 2.5 and 3.0 x 10^8 ml^-1 of plasma (Fatima Shad and Saeed 2007).

**Measurement of platelet aggregation**

Aggregation was measured by Dual-channel Lumi-aggregometer (Model 400 Chronolog Corporation, Chicago, USA) using 0.45 ml aliquots of PRP (Shah and Saeed, 1995; Shah et al., 1996). The final volume was made up to 0.5 ml with the plant fraction, dissolved either in normal saline or appropriate vehicle known to be devoid of any effect on aggregation. Aggregation was induced by using AA/PAF. The anti-aggregatory effects of plant fractions were studied by incubating PRP with various plant fractions for 1 min followed by the addition of aggregating agents. The resulting aggregation was recorded for 5 minutes after the challenge, by the change in light transmission as a function of time. Once the anti-platelet activity of various fractions against agonists was established, dose-response curves were constructed to calculate the IC_{50} values of the various plant fractions.

**Measurement of Total Antioxidant Status (TAS)**

TAS was measured in human plasma using commercially available kits from RANDOX, UK and assays were carried out on spectrophotometer DU 800 (Beckmann, USA). The assay to measure TAS levels was conducted as described by Mitrevky and his Colleagues (Mitrevski et al., 1996). After adding all the chemicals, test fraction was added before the addition of substrate at the end. The concentration was then observed by measuring absorbance at 600nm. This assay relies on the ability of antioxidants in the plasma to inhibit oxidation of 2,2’ azino-bis-[3-ethylbenz-thiazoline-6-sulfonic acid] (ABTS) to ABTS+ by metmyoglobin. The amount of ABTS+ produced was monitored by reading the absorbance at 600 nm. Under these reaction conditions, the antioxidants in the plasma cause suppression of the absorbance at 600 nm to a degree that is proportional to their concentration. The final plasma antioxidant concentration was obtained using the following formula:

\[
\text{Antioxidant concentration (mmol/L)} = \text{Factor} \times \frac{\text{absorbance of blank}}{\text{absorbance of sample}}
\]
Where,
\[
\text{Factor} = \frac{\text{concentration of standard}}{\text{absorbance of blank}} - \text{absorbance of standard}
\]

**Measurement of Glutathione Peroxidase Activity**

GPx levels were determined using commercially available kits from RANDOX, UK and assays were carried out on spectrophotometer DU 800 (Beckmann, USA). After adding all the chemicals, test fraction is added before the addition of substrate at the end. The absorbance at 340nm was then measured. In this assay, GPx was measured by coupling the peroxidase reaction with the reduction of oxidized glutathione by glutathione reductase and NADPH (Paglia et al., 1967). t-Butyl-hydroperoxide (hydrogen peroxide or tert-butyl hydroperoxide) reduction was followed by the decrease in absorbance of NADPH at 340 nm. Activity was evaluated using GSH as the cosubstrate (Paglia et al., 1967).

**Measurement of Superoxide Dismutase Activity**

SOD activity was determined using commercially available kits from RANDOX, UK and assays were carried out on spectrophotometer DU 800 (Beckmann, USA). SOD activity assay was conducted as described by Oyanagui and his colleagues (Oyanagui et al. 1984). After adding all the chemicals, test fraction was added before the addition of substrate at the end. The absorbance at 505nm was then measured. The method employed xanthine and xanthine oxidase to generate superoxide radicals, which reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a formazane dye. The SOD activity was then measured by the degree of inhibition of this reaction. One unit of SOD caused a 50% inhibition of the rate of reduction of the INT under the condition of the assay.

**Tissue preparation Experiments for Calcium Channel Blocking Activities:**

The tissue experiments were carried out as described previously (Kulkarni 2004). The animals had free access to water but were fasted for 24 h before the experiment. The animals were killed by cervical dislocation, the abdomen was cut open and the ilium was isolated. Preparations were mounted in 10
mL tissue baths containing Tyrode’s solution maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen (carbogen). The composition of Tyrode’s, in mM, was: KCl 2.7, NaCl 136.9, MgCl2 1.1, NaHCO3 11.9, NaH2PO4 0.4, glucose 5.6 and CaCl2 1.8 (pH 7.4). A preload of 1 g was applied and the tissues were incubated for 30 min, after which control responses to a sub maximal dose of acetylcholine (0.3μM) were obtained and the tissue was presumed stable only after two consecutive doses of acetylcholine produced equal responses. After stabilization, plant fractions were investigated for calcium channel blocking activity; KCl was used as agonist (80 mM final bath concentration).

Statistical analysis

All the data expressed was analyzed using the mean ± standard deviation (SD). The statistical parameter applied was $p < 0.05$, noted as significantly different.

RESULTS

Anti-Platelet Activity:

Each solvent fraction (Aqueous [Aq] fr., 55% Chloroform [Chlm] fr 25%. Ethyl acetate [EtAc] fr 20%) from fruits of *S. Melongena* was tested for anti platelet activity. Platelet aggregation was induced by AA and PAF, both are strong platelet aggregators.

When Aq fraction of *S. Melongena* was screened for antiplatelet activity, it inhibited AA-induced platelet aggregation in a dose-dependent manner showing a value of 88% inhibition at 10 μg/ml dose with an IC$_{50}$ of 3.24±0.35 μg/ml. For PAF-induced aggregation, this fraction was slightly less potent exhibiting 91% inhibition at a dose of 40 μg/ml with IC$_{50}$ of 18.35±2.735 μg/ml. (Table1). Similarly, the Chlm fraction of *S. Melongena* showed strong antiplatelet activity induced by AA in a dose-dependent manner with IC$_{50}$ of 12.78±3.25 μg/ml with a maximum inhibition of 86% at 50 μg/ml. Chlm fraction did not exhibit any inhibition up to maximum concentration in PAF-induced aggregation. Whereas, EtAc exhibited inhibition of AA-induced platelet aggregation between the range of 100 – 500 μg/ml with IC$_{50}$ value of 183±31 μg/ml And has no effect on PAF induced aggregation.
Anti Oxidant Activity:

All fractions of *S. Melongena* were tested for their antioxidant activities including TAS, GPx and SOD. Before testing the extracts, TAS, GPx and SOD were measured in normal plasma, stress and with standard (Table 2(a)). After that, were measured the values of TAS, GPx and SOD in different fractions of the *S. Melongena* as shown in Table 2(b). All fractions doses dependently increased the antioxidant enzyme activities of *S. Melongena*, particularly; ethyl acetate fraction showed relatively higher antioxidant enzymes activity as compared to other fractions. At a maximum dose of 5 mg/ml, total fraction of EtAc activated TAS; GPX and SOD levels were 1.76 mmol/L, 8469 U/L and 218 U/ml, respectively. Whereas, for other fractions, maximum dose was 20 mg/ml for Chlm giving the values of 1.61 mmol/L, 7852 U/L and 197 U/ml for TAS, GPX and SOD respectively. For Aq fraction, TAS, GPx and SOD, activities at maximum concentration of 10mg/ml gave the values of 1.65 mmol/L , 8216 U/L and 203 U/ml, respectively.

While comparing the activities of all fractions at 5 mg/ml, SOD Aq fraction was 10% and Chlm fraction was 25% less effective than EtAc. Similar results were found with GPx and TAS.

Calcium Channel Blocking Activities:

Calcium channel blocking activities could not be measured in the plasma due to the lack of spectrophotometer sensitivity and unavailability of the specific filters. For that reason, these activities were measured in smooth muscle tissue using organ bath. The inhibitory effects of plant fractions (Aq, Chlm & EtAc) on calcium channel activity were studied against agonist-induced contractile responses. KCl produced submaximal contractions at (0.3μM). Pretreatment of tissue with plant fractions (0.3 mg/ml) caused approximately 5% and 85% inhibition (in Aq & Chlm respectively) of KCl responses (Table. 4). Next higher concentration (1 mg/ml) further suppressed the agonist contractile responses to 7% and 85% inhibition (in Aq & Chlm respectively). Similarly, pretreatment of tissue with plant fractions at 5 mg/ml & 10 mg/ml concentrations caused 16% and 23% inhibition, respectively in Aq fraction and 87% and 89% inhibition in Chlm fraction respectively. There was no inhibition with EtAc fraction at any contraction. This inhibitory effect of the plant extract was reversible on wash out as the tissue regained its initial
sensitivity to agonist after 3-5 washings and testing the agonist response between washings.

**DISCUSSION**

Blood platelets are involved in many physiological events, including hemostasis. However, their dysfunction contributes to the development and progression of a number of cardiovascular diseases, including arterial hypertension, atherosclerosis, and thrombosis. Our results indicated that all fractions of *S. melongena* significantly reduced AA-evoked platelet aggregation in a concentration-dependent manner. Among the different fractions of *S. melongena*, the aq. fraction showed the highest inhibitory effect against AA-induced aggregation as compared to Chlm and EtAc fractions. Aq. fraction also inhibited the PAF-induced platelet aggregation while Chlm and EtAc fraction were ineffective. This is in line with many other studies where the Aq. fraction of plant demonstrated antiplatelet activity against PAF while other fractions of the same plant failed to inhibit PAF-induced platelet aggregation (Lee HS 2005). It is interesting to note that all three fractions were much more potent than standard (Aspirin) as anti-aggregatory agents. Other studies have also shown the presence of plant constituents more potent than Aspirin (Lee HS 2005).

Inhibition of both AA and PAF platelet aggregation by Aq. fraction suggested the presence of at least two compounds or two types of compounds i.e. one inhibiting AA-induced aggregation and the other PAF-induced aggregation. Alternatively, it is also possible that both of these inhibiting activities were performed by a single compound, as both AA and PAF activate PKC and IP$_3$ downstream of their receptor and, therefore, any compound blocking this pathway will inhibit both AA- and PAF-induced aggregation.

All three fractions of *S. melongena* exhibited antioxidant activities as indicated by the elevated levels of GPx, SOD, and TAS with EtAc fraction being the most potent. These activities might be due to phenolic compounds and flavonoids, which have previously been reported in *S. melongena* (Nisha et al 2009; & Noda et al 2000). Studies with other plants also revealed the presence of antioxidants in the EtAc fractions (Kurian et al. 2010; Farhana et al. 2009). Aq. and Chlm fractions, though not as potent as EtAc fraction elevated the antioxidant enzyme levels with maximum effects on 10 mg/ml and 20 mg/ml, respectively. Many reports have shown the antioxidant activities of this
Many pathophysiological conditions like achalasia, myocardial ischemia, and bronchial asthma are associated with aberrant smooth muscle contraction. This study demonstrated the spasmolytic activities of different fractions of *S. melongena* using guinea pig ilium. To reveal the possible mechanism for this spasmolytic effect, a high dose of K⁺ (80mM) was used to obtain sustained contractions, which were inhibited by different fractions of *S. melongena* at a dose between 0.03–10 mg/ml. This inhibition of K⁺-induced contraction in guinea pig ilium (K⁺-induced contraction is a result of the opening of calcium channels) was similar to that of verapamil, a standard calcium channel blocker. It is well known that the contraction of smooth muscle is dependent upon an increase in the cytoplasmic free [Ca²⁺], which activates the contractile elements (Karaki and Wiess 1988; Karaki et al. 1997).

The increase in intracellular Ca²⁺ may occur either via its release from intracellular stores in the sarcoplasmic reticulum (Godfraind et al. 1986). As discussed in the section in platelet aggregation, *S. melongena* may possess compounds that inhibit PKC and IP₃ pathway, the same phenomenon may explain the calcium channel blocking effects in guinea pig ilium. Alternatively, the inhibition of the spontaneous contractions by the plant fractions might have occurred via blockade of calcium entry through the VDCs.

As revealed in this study, each of the three fractions possessed components having at least one potent activity. Aqueous fraction seemed to possess the most potent antiplatelet activity against both AA- and PAF-induced platelet aggregations. Ethyl acetate is most effective in elevating the levels of antioxidant enzymes while chloroform fraction the most potent spasmolytic activity in guinea pig ileum.

**CONCLUSION**

In conclusion, the results of this study suggest that the fractions of *S. melongena* (aqueous, chloroform and ethyl acetate) possess antiplatelet, antioxidant, and calcium channel blocking activities. However, we do not know which compounds in the plant fractions show these activities or which signaling pathways are involved. Detailed studies on chemical composition of the plant fractions, as well as different signaling pathways, are necessary to further explain other mechanism. The findings of this study support the view
that S. melongena is a promising source of antioxidants and may be efficient as preventive agent in some diseases. The generated data may enhance the basic understanding of S. melongena extract as antioxidant, antiplatelet, and calcium channel blocker.

SIGNIFICANCE, APPLICATION AND IMPLICATIONS

Cardiovascular diseases such as thrombosis, myocardial infraction, and atherosclerosis are the major cause of morbidity and mortality in mankind and no longer exclusive to the industrialized nations but also affect developing countries such as Pakistan. Indeed, the migrant population of Indo-Pak Sub-Continent was found to be at high risk of cardiovascular diseases compared to the indigenous Europeans in a recent survey done in U.K. (Laws et al. 1994). Pharmacotherapy of such diseases usually requires life-long treatment, which is not only expensive and beyond the reach of a common man but also fails to provide complete cure against certain disorders. Therefore, we investigated these biological activities on this indigenous medicinal plant.

NOTE:

Pursuant to the international character of this publication, the journal is indexed by the following agencies: (1) Public Knowledge Project, a consortium of Simon Fraser University Library, the School of Education of Stanford University, and the British Columbia University, Canada; (2) E - International Scientific Research Journal Consortium; (3) Journal Seek - Genamics, Hamilton, New Zealand; (4) Google Scholar; (5) Philippine Electronic Journals (PEJ); and, (6) PhilJol by INASP.

LITERATURE CITED

Baines, M. and A. Shenkin


Flow Chart: Extraction/Fractionation Procedure

1. **Fruit Powder 5Kg**
   - Soaked in 5 liters 70% aqueous methanol for 3 days

2. **Semi solid brown Crude Extract**

3. **50 gm crude extract dissolved in 50 ml distilled water**

4. **Introduced into separating funnel**

5. **50 ml Ethyl acetate fraction was added**
   - After 30 min the upper layer was collected and the same procedure was repeated twice

6. **Ethyl acetate fraction**

7. **50 ml Chloroform was added in the remaining layer**
   - After 30 min the upper layer was collected and the same procedure was repeated twice

8. **Chloroform fraction**

9. **Remaining layer in the separating funnel**

**Table 1: Anti Platelet Activities of Different Fractions of S. Melongena**

<table>
<thead>
<tr>
<th>CHEMICALS</th>
<th>ARACHIDONIC ACID (AA)</th>
<th>PLATELET ACTIVATING FACTOR (PAF)</th>
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<tbody>
<tr>
<td></td>
<td>n=5</td>
<td></td>
</tr>
<tr>
<td>Concentrations</td>
<td>Inhibition percentage (%)</td>
<td>IC_{50} Value</td>
</tr>
<tr>
<td>Aspirin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/ml</td>
<td>5%</td>
<td>13.12 ± 2.19 µg/ml</td>
</tr>
<tr>
<td>20 mg/ml</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td>40 mg/ml</td>
<td>98%</td>
<td></td>
</tr>
<tr>
<td>Aq fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 µg/ml</td>
<td>41%</td>
<td>3.24 ± 0.35 µg/ml</td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>81%</td>
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</tr>
<tr>
<td>Chlor fraction</td>
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<td></td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>28%</td>
<td>12.78 ± 3.25 µg/ml</td>
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<tr>
<td>10 µg/ml</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>EAce fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>29%</td>
<td>183 ± 31 µg/ml</td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>71%</td>
<td></td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>90%</td>
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Investigating the Protective Effect of *Solanum melongena*

S. Gul, S. Ahmed, H. Gul and F.S. Kaneez

Fig: 1a. Anti Aggregatory Effect of Different Fractions of *S. Melongena*

(i) Aqueous Fraction (AA induced)

(ii) Chloroform fraction (AA induced)

(iii) Ethyl acetate fraction (AA induced)

(iv) Aqueous Fraction (PAF induced)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>ENZYMES ACTIVITIES</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAS (mmol/L)</td>
<td>GPx (U/L)</td>
<td>SOD (U/mg)</td>
<td></td>
<td></td>
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<tr>
<td>Normal</td>
<td>1.20±0.18</td>
<td>518±11.509</td>
<td>170±3.66</td>
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<tr>
<td>Standard (vit C, 100mg/kg)</td>
<td>1.80±0.14</td>
<td>8769±6832</td>
<td>223±19</td>
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<tr>
<td>Stress (H2O2, 0.3 μM)</td>
<td>1.02±0.15</td>
<td>3923±520</td>
<td>137±24</td>
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</table>

Table 2a. Measurements of Anti Oxidant Activities in Plasma Using Randox Kit

<table>
<thead>
<tr>
<th>FRACTIONS</th>
<th>Concentration mg/ml</th>
<th>ICP50 Value mg/ml</th>
<th>IC50 Value mg/ml</th>
<th>GPx U/L</th>
<th>SOD U/mg</th>
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</thead>
<tbody>
<tr>
<td>Aqueous Fraction</td>
<td>1</td>
<td>1.31±0.23</td>
<td>4.1±0.9</td>
<td>6075±0.75</td>
<td>3.9±0.9</td>
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<td></td>
<td>5</td>
<td>1.69±0.19</td>
<td>10.0±2.1</td>
<td>7380±189</td>
<td>7.0±0.9</td>
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<tr>
<td></td>
<td>10</td>
<td>1.65±0.20</td>
<td></td>
<td>8160±223</td>
<td></td>
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<tr>
<td>Chloroform Fraction</td>
<td>5</td>
<td>1.39±0.17</td>
<td></td>
<td>5865±534</td>
<td>9.2±1.7</td>
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<tr>
<td></td>
<td>10</td>
<td>1.60±0.20</td>
<td></td>
<td>6959±317</td>
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<tr>
<td></td>
<td>20</td>
<td>1.61±0.28</td>
<td></td>
<td>7853±820</td>
<td></td>
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<tr>
<td>Ethyl acetate Fraction</td>
<td>1</td>
<td>1.41±0.14</td>
<td>1.31±0.36</td>
<td>6208±423</td>
<td>1.46±0.37</td>
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<td></td>
<td>2</td>
<td>1.63±0.31</td>
<td></td>
<td>7570±650</td>
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<tr>
<td></td>
<td>5</td>
<td>1.76±0.36</td>
<td></td>
<td>8469±578</td>
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Table: Calcium Channel Blocking Activities in Smooth Muscle in different fractions of *S. Melongena* Using Organ bath

<table>
<thead>
<tr>
<th>FRACTIONS</th>
<th>CHEMICAL/DOSIS</th>
<th>RESPONSE (mm)</th>
<th>Inhibition percentage (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aqueous Fraction:</strong></td>
<td>KCl 30mM</td>
<td>43 mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Verapamil 1mM</td>
<td>1.2 mm</td>
<td>97.3 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg/ml</td>
<td>41 mm</td>
<td>4.65 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1 mg/ml</td>
<td>50 mm</td>
<td>7 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 mg/ml</td>
<td>36 mm</td>
<td>16.27 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01 mg/ml</td>
<td>33 mm</td>
<td>23.25 %</td>
<td></td>
</tr>
<tr>
<td><strong>Chloroform Fraction:</strong></td>
<td>KCl 20mM</td>
<td>42.2 mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Verapamil 1mM</td>
<td>1.2 mm</td>
<td>95.74 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg/ml</td>
<td>6.4 mm</td>
<td>84.334 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1 mg/ml</td>
<td>6.6 mm</td>
<td>84.834 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 mg/ml</td>
<td>5.5 mm</td>
<td>86.967 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01 mg/ml</td>
<td>4.8 mm</td>
<td>89.999 %</td>
<td></td>
</tr>
<tr>
<td><strong>Ethanolic Fraction:</strong></td>
<td>KCl 20mM</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Verapamil 1mM</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0.3 mg/ml</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0.1 mg/ml</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0.05 mg/ml</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0.01 mg/ml</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
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</table>

Typical tracing showing inhibitory effect of *S. Melongena* on the contractions in isolated guinea pig ilium preparations.
Flow Chart: Extraction / Fractionation procedure
Flow chart regarding phytochemical procedure for the extraction of *S. Melongena* by using different organic compounds we obtain the 20% Ethyl acetate fraction, 25% Chloroform fraction and 55% Aqueous fraction.

**Table: 1. Anti Platelet Activities of Different Fractions of *S. Melongena***
This table exhibits concentration dependent inhibition of platelet aggregation induced by AA & PAF by using different fractions of *S. Melongena*. When compared with the standard (aspirin), we found that aqueous (Aq) fraction is of highest efficacy exhibiting IC$_{50}$ Value of 3.24±0.35 μg/ml followed by ethyl acetate (EtAt) fraction with an IC$_{50}$ of 183±31 μg/ml and chloroform (Chlm) fraction with an IC$_{50}$ of 12.78±3.25 μg/ml in the AA induced aggregation. Whereas PAF induced patelet aggregation the only fraction which exhibits
any antiplatelet activity is of aqueous (Aq) fraction which gives the IC$_{50}$ of 18.35±2.735 μg/ml

**Fig: 1a. Anti Aggregatory Effect of Different Fractions of S. Melongena**
The chart record exhibits the concentration dependant inhibitory effects of different fractions of *S. Melongena*. (i) Aq (ii) Chlm (iii) EtAc in AA induced aggregation. Whereas in (iv) we are looking at the anti aggregatory effect of Aq fraction on PAF induced platelet aggregation. Other fraction does not show any effect on PAF induced platelet aggregation.

**Fig: 1b. Anti Aggregatory Effect of Different Fractions of S. Melongena**
Similar results like that of fig (a) in the form of Bar diagram. AA and PAF induced activity of Aq, Chlm and EtAc fractions at different concentrations (1 - 500 μg/ml) using platelet aggregation assay. Data represent the mean ± SD (n = 5).

**Table: 2a. Measurements of Anti Oxidant Activities in Plasma Using RANDOX Kit**
Table shows the antioxidant enzyme activities in normal, standard and stress condition by using RANDX kit.

**Table: 2b. Anti Oxidant Activities of Different Fractions of S. Melongena in Plasma Using RANDOX kit**
Table shows the inhibition of different enzyme activities at various concentrations by Aq, Chlm, & EtAc. Fraction of *S. Melongena* with IC$_{50}$ Value of each fraction for each antioxidant activity. Significant differences are found to be present at 5mg/ml EtAc being most potent and close to standard.

**Table: 3. Calcium Channel Blocking Activities in Smooth Muscle in different fractions of S. Melongena Using Organ bath**
Table exhibits calcium channel blocking activities induced by KCl using standard (verapamil) and different fractions of *S. Melongena*. Out of 3 fractions Chlm fraction shows maximum potency which is close to standard (verapamil). n = 5. Inset exhibits a typical trace showing the inhibitory effect of Chlm fraction of *S. Melongena* on the contraction KCl induced calcium channel blocking activity.