ANTIDIABETIC ACTIVITY OF PONGAMIA PINNATA SEED EXTRACT

* Amit Kumar and ** Dr. Anil Middha and *** Rajesh Kumar

* Research Scholar, OPJS University, INDIA
** Department of Pharmacy OPJS University, INDIA
*** Research Scholar, OPJS University, INDIA

Abstract

In recent year several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Previous studies confirmed the efficacy of several medicinal plants in the modulation of oxidative stress associated with diabetes mellitus. Effect of hydroalcoholic extract of plants on serum glucose, lipid profile and antioxidant status in STZ induced diabetic rats was studied. Based on this, potentiation of dreaded disease like diabetes mellitus may shows a ray for better protocol for future treatment. The efficacy of Pongamia pinnata in experiment showed the significant decrease in the blood glucose level.

Introduction

Natural products have a history of therapy in the form of folk remedies, but little of today’s drug therapy is based on these remedies. Some of the natural products currently used, either as such or as derivative may often be used originally for other purposes, such as arrow poisons, part of religious or other rituals, and even cosmetics. Examples of such products include opium, belladonna, cinchona bark, ergot, curare, nutmeg, calabar bean, foxglove and squill. Many drugs originally used as folk medicines, have been abandoned. The knowledge of anatomy and physiology play an important role in the development of drug therapy (1)

Plant profile of Pongamia pinnata Linn. (2)

Botanical name: Pongamia pinnata Linn. Family: LegoseaumSynonyms:pongamia glabra (Vent.,)Derris indica(Lamk.,)Local/common name: English - Indian beechHindi -Dithouri, Karuaini Gujrati- Kanajo, Karanji Sanskrit-Karaµjaka, Naktamila, Naktıhva, Ghµtakaraµja Tamil -Pungan, Pongana
Plant Monograph

Occurrence & Description:

Pongamia is derived from the termal name Pongama or Ponga and pinnata (Latin) means leaflets arrange on either side of the stalk , and Glabera (Latin) means without hairs. Plant is distributed throughout India. *Pongamia pinnata* Linn. is a medium sized semi-evergreen glabrous tree with a short bole and spreading crown up to 18m or more in height and 1.5m in girth. The flowers appear in April-June and its pods ripen during March-May of following years.

**Description:**
- **Baek:** Green or brown
- **Leaves:** Compound, leaflets - 5-7 ovate or elliptic
- **Flower:** Pinkish-white
- **Fruit:** Pod which is thick, woody, smooth, compressed with a short curved beak
- **Seeds:** 1 or 2 per pod, smooth or wrinkled testa and reddish brown leathery.

**Traditional Uses:**
- **Root bark:** Cooling, beneficial in gonorrhea, rheumatoid arthritis, wound scabies
- **Bark:** Used internally in bleeding piles
- **Leaf decoction:** In beri-beri
- **Leaf juice:** In flatulent, dyspepsia, diarrhea, cough and leprosy
- **Leaf paste:** Ointment for leprosy
- **Flower:** Cures blood sugar
- **Seed kernel:** Beneficial in whooping cough and leprosy.

**EXPERIMENTAL**

Collection of plant material

Seeds of *Pongamia pinnata* Linn. were collected in the month of August. The plant was authenticated by Dr. Anil Middha, Department of pharmacy, OPJS University Churu Rajasthan.
Preparation of the extract  The Seeds of *Pongamia pinnata* Linn. was dried under shade and powdered mechanically and sieved using a mesh No.60.

Solvents used for extraction  Hexane, Chloroform, Ethanol(50% v/v), Distilled water

Method of Extraction

The extraction of seeds were carried out from less polar to more polar solvents by using a Percorater apparatus for continuous cool percolation procedure.

Ethanolic extract

The marc was dried and then extracted with Ethanol 50% v/v for 72 hrs, ethanol is more polar solvent, active constituents of the seed were extracted in this extraction. The filtered extract was dried under reduced pressure 400c using a rotary evaporator. The dried ethanolic extract was transferred into air tight container. The percentage yields of the above extract were expressed in Table No.1

Physico-chemical standardization\(^{(4)}\)

Estimation of sugar/starch\(^{(4)}\) Shown in table no.3 Estimation of total alkaloid: \(^{(4)}\) Shown in table no.3

Estimation of Total Phenols \(^{(4)}\)

Phytochemical screening\(^{(5)}\).

Chemical evaluation comprises of different chemical tests and chemical assays, the isolation, purification and identification of active constituents. Shown in table no.2

Test for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of Carbohydrate and glycosides.

(a) Molisch’s test
The filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated H2SO4 was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

(b) Fehling’s test

The filtrate was treated with 1 ml of Fehling’s solution A and B and heated on the water bath. A reddish precipitate was obtained shows the presence of carbohydrate.

Test for fixed oils and fats

(a) Spot test

Small quantity of extract was pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

(b) Saponification test

Few drops of 0.5% alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for proteins and free amino acid

Small quantity of the extract was dissolved in few ml of distilled water and treated with following reagents.

(a) Millon’s test – Appearance of red color shows the presence of proteins and free amino acids.

(b) Ninhydrin reagent – Appearance of purple color shows the presence of proteins and free amino acids.

(c) Biuret test – Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.
Test for saponins

**Foam test** – The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Test for phenolic compounds and tannins

Small quantity of the extract was taken in distilled water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

(a) **Dilute ferric chloride solution (5% w/v)** - Violet color.

(b) **10% lead acetate solution** - White precipitate.

Test for phytosterols

Small quantity of the extract was dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosteroles.

(a) **Libermann-Burchard’s test**

The above prepared chloroform solution was treated with few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3 ml of acetic anhydride. A bluish green color appeared indicates the presence of phytosterols.

(b) **Salkowski reaction**

To 1 ml of the above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown color produced shows the presence of phytosterols.

Test for Alkaloids

Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the following tests.

(a) **Mayer’s reagent** – cream precipitate

(b) **Dragendorff’s reagent** – Orange brown precipitate

(c) **Hager’s test** – yellow precipitate
Test for flavonoids

(a) With aqueous NaOH solution

Small quantity of the extract was dissolved in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.

(b) With conc. sulphuric acid

To a small portion of extract, concentrated sulphuric acid was added. Yellow orange color was obtained shows the presence of flavonoids.

Pharmacological screening

Animals

Sprague-Dawley rats (150-200g) The animal were exposed to alternated cycle of 12 hrs of dark and light each, before each test, the animal were fasted for at lest 12 hrs. The toxic dose were selected based on the Guideline 423(6), the result were shown in table no.4

In-vivo Antidiabetic studies(7)

Material requirements

Animal : Sprague-Dawley rats (150-300g)

Chemical: Streptozotocin (55 mg/kg)

Dose: Ethanol(50%) extract of seeds of Pongamia pinnata Linn. Glibenclamide

Experimental Design

Group I - Control rats received vehicle solution (1% gum acacia)
Group II - Diabetic control rats received vehicle solution (1% gum acacia)
Group III - Diabetic rats treated with extract 100 mg/kg body weight in 1% gum acacia
Group IV - Diabetic rats treated with extract 200 mg/kg body weight in 1% gum acacia
Group V - Diabetic rats treated with extract 400 mg/kg body weight in 1% gum acacia
Group VI - Diabetic rats treated with Glibenclamide 600 µg/kg body weight in aqueous solution.
Histopathological studies

The slides of kidney and pancreas fixed for 12 hours in 10% formaldehyde solution were processed for paraffin embedding following standard micro technique. By the use of eosin the changes in slide are shown in fig.no3-14.

RESULT & DISCUSSION

The phytoconstituents were extracted by using different solvent of increasing polarity like Hexane, Chloroform, Ethanolic(50%), Aqueous. The extractive values were presented in Table-1

Percentage yield of different extracts of dried seed of *Pongamia pinnata* Linn.:-

<table>
<thead>
<tr>
<th>Plant used</th>
<th>Part used</th>
<th>Method of extraction</th>
<th>% Yield (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pongamia pinnata</em> (Linn.)</td>
<td>Seeds</td>
<td>Cold Percolation</td>
<td>Hexane</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.5</td>
</tr>
</tbody>
</table>

Phytochemical evaluation

The phytoconstituents were identified by chemical test which showed the various phytoconstituents (Table No.2) mainly in the following extract

Table:2 Phytochemical screening of different extract of dried seed of *Pongamia pinnata* Linn.:-

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Constituents</th>
<th>Tests</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>50% Ethanol</th>
<th>Aqu.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrate</td>
<td>Molish’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anthrone test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table No:3  Quantitative analysis of dried seeds of *Pongamia pinnata* Linn.:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range (%)</th>
<th>Mean ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sugar</td>
<td>0.560 - 0.571</td>
<td>0.565 ± 0.007</td>
</tr>
<tr>
<td>Total starch</td>
<td>1.237 - 1.242</td>
<td>1.24 ± 0.003</td>
</tr>
<tr>
<td>Total phenolics</td>
<td>0.189 - 0.186</td>
<td>0.187 ± 0.002</td>
</tr>
<tr>
<td>Total alkaloid</td>
<td>0.33 - 0.28</td>
<td>0.305 ± 0.035</td>
</tr>
</tbody>
</table>
Table No.4 LD$_{50}$ value of 50% hydroalcoholic extract of dried seed of *Pongamia pinnata* Linn.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>No. of animal per group</th>
<th>Dose(mg/kg)</th>
<th>No. of death of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2000</td>
<td>2</td>
</tr>
</tbody>
</table>

Animal - Rat  Weight of animal- 150-300  No of animal per group - 3

Rout- Oral  **LD 5O value**  = 2000 mg/kg  **ED 50 value**  = 200 mg/kg

**IN -VIVO ANTIDIABETIC**

Per se effect of ethanolic(50%) extract of *Pongamia pinnata* Linn. on the glucose level and body weight of rats at a dose of 100, 200, 400 mg per body weight, not significant difference on body weight (179.51-195.38) and There is significantly decrease the glucose level (94.86-72.94, p<0.01, p<0.001) and shown in (Table No:5)

**Table .No.5**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>No. of animal per group</th>
<th>Dose(mg/kg)</th>
<th>Hypoglycemic effect (gm/dl)</th>
<th>Body weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>10 ml</td>
<td>94.86 ± 0.861</td>
<td>179.51 ±1.792</td>
</tr>
<tr>
<td>II</td>
<td>STZ+Extract of <em>Pongamia pinnata</em></td>
<td>55+100</td>
<td>88.03± 0.648</td>
<td>186.21± 1.782</td>
</tr>
</tbody>
</table>
Values are expressed as Mean ± SEM of 6 rats in each group

P value: \(^a<0.01, ^b<0.001\) compared with control group.

**Histopathological studies**

Kidney section of rat of normal group (fig.no.3) showed that glomeruli appeared normal.

Kidney section of diabetic control rat (fig.no.4) showed moderate increase in mesangial cellularity & matrix.

Kidney section of rat treated with 100 mg/kg ethanolic(50%) extract of dried seed of *Pongamia pinnata* Linn. (fig.no.5) showed that the glomerulus showed slow increase in mesangial cellularity.

Kidney section of rat treated with 200 mg/kg ethanolic(50%) extract of dried seed of *Pongamia pinnata* Linn. (fig.no.6) showed slow moderate increase in mesangial matrix & cellularity.

Kidney section of rat treated with 400 mg/kg ethanolic(50%) extract of dried seed of *Pongamia pinnata* Linn. (fig.no.7) showed that glomeruli showed mild increase in mesangial & cellularity. The tubules appeared normal.

Kidney section of rat treated with 600 µg/kg of Glibenclamide(fig.no.8) showed that glomeruli showed mild increase increase in mesangial matrix.

Pancreas section of rat of normal group (fig.no.9) showed normal architecture of pancreas.
Pancreas section of rat of diabetic control group (fig.no.10) showed that normal architecture of pancreas.

Pancreas section of rat treated with 100 mg/kg ethanolic (50%) extract of dried seed of *Pongamia pinnata* Linn. (fig.no.11) showed that normal architecture of pancreas.

Pancreas section of rat treated with 200 mg/kg ethanolic (50%) extract of dried seed of *Pongamia pinnata* Linn. (fig.no.12) showed that normal architecture of pancreas.

Pancreas section of rat treated with 400 mg/kg ethanolic (50%) extract of dried seed of *Pongamia pinnata* Linn. (fig.no.13) showed that normal architecture of pancreas.

Pancreas section of rat treated with 600 µg/kg of Glibenclamide (fig.no.14) showed that normal architecture of pancreas.

### Photographs of Kidneys

**Fig.no.3 Normal Controle**

Kidney showing normal glomeruli with tubules stroma

**Fig.no.4 Diabetic Controle**

Kidney showing moderate increase in mesangial cellularity & matrix

**Fig.no.5 100 mg/kg of Etoh (50%) extract of dried seed of *Pongamia pinnata* Linn.**

Kidney showing mild increase in mesangial cellularity

**Fig.no.6 200 mg/kg of Etoh (50%) extract of dried seed of *Pongamia pinnata* Linn.**

Kidney showing moderate increase in mesangial matrix & cellularity
Photographs of Pancreas

Fig.no.9 Normal Pancreas showing normal architecture of acini, no fibrosis or inflammation

Fig.no.10 Diabetic Control Pancreas showing focal lymphocytic infiltrate present in stroma

Fig.no.11 100 mg/kg of Etoh(50%) extract of dried seed of *Pongamia pinnata* Linn. Pancreas showing normal architecture of acini, no fibrosis or inflammation

Fig.no.12 200 mg/kg of Etoh(50%) extract of dried seed of *Pongamia pinnata* Pancreas showing normal architecture of acini, no fibrosis or inflammation

Fig.no.13 400 mg/kg of Etoh(50%) extract of dried seed of *Pongamia pinnata* Linn. Pancreas showing normal architecture of acini, no fibrosis or inflammation

Fig.no.14 600 µg/kg of Glibenclamide Pancreas showing normal architecture of acini, no fibrosis or inflammation
CONCLUSION

In spite of overwhelming influence of modern medicine and tremendous advances made in the production of synthetic drugs, traditional medicaments referred to now-a-days as herbal drugs in different places in the literature, have retained their place in therapy. Their effectiveness, low cost and comparative freedom from serious toxic effects make these medicaments not only popular but also an accepted mode of treating disease even in developed countries.

The major goal in treating diabetes is controlling elevated blood sugars (glucose) without causing abnormally low levels of blood sugar. Type I diabetes is treated with insulin, exercise, and a diabetic diet. Type II diabetes is first treated with weight reduction, a diabetic diet, and exercise. When these measures fail to control the elevated blood sugars, oral medications are used. If oral medications are still insufficient, insulin medications are considered. Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. In recent year several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Previous studies confirmed the efficacy of several medicinal plants in the diabetes mellitus. Effect of 50 % hydroalcoholic extract of plants on serum glucose, lipid profile in STZ induced diabetic rats was studied. Based on this, potentiation of dreaded disease like diabetes mellitus may shows a ray for better protocol for future treatment. The efficacy of Pongamia pinnata in experiment showed the significant decrease in the blood glucose level, in streptozotocin induced diabetes. The oral administrations of the 50% hydroalcoholic extract decrease the blood glucose level.

The present study showed that the 50% hydroalcoholic extract of Pongamia pinnata possesses hyperglycemic properties in diabetes condition, which was confirmed by glucose level and pancreatic secretion. These observation and description of mechanism of Pongamia pinnata, which interplay with diabetes biology and pharmacology lead to rapid development in diabetes treatment. In addition to this, studies on molecular aspect of diabetic therapy will give mechanistic information in diabetes therapy and also critical balance should be there between the animal model and clinical research. This holds great promise for future research in human beings.
REFERENCES


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