Antihyperglycemic and neuroprotective activity of *Adenanthera pavonina* bark against streptozotocin induced diabetic rats

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**ABSTRACT:** Worldwide diabetes and diabetic neuropathy is the highly prevalent healthcare problem. Herbal based therapy is an alternative and safe management of this problem. *Adenanthera pavonina* is an important medicinal plant having many of the medicinal values. This research aimed to evaluate the antihyperglycemic and neuroprotective activity of bark of *Adenanthera pavonina*. Hydro alcohol (50% methanol) was used as the solvent for extraction. Streptozotocin (55 mg/kg, i.p) induced diabetic rats were used for the evaluation of antihyperglycemic and neuroprotective activity. Diabetic rats were treated with hydro alcoholic bark extract of *Adenanthera pavonina* (HABEA) for 21 days. After treatment period blood glucose levels, SGOT, SGPT, Glutamate and Lipid peroxidase levels were decreased significantly in HABEA (200 and 400 mg/kg, p.o) treated rats. However, body weight, Serotonin, GABA, total protein and reduced glutathione levels were increased. And also behavioral parameter such as pain behavior (Hot Plate Method and Tail Immersion Method) and locomotor behavior (actophotometer) were significantly improved in HABEA (200 and 400 mg/kg, p.o) treated rats. These are the findings confirms that, the HABEA has significant antihyperglycemic and neuroprotective activity. Alkaloids, glycosides, flavonoids, saponin, phenolics and phytosterol present in the bark of *Adenanthera pavonina* may be the reason for its antihyperglycemic and neuroprotective activity. Further, this research will useful for the isolation of active principles from HABEA and determining its molecular mechanisms.

**KEYWORDS:** Diabetes; glutamate; behavioral; herbal medicine; diabetic neuropathy.

1. **INTRODUCTION**

Diabetes is a metabolic problem of multiple etiologies categorized by prolonged hyper-glycaemia resultant from failings in insulin production, insulin activity, or both [1]. Diabetic patients are predisposed to many problems associated with their disease and most prevalent problems in diabetes are diabetic neuropathy which affects about 50% of diabetic patients [2]. Diabetic neuropathy distresses pain fibres and motor neurons, causing foot ulcers otherwise damage. This damage is prominent to amputation in excess of 80% of cases [3]. Numerous pathways inventing from diabetes prompt oxidative stress causing diabetic neuropathy and formation of polyol pathway initiation, aldol reductase initiation, AGEs (advanced glycation end products) and initiation of PKC (protein kinase C) [4]. Due to its multifactorial nature, management of this problem is difficult and treatment options are limited. This has led to increased exploration of alternative drugs from natural sources, having potent antidiabetic as well as neuroprotective activity. *Adenanthera pavonina* (AP) is an important medicinal plant widely distributed in Asia and America. Various parts of AP are traditionally used for the management of various health illnesses such as boils, inflammations, arthritis, rheumatism, blood disorders, cholera, epilepsy, convulsion, paralysis, spasm, indigestion, pulmonary infection, chronic rheumatism, gout, haematuria, haematemesis, ulcer and diarrhoea [5]. And also bark of AP have been scientifically evaluated for its anti-inflammatory [6], anticancer [7], antibacterial [8], antifungal [9], anthelminthic [10], antioxidant [11] and antihyperlipidemic activities [12]. The bark of AP contains the secondary metabolites including alkaloids, glycosides, flavonoids, saponin, and...
phenolics, phytosterol, tannins and terpenoids [13]. Commonly alkaloids, flavonoids and phenolics are associated with anti hyperglycemic activity as well as neuroprotective activity [14, 15, 16]. However, there is no scientific validation for anti hyperglycemic and neuroprotective activities of AP bark extract. So that, this research was aimed to evaluate the anti hyperglycemic and neuroprotective activity of AP bark extract using streptozotocin (STZ) persuaded diabetic rats.

2. RESULTS

The body weight of the normal and diabetic rats are shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Before induction</th>
<th>1st day treatment</th>
<th>7th day treatment</th>
<th>14th day treatment</th>
<th>21st day treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>164.66±13.77</td>
<td>165.33±13.38</td>
<td>164.66±12.99</td>
<td>165.66±13.77</td>
<td>168.33±13.64</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>168.33±3.38</td>
<td>145.66±7.623*</td>
<td>140.66±7.51*</td>
<td>130.33±5.78**</td>
<td>115.66±5.78***</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>152±4.61</td>
<td>146.33±4.88</td>
<td>150.33±4.09</td>
<td>156±3.78*</td>
<td>159±3.78***</td>
<td></td>
</tr>
<tr>
<td>Low dose</td>
<td>171.66±3.75</td>
<td>153.33±2.91</td>
<td>159.66±0.88</td>
<td>177.66±12.25***</td>
<td>180.66±12.44***</td>
<td></td>
</tr>
<tr>
<td>High dose</td>
<td>154±2.64</td>
<td>122±211</td>
<td>135.66±1.212</td>
<td>143±215**</td>
<td>161±7.230***</td>
<td></td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SEM, n=6, One way ANOVA followed by Dunnett’s test Compared with normal; # p<0.05, ## p<0.01, ### p<0.001, Compared with Disease control; * p<0.05, ** p<0.01, *** p<0.001.

The body weights of diabetic control rats and all the treatments rats were significantly lower than that of normal group rats at first day of treatment. At the 21st day, the body weights of Metformin (65 mg/kg, p.o) and hydro alcoholic bark extract of Adenanthera pavonina (HABEA) 200 and 400 mg/kg, p.o., treated rats were significantly increased when compared with diabetic control rats. Previous studies reported that, decreased body weight is due to muscle degeneration in diabetic rats [17]. The increased body weight treatment groups may be the reason for prevention of muscle wasting in diabetic rats. The blood glucose levels of the normal and diabetic rats are shown in Figure 1.

The blood glucose levels of diabetic control rats and all the treatments rats were significantly increased when compared with normal group rats at first day of treatment. At the 21st day, the blood glucose levels were significantly declined in Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) treated rats. The increased in serum glucose levels due to diabetes induced by STZ [18]. The decreased of blood glucose level maybe the reason of anti-hyperglycemic effect of HABEA. Hemoglobin, Glycosylated hemoglobin (HbA1C), SGOT and SGPT levels in normal and diabetic rats are shown in Table 2.
Table 2. Effect of HABEA on Hemoglobin, Glycosylated hemoglobin, SGOT and SGPT levels in normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hemoglobin (g/dl)</th>
<th>HbA1C (%)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15.05±0.050</td>
<td>5.04±0.33</td>
<td>128.50±3.5</td>
<td>60.50±3.5</td>
</tr>
<tr>
<td>Control</td>
<td>8.95±0.280 ***</td>
<td>7.185±0.035</td>
<td></td>
<td>285.4±12.5</td>
</tr>
<tr>
<td>Standard</td>
<td>12.025±0.055 **</td>
<td>6.165±0.065</td>
<td>133.0±3.00</td>
<td>80.0±4 **</td>
</tr>
<tr>
<td>Low dose</td>
<td>12.025±0.055 *</td>
<td>6.805±0.175</td>
<td>190.0±9.00</td>
<td>117.5±2.5 **</td>
</tr>
<tr>
<td>High dose</td>
<td>11.88±0.600 **</td>
<td>6.805±0.175</td>
<td>121±21.00</td>
<td>80.0±4 **</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SEM, n=6, One way ANOVA followed by Dunnett’s test Compared with normal; # p<0.05, ## p<0.01, ### p<0.001, Compared with Disease control; * p<0.05, ** p<0.01, *** p<0.001.

The hemoglobin levels in diabetic control group were significantly declined when compared with normal group. Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) treated rats were significantly increased the hemoglobin levels. Consequently Glycosylated hemoglobin levels were significantly increased in diabetic rats and declined in Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) treated diabetic rats. The extreme glucose present in the blood bound to the hemoglobin so that hemoglobin level was decreased in diabetic rats. Glycosylated hemoglobin was increased in diabetic rats due to the non-enzymatic reaction between glucose and free amino groups of haemoglobin [19].

The SGOT and SGPT levels were significantly increased in diabetic control group when compared with normal group. These elevated levels were significantly declined in Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) treated diabetic rats. Increased levels of SGOT and SGPT are a common indication of liver illness [20]. In STZ induced diabetic rats, the leakage of SGOT and SGPT enzymes from liver to blood stream consequently of the hepatotoxic outcome of STZ. The behavioral parameters of the normal and diabetic rats are shown in Figure 2.

Figure 2. Effect of HABEA on behavioral parameters of the normal and diabetic rats. Data is expressed as mean ± SEM, n=6, One way ANOVA followed by Dunnett’s test Compared with normal; # p<0.05, ## p<0.01, ### p<0.001, Compared with Disease control; * p<0.05, ** p<0.01, *** p<0.001.

Paw licking time (Hot plate method) and tail withdrawal time (Tail immersion method) in all the treatment groups except normal control rats were significantly increased in 1st day of treatment. At the 21st day, the rats treated with Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) were decreased the paw licking time and tail withdrawal time when compared with STZ diabetic rats. Diabetic neuropathy distresses the peripheral nerves such as motor neurons and pain fibres [21]. The higher response time in hot plate and tail immersion method in diabetic rats is due to the loss of pain perception caused by nerve damage [22]. The declined response time in hot plate and tail immersion method in Metformin (65 mg/kg,
p.o) and HABEA (200 and 400 mg/kg, p.o) treated rats may due to prevention of nerve damage followed by pain perception.

The locomotor activity (Actophotometer) counts were significantly decreased in all the group of diabetic rats except normal rats on 1st day of treatment. At the 21st day of treatment period Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) treated rats were significantly increased the locomotor activity when compared to diabetic control rats. The decreased locomotor activity is due to the neuronal damage caused by STZ. The increased locomotor activity is due to the neuroprotective effect of HABEA. The neurotransmitter (Glutamate, serotonin and GABA) levels of the normal and diabetic rats are shown in Figure 3.

![Figure 3](image_url)

**Figure 3.** Effect of HABEA on Glutamate, Serotonin, GABA of the normal and diabetic rats. Data is expressed as mean ± SEM, n=6, One way ANOVA followed by Dunnett’s test Compared with normal; # p<0.05, ## p<0.01, ### p<0.001, Compared with Disease control;* p<0.05, ** p<0.01, *** p<0.001.

The serotonin and GABA levels were significantly decreased in diabetic control rats when compared with normal group rats. However, Glutamate levels were increased in diabetic control rats. Treatment with Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) rats shows significant increased levels of serotonin and GABA and also Glutamate were decreased when compared with diabetic control rats. The serotonin and GABA are the neurotransmitters that acts chief role in nerve pathways in the brain [23, 24]. These neurotransmitters are accountable for cognitive mechanisms. The increased levels of HABEA (200 and 400 mg/kg, p.o) is due to the neuroprotective activity of HABEA. Additional release of glutamate can lead to excite-toxic injuries in the brain due to the epilepsy, ischemia and neurodegenerative diseases [25]. Decreased levels of glutamate levels confirm the neuroprotective activity of HABEA against STZ induced diabetic rats. The antioxidant levels of the normal and diabetic rats are shown in Table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (mg/dl)</th>
<th>GSH (mM/mg of tissue extract)</th>
<th>LPO (nMoles of MDA released/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Brain</td>
<td>Liver</td>
</tr>
<tr>
<td>Normal</td>
<td>4.66±0.267</td>
<td>3.237±0.0142</td>
<td>9.303±0.0303</td>
</tr>
<tr>
<td>Control</td>
<td>8.117±0.505***</td>
<td>5.328±0.0741***</td>
<td>3.973±0.142***</td>
</tr>
<tr>
<td>Standard</td>
<td>6.233±0.184***</td>
<td>3.025±0.0147***</td>
<td>8.287±0.318**</td>
</tr>
<tr>
<td>Low dose</td>
<td>7.050±0.095***</td>
<td>3.201±0.014**</td>
<td>6.400±0.488***</td>
</tr>
<tr>
<td>High dose</td>
<td>6.70±0.370***</td>
<td>3.582±0.0245***</td>
<td>7.19±0.0310***</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SEM, n=6, One way ANOVA followed by Dunnett’s test Compared with normal; # p<0.05, ## p<0.01, ### p<0.001, Compared with Disease control;* p<0.05, ** p<0.01, *** p<0.001.
The GSH levels were significantly decreased in liver and brain of diabetic control rats when compared with normal group rats. However, total protein and lipid peroxides (LPO) levels were increased in liver and brain of diabetic control rats. Treatment with Metformin (65 mg/kg, p.o) and HABEA (200 and 400 mg/kg, p.o) rats shows significant increased levels of GSH, and decreased levels of total protein and LPO when compared with diabetic control rats. GSH is one of the major scavenging enzymes that remove toxic free radicals and play important role in protecting the cell against the potentially deleterious effects of reactive oxygen species [26].

3. CONCLUSION

Results of this study confirm the anti-hyperglycemic and neuroprotective effects of hydroalcoholic bark extract of Adenanthera pavonina. Further studies needed to elucidate the mechanism of action and identification of active principles.

4. MATERIALS AND METHODS

4.1. Materials

The bark of AP was collected from Kalapatti road sides, Coimbatore district, Tamil Nadu, India on January 2019. STZ, metformin were purchased from Sigma Aldrich, USA. Chloroform, methanol was obtained from SD Fine Chemicals Pvt. Ltd, Mumbai, India. Ketamine hydrochloride was obtained from KMCH Pharmacy, Coimbatore. The experimental animals were procured from Sri Venkateshwara Enterprises, Bangalore, India. EDTA, HCl- heptane potassium phosphate buffer pH 7.2, Serotonin, Glutamate, Tris-HCl buffer, n- Butanol, copper sulphate, carbonate-bicarbonate buffer, pH 9.95, copper tartarate reagent, Folin’s reagent, Trichloro acetic acid (TCA), Ellman’s reagent, Glutathione reduced, sodium dodecyl sulfate, acetic acid, thiobarbituric acid and 1,1,3,3 – tetraethoxy propane were procured from Merck (India) Ltd., Mumbai. SGOT and SGPT kits were purchased from Span diagnostics, Surat, India.

4.2. Preparation of plant material and extraction

The plant was authenticated by Botanical survey of India (BSI), Southern circle, Coimbatore, Tamil Nadu (No. BSI/SRC/5/23/2019/Tech/3011). The collected bark material was washed with distilled water, dried under sunshade in dark room, powdered by using mechanical mixer and sieved under sieve No. 40 and sieve No.20. The powdered material was macerated in hydroalcohol (50 % methanol) for 15 days. Then the extract was filtered with whatman no. 1 filter paper. The obtained extract was concentrated distillation at 45°C and stored in air tight glass container at 4°C [27].

4.3. Experimental animals

About 30 Male Sprague Dawley rats (age: 6 months, weight: 180 ± 20 g) were purchased from Biogen-laboratory animal facility, Bangalore. The rats were housed in clean polypropylene cages and maintained under room temperature (27 ± 2°C) with 12 h light and 12 h dark cycle. The rats were given standard pellets diet and water ad libitum throughout the experimental period. The animal ethical was getting from institutional animal ethics committee of KMCH College of Pharmacy (Approval No. KMCH/M.Pharm/10/2019-20).

4.4. Grouping of animals

The rats were divided into five groups, six rats in each group and treated as below and The doses for HABEA were selected as per the literature [40]:

- Group I: Normal control rats received vehicle solution (normal saline) for 21 days.
- Group II: Diabetic control rats injected with STZ (55 mg/kg, i.p) at the day of induction and received vehicle solution (normal saline) for 21 days.
- Group III: Standard group rats injected with STZ (55 mg/kg, i.p) at the day of induction and received Metformin (65 mg/kg, p.o) for 21 days.
- Group IV: Low dose group rats injected with STZ (55 mg/kg, i.p) at the day of induction and received HABEA (200 mg/kg, p.o) for 21 days.
- Group V: High dose group rats injected with STZ (55 mg/kg, i.p) at the day of induction and received HABEA (400 mg/kg, p.o) for 21 days.
4.5. Experimental induction of diabetes

The all group rats except group I were fasted overnight and diabetes was induced by a single injection of a freshly prepared solution of STZ (55 mg/kg, i.p). After 72 hour of induction, all the rats were checked for its glucose level using AccuCheck glucometer. The rats were considered as diabetic, if their blood glucose values above 250 mg/dl. After 7 days of stabilization, the plant extracts and standard drugs were given to respective groups for 21 days [2].

4.6. Estimation of body weight and blood glucose levels

The body weight and blood glucose levels of each group of animals were estimated at the 1th day, 7th day, 14th day and 21st day of treatment. The blood was collected at tail tip for the estimation of blood glucose levels and estimated using AccuCheck glucometer.

4.7. Estimation of behavioral changes

Behavioural changes were estimated at 1th day, 7th day, 14th day and 21st day of treatment using pain behavior method (Hot plate method and tail immersion method) and locomotor behavior method (Actophotometer test).

4.7.1. Hot plate method

Eddy’s hot plate was used for the hot plate method. About 50-55°C were maintained in hot plate. Each animal were placed in the hot plate and observed the paw licking response time and 10 seconds was cut off time. The reaction time was noted after the oral administration of the drugs and test compounds. The responses were recorded 30 min after administration of standard and plant extracts [28].

4.7.2. Tail immersion method

Tail immersion was performed through immersing tail in hot water (52.5±2°C). The tail withdrawal times were recorded and 15 seconds was cut off time. After recording, the tail of rats was sensibly dried. The responses were recorded 30 min after administration of standard and plant extracts [29].

4.7.3. Locomotor behavior method

Actophotometer was used for the testing of locomotor behavior of each rat. Animals were hired in Actophotometer. The counts were documented for 5 min in all animals. Each animal was treated with respective drug and activity score was recorded after 30 min Decreased activity score was taken as index of CNS depression. The responses were recorded 30 min after administration of standard and plant extracts [30].

4.8. Estimation of serum biochemical parameters and hematological parameters

At the end of the treatment period of 21 days, the blood samples were collected from retro orbital sinus using centrifugation tubes contains EDTA for hematological analysis and without EDTA for serum biochemical estimation. The hematological parameters such as hemoglobin and HbA1C were estimated using standard methods described by Nayak and Pattabiraman [31], Reitmann and Franke [32]. The serum was isolated from blood using centrifugation method. And serum SGOT and SGPT were estimated using standard enzymatic kits.

4.9. Estimation of neurotransmitters

At the end of the experimental period, all the rats were euthanized using ketamine hydrochloride (87mg/kg, ip) and the brain tissues were excised at 0°C. The 5 % brain homogenate was prepared with HCl-heptane potassium phosphate buffer pH 7.2, centrifuged at 2000 rpm for 10 min and the supernatant was used for the estimation of GABA, Glutamate and Serotonin levels.

4.9.1. Estimation of glutamate

About 1.4 ml of sample were added with 1.75 ml of OPT reagent and heated at 100°C for 10 min. After fluorophore development, readings were measured at 515 nm using spectrofluorimeter. Glutamate was used as standard.
4.9.2. Estimation of serotonin

About 1.4 ml of sample were added with 1.75 ml of OPT reagent and heated at 100°C for 10 min. After fluorophore development, readings were measured at 360-470 nm using spectrofluorimeter. Serotonin was used as standard [33].

4.9.3. Estimation of brain GABA content

About 0.1 mL of sample was added with 0.2 ml of 0.14 M ninhydrin (Prepared with 0.5M carbonate-bicarbonate buffer, pH 9.95). This mixture was heated for 30 min at 60°C. After heating, the mixture was allowed to cool and 5 ml of copper tartarate reagent was added. Then the fluorescence was measured at 377/455 nm in a spectrophotometer after 10min [34].

4.10. Estimation of antioxidant parameters

After treatment period, the rats were euthanized using ketamine hydrochloride (87mg/kg, ip), liver and brain of rats were isolated and washed with normal saline. The separated liver and brain was homogenized with 0.1 M Tris-HCl buffer (pH 7.4) and n-Butanol buffer using Teflon coated homogenizer. The homogenate was centrifuged for 10 min at 10000 rpm. The supernatant was collected and used for the estimation of total protein, Reduced Glutathione (GSH) and Lipid peroxidase levels.

4.10.1. Estimation of total protein

Total protein was estimated using Lowry method [35]. About 0.1 ml of sample was added with 0.9 ml of water and 4.5 ml of copper sulphate. The mixture was added with 0.5 ml of Folin’s reagent after 10 min at room temperature. The blue color was formed after 20 min, and measured at 640 nm using spectrophotometer.

4.10.2. Estimation of GSH

GSH was measured using Ellman’s procedure [36]. About 250 μl of tissue homogenate was added with 1 ml of 5% TCA and centrifuged for 10 min at 3000 rpm. The supernatant was added with 1.5 ml of 0.2 M phosphate buffer and 250 μl of 0.6 mM Ellman’s reagent. The values were analyzed at 412 nm. Glutathione reduced was uses as standard [37].

4.10.3. Estimation of LPO

Lipid peroxidation levels were measured using Okhawa et al. [38], method. About 1 ml of tissue homogenate were added with 4 % (w/v) sodium dodecyl sulfate, 20% acetic acid (prepared in 0.27 M hydrochloric acid, pH 3.5) and 0.8% thiobarbituric acid, pH 7.4 and heated for 1 h at 85°C. The pink color was formed and measured at 532 nm. The 1,1,3,3- tetraethoxy propane was used as the standard [39].

4.11. Statistical analysis

The data obtained from the results were analyzed by using one way ANOVA test using graph pad prism software. All data were expressed as the mean SEM of their parameter.


Conflict of interest statement: The authors declared no conflict of interest.

Ethics committee approval: Experimental studies were approved by IAEC with registration number of 685/OPO/Re/S/A/CPCSEA (IAEC No. KMCRET/M.Pharm/10/2019-20).

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