Antihyperglycemic effect of Aqueous and Ethanol extract of Aerial part of Osbeckia nepalensis Hook in Alloxan induced Diabetic rats

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Abstract: Aqueous and ethanolic extract of Osbeckia nepalensis Hook was evaluated for the antihyperglycemic activity in alloxan induced diabetic rats. Hyperglycemic was confirmed to Albino rats (150- 200 g) by treated with alloxan upto 320.6 ± 3.59 at 1 hr, 445.6 ± 4.23 at 5 days from non treated rats of 316 ± 3.56± mg/ dL. A significant hypoglycemic activity of aqueous and ethanolic extract was confirmed in alloxan induced diabetic rats (P<0.001). The serum glucose level of alloxan induced diabetic rats reduced upto 312.2 ± 3.65 and 232 ± 3.05 mg/dL at 1 hr and 120 hrs from 359±3.8 mg/ dL in aqueous extract and 320.6 ± 3.59, 445.6 ±4.24 mg /dL at 1 hr and 120 hrs. after treatment from 330 ± 3.64 mg/ dL before treatment in ethanolic extract of the test herbal. The aqueous and ethanolic extract of Osbeckia nepalensis enhance the checking of reduction in weight in comparision to normal and diabetic control animals. Further in the present work, phytochemicals of antioxidant (IC₅₀ 23 µg/ml ); saponin (20.0 ± 0.89 mg/g); flavonoid (38.28 mg/g) and minerals of K, (6.02 ± 0.51); N(22.1± 0.94); P(0.53±0.14) and Co (N.D) have screened out from the methanolic extract of Osbeckia nepalensis suggesting a unique supportive to the health care ingredients. The extracts both in aqueous and ethanolic substantiates for pharmaceutical formulation upto 4000 mg/kg oral toxicity.

Key words: Osbeckia nepalensis Hook, Antihyperglycemic activity, Phytochemical constituents, Minerals.

INTRODUCTION

Due to the change in lifestyle, the number of the people in the world with diabetes has increased dramatically over recent years. It is reported that the diabetic population will increased continuously around the world ¹. Diabetes mellitus is one of the most serious, chronic diseases i.e. developing with an increase in obesity and ageing in the general population.

Furthermore, diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism¹². The non-insulin dependent diabetes mellitus (NIDDM) conditions, which has assumed epidemic proportion today¹⁸, is characterized by reduced circulating
concentration of insulin, poor insulin sensitivity or insulin resistant, poor glucose tolerance resulting in high sugar in plasma. Again, prolonged hyperglycemia leads to other micro vascular and macro vascular complication. Current drugs used for treatment of diabetes including few groups of chemical compounds such as the drug biguanide (metformin), which although anti hyperglycemic does not affect hypoglycemia in the normal subject and involves extra-pancreatic mechanisms. Further, it is apparent that different mechanisms are involved in bringing down blood sugar level in normal or hyperglycemic conditions. However, some, such as biguanides, reportedly have undesirable side effects, and hence there is need for effective, safe and better oral hypoglycemic agents.

*Osbeckia nepalensis* Hook, a shrub growing to 1 m or more, with 4-angled branches covered with addressed hairs. Lance like leaves with entire margins have 3-5 parallel veins. Handsome flowers are mauve-purple or white, 3-5 cm across, in rather dense branched clusters at the end of branches. Sepals are covered with large flat scales, fringed with bristles. Stamens are straight, and all similar. The anthers also do not have apical beaks. It is found at altitudes of 600-2300 m. flowering in July-November. Traditionally the plant is used in health care system since immemorial time in management of diabetes in Manipur. There were scanty reports of *Osbeckia nepalensis* on diabetes. Hence, the present work was undertaken to investigate on its antihyperglycemic activity of therapeutic compounds from aqueous and ethanolic extract of *Osbeckia nepalensis* in alloxan induced diabetic rats.

**MATERIALS AND METHODS**

**Chemicals and instruments used**

The following chemicals used in the study: Alloxan (Labo. Chemic Bombay, India).

**Plant material**

For detecting the organic phytochemicals of therapeutic compounds, all the aerial parts of the plant of the fresh *Osbeckia nepalensis* Hook was collected freshly from Manipur near Kakching, Thoubal District during the month of July - August 2009 and its authentication has been confirmed by BSI, Shillong and deposited the plant in the Post Graduate Study centre, HRDRI, Canchipur. HRDRI/NES-10 for future reference.

**Preparation of plant extracts**

The dried plant material (1.5 kg) was ground in a warming blender and shifted through a wire screen. The powdered material (500 g) was extracted exhaustively first in soxhlet apparatus with ethanol and water for 48 hrs. The extracts was filtered and concentrated on a rotary vacuum evaporator. These are stored in room temperature and then refrigerated. The ethanol extract was used for acute toxicity and antidiabetic investigation was made under standardized condition.

Determination of free radial scavenging of plant extracts by the use of DPPH radical, Estimation of total flavonoid content, Saponin.

Mineral element determination of Potassium is determined using flame photometer, phosphorous by vanado-molybdate yellow method and nitrogen by Kjelhdal method.

**Experimental animals**

Albino rats of both sexes (150- 200 g) were kept in approved standard animal house conditions, fed standard pellet diet (Hindustan Lever Ltd., Bombay). Fasted animal were deprived of food for at least 16 hr. but were allowed free access water. The study was carried out with prior approval obtained from the institutional animal ethical committee of Institute of Bioresource and Sustanable Development (IBSD), Takyelpat, Imphal.

**Acute toxicity and selection of doses**

The acute toxicity studies was carried out in adult female Wister albino mice weighing about 200g by up and down method as per OECD 425 guidelines (OECD 425 guidelines). Overnight fasted animals received test drug at a dose of 2000 mg/kg body weight orally. Then the animals were observed continuously once in half an hour for next 4hrs and then after 24hrs from the time of administration of extract for general behavioral, neurological, autonomic profiles and find out mortality. The extracts found safe up to a dose of 200 mg/kg body weight.

**Biochemical parameters test in Alloxan induced diabetic rats**

For analysis of haematological and biochemical changes, blood samples were centrifuged at 4000rpm for 15minutes, then collected serum was determined according to the ERBA diagnostic kit instruction manual. The fasting glucose level was determined by Accu chek Narmoglycemic study Goglucan etc. some plasma used for biochemical parameters test in Alloxan induced diabetic rat estimation of plasma cholesterol triglycerides creatinine and urea by spectrophotometric assays.
According to the method presented in the Erba diagnostic kit instruction manual.

For hormglycemic study rats were divided into three groups (n=3) and were administered 2% gum acacia solution, since alloxan is comparable of producing fatal hypoglycemic as a result of massive pancreatic insulin release rats were treated with 20% glucose solution (15-20 ml).

Induction of experimental diabetes

Diabetes has induced in overnight fasted rats by single intraperitoneal injection of 150mg/kg alloxan monohydrate saline (2%IP). Hyperglycemia was confirmed by the elevated blood glucose level determined at 48 hrs after the dose. Animal that exhibited glycosuria after 48 hrs tested by urine test strips (Uristix, Bayer diagnostic Ltd., India) were considered as diabetic.

Experimental and design

The animals were divided into four groups of five animals in each group.

Group I: Normal healthy control.

Group II: Diabetic control (alloxan monohydrate 150 mg/kg in saline 2% IP).

Group III: Diabetic + Aqueous extract 200 mg/kg BW/day orally.

Group IV: Diabetic + Ethanol extract 200 mg/kg BW/day dose orally.

The extract treatments were carried out for a period of 7 days. During the period, animals for all groups had free access to standard diet and water. Body weight and blood glucose level were estimates on first day before and after treatment and 7th days of extract treatment. On the 7th day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for hematological and biochemical analysis.

Statistical analysis

The result were expressed as mean ± SEM. Statistical analysis were carried out using paired t-test and one way ANOVA following Bonferroni Multiple Comparisons test using graph pad install version 3 (Graph Pad software). All graphs prepared were made by graph and prism software. Differences below p <0.05 implied statistically significance.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>IC50 (µg/ml)</th>
<th>Saponin (µg/ml)</th>
<th>Flavonoid (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osbeckia nepalensis</td>
<td>23</td>
<td>20.00±0.89</td>
<td>38.28±1.24</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM; n = 3 in triplicate for each data.

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Fig.A.1. Phytochemical composition of Osbeckia nepalensis

Fig.A.2. Mineral composition of Osbeckia nepalensis.
Fig. A.3. DPPH free radical scavenging activity of methanolic extract Osbeckia nepalensis added to methanolic solution of DPPH as compared to standard Ascorbic acid.

Table A.2. Composition of Mineral elements of Osbeckia nepalensis. (Average of three replications).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>K</th>
<th>N</th>
<th>P</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osbeckia nepalensis</td>
<td>6.72±0.51</td>
<td>22.1±0.94</td>
<td>0.53±0.14</td>
<td>ND</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM; n = 3 in triplicate for each data.

Table B.1. Effect of aqueous and ethanol extract from Osbeckia nepalensis on serum glucose level (mg/dL) in alloxan diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After 1 hr of treatment</td>
</tr>
<tr>
<td>I</td>
<td>Diabetic control (Aloxan only)</td>
<td>316±3.56</td>
</tr>
<tr>
<td>II</td>
<td>Aqueous extract + Alloxan</td>
<td>359.4±3.8</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract + Alloxan</td>
<td>330.0±3.64</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM; n = 5 in duplicate for each treatment.

Table B.2. Effect of Alloxan on experimental rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After 1 hr of treatment</td>
</tr>
<tr>
<td>I</td>
<td>Normal control (1 ml distilled water P.O./day + 2 ml/kg saline I.P.)</td>
<td>89.20 ± 1.89</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control (1 ml distilled water P.O./day + 150 ml/kg saline I.P.)</td>
<td>89.20±1.89</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM; n = 5 in duplicate for each treatment.
Table B.3. Effect of aqueous and ethanol extract from *Osbeckia nepalensis* on serum glucose level (mg/dL) in normal fasted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Glucose level (mg/dL)</th>
<th></th>
<th></th>
<th></th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O₁</td>
<td>O₂</td>
<td>O₃</td>
<td>O₄</td>
<td>O₅</td>
</tr>
<tr>
<td>I</td>
<td>Normal control /Diabetic control</td>
<td>85</td>
<td>103</td>
<td>78</td>
<td>87</td>
<td>93</td>
</tr>
<tr>
<td>II</td>
<td>Sample aqueous extract</td>
<td>85</td>
<td>101</td>
<td>78</td>
<td>87</td>
<td>90</td>
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<tr>
<td>III</td>
<td>Sample ethanol extract</td>
<td>85</td>
<td>100</td>
<td>77</td>
<td>86</td>
<td>89</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM (Standard error Mean)

![Fig B.1. Effects of aqueous and Ethanol Extract from *Osbeckia nepalensis* on blood glucose level in Alloxan monohydrate induced diabetic rats](image1)

![Fig B.2. Effects of aqueous and Ethanol Extract from *Osbeckia nepalensis* on Cholesterol & urea in Alloxan monohydrate induced diabetic rats](image2)
Table B.4. Effect of unknown sample on body weight (gm) of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial (0) day (g)</th>
<th>Final 7 Days (g)</th>
<th>Remark Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>±</td>
<td>±</td>
<td>±increases</td>
</tr>
<tr>
<td>I</td>
<td>Normal control (Vehicle)</td>
<td>200 ± 2.85</td>
<td>208 ± 2.9</td>
<td>+8</td>
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<tr>
<td></td>
<td></td>
<td>±</td>
<td>±</td>
<td>±decreases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>260</td>
<td>+10</td>
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<tr>
<td></td>
<td></td>
<td>170</td>
<td>150</td>
<td>+10</td>
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<td>190</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>207</td>
<td>+7</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>200 ± 2.83</td>
<td>185 ± 2.71</td>
<td>-15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±</td>
<td>±</td>
<td>-10</td>
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<tr>
<td></td>
<td></td>
<td>210</td>
<td>200</td>
<td>-20</td>
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<td></td>
<td>±</td>
<td>±</td>
<td>-10</td>
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<td></td>
<td></td>
<td>210</td>
<td>190</td>
<td>-30</td>
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<td>190</td>
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<td></td>
<td>180</td>
<td>150</td>
<td>-10</td>
</tr>
<tr>
<td>III</td>
<td>Sample aqueous extract</td>
<td>190 ± 2.82</td>
<td>190 ± 2.77</td>
<td>-10</td>
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<tr>
<td></td>
<td>200mg/kg BW/day dose</td>
<td>±</td>
<td>±</td>
<td>-10</td>
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<td></td>
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<td>210</td>
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<td></td>
<td>200</td>
<td>170</td>
<td>-10</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanol extract</td>
<td>200 ± 2.86</td>
<td>195 ± 2.74</td>
<td>-10</td>
</tr>
<tr>
<td></td>
<td>200mg/kg BW/day dose</td>
<td>±</td>
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<td>-10</td>
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<td></td>
<td>210</td>
<td>200</td>
<td>-10</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM; n = 5 in duplicate for each treatment.

RESULTS AND DISCUSSION

The phytochemical investigation of Osbeckia nepalensis, revealed the presence of with constituents of saponin and flavonoid (Table A.1.). Investigation on quantitative phytochemical constituents of Osbeckia nepalensis yields a number of organic compounds inclusive of antioxidant (Table A.1.). Graphically it is represented in fig. A.1. and fig. A.3. The potential of concentration of antioxidant ranged to 23 μg/ml, saponin 20m/g and flavonoid 38.28 mg/g.

The present phytochemical test of plant extract estimated for antioxidants accord IC₅₀ 23 μg/ml, under free radical scavenging activity technique and confirmed the unique presence of
antioxidants and patented in the test herbal *Osbeckia nepalensis*. It is shown in table A.1. and fig. A.3.

Blood glucose levels of alloxan induced diabetic rats accord 316 ± 3.56, 320.6 ± 3.59, 445.6± 4.23 mg/dL while the control accord 89.2±1.89, 89.40 ±1.89, 89.4±1.89 mg/ dL respectively at 48 hrs, 49 hrs and 5 days after injection(Table B.1.,B.2. and Fig. B.1.).

Blood glucose level of animal treated with aqueous extract of *Osbeckia nepalensis* accord 88.20 ± 1.88mg/dL and 87.40±1.87mg/dL with ethanol extract of the same test plant while the control (without any extract) accord 89.20 ± 1.89. (Table B. 3. and Fig. B.3.).

In the experimentation on the effect of *Osbeckia nepalensis* extracts in aqueous and ethanol, on blood glucose level of alloxan induced diabetic rats accord 359.4 ± 3.8, 332.2 ±3.65, 232.4 ±3.65mg/dL in aqueous extract and 330.0 ±3.64, 261.0 ±3.23, 110.8 ±2.11mg/dL in ethanol extract at 0hrs and 120 hrs after treatments respectively(Table B.1. and Fig. B.1.).

Experimentation on effect of *Osbeckia nepalensis* extract in aqueous and ethanol for body weight (gm) of alloxan induced diabetic rats strike with 198±2.82gm and 191±2.77gm in aqueous extracts and 196±2.86 gm, 187±2.74 gm in ethanol extract at 0 and 7th day respectively after treatment. While diabetic control accord 200±2.83gm and 183±2.7gm and normal control accord 202±2.85gm and 210±2.9gm at 0 and 7th day after treatment. (Table B.4.).

Effect of test herbal *Osbeckia nepalensis* extract in aqueous and ethanol on the biochemical parameters (mg/dL) of cholesterol, creatinine and urea of alloxan induced diabetic rats has detected. The aqueous extract hits 56.00 ± 1.15, 0.4780±0.13 96.21±1.96 and ethanol extract strike 57.79 ± 1.5, 0.4220 ± 0.13, 81.27±1.8mg/dL, for cholesterol, creatinine and urea respectively as against the record of the diabetic control rats with 143.5±2.4, 1.106±0.21, 193.3±2.78 mg/dL for cholesterol, creatinine and urea respectively. While the normal control rats accord 82.68 ± 1.82, 0.448 ±0.13, 33.86±1.16 mg/dL for cholesterol creatinine and urea respectively. (Table B.5. and Fig. B.2.; B.3.).

<table>
<thead>
<tr>
<th>Table. B.5. Effect of aqueous and ethanol extract from <em>Osbeckia nepalensis</em> on biochemical parameter in alloxane induced diabetes rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>Normal control (1ml distilled water P.O./day + 2 ml/kg saline I.P.)</td>
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<td></td>
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<tr>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Diabetic control (1ml distilled water P.O. + 150 mg/kg alloxane monohydrate in saline(2%, I.P.)</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Mean ± SEM</td>
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<tr>
<td>Sample aqueous extract 200mg/kg BW/day dose</td>
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<td>Mean ± SEM</td>
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<td>extract 200mg/kg BW/day dose</td>
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<tr>
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</tr>
<tr>
<td>Mean ± SEM</td>
</tr>
</tbody>
</table>
ONE –WAY ANALYSIS OF VARIANCE (ANOVA)
GLUCOSE COMPARISON DATA (7TH DAY)
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference</th>
<th>t</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic vs Aq. Extract</td>
<td>213.20</td>
<td>9.718</td>
<td>***P&lt;0.001</td>
</tr>
<tr>
<td>Diabetic vs Ethanol extract</td>
<td>334.80</td>
<td>15.261</td>
<td>***P&lt;0.001</td>
</tr>
</tbody>
</table>

Differences
<table>
<thead>
<tr>
<th>Mean difference</th>
<th>95% confidence Interval From</th>
<th>To</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic –Aq. Extract</td>
<td>213.20</td>
<td>158.95-</td>
</tr>
<tr>
<td>Diabetic -Ethanol extract</td>
<td>334.80</td>
<td>280.55-</td>
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</tbody>
</table>

ONE –WAY ANALYSIS OF VARIANCE (ANOVA)
CHOLESTROL DATA
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference</th>
<th>t</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic vs Aq. Extract</td>
<td>87.500</td>
<td>10.202</td>
<td>***P&lt;0.001</td>
</tr>
<tr>
<td>Diabetic vs Ethanol extract</td>
<td>85.710</td>
<td>9.993</td>
<td>***P&lt;0.001</td>
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</tbody>
</table>

Differences
<table>
<thead>
<tr>
<th>Mean difference</th>
<th>95% confidence Interval From</th>
<th>To</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic –Aq. Extract</td>
<td>87.500</td>
<td>66.291-</td>
</tr>
<tr>
<td>Diabetic -Ethanol extract</td>
<td>85.710</td>
<td>64.501-</td>
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</tbody>
</table>

ONE –WAY ANALYSIS OF VARIANCE (ANOVA)
UREA DATA
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference</th>
<th>t</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic vs Aq. Extract</td>
<td>97.090</td>
<td>8.923</td>
<td>***P&lt;0.001</td>
</tr>
<tr>
<td>Diabetic vs Ethanol extract</td>
<td>112.03</td>
<td>10.296</td>
<td>***P&lt;0.001</td>
</tr>
</tbody>
</table>
Differences Mean difference 95% confidence Interval
From To
Diabetic – Aq. Extract 97.090 70.182- 124.00
Diabetic - Ethanol extract 112.03 85.122- 138.94

ONE WAY ANALYSIS OF VARIANCE (ANOVA) CREATININE DATA
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

<table>
<thead>
<tr>
<th>Comparision</th>
<th>Mean difference</th>
<th>t</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic vs Aq. Extract</td>
<td>0.6280</td>
<td>14.021</td>
<td>***P&lt;0.001</td>
</tr>
<tr>
<td>Diabetic vs Ethanol extract</td>
<td>0.6840</td>
<td>15.272</td>
<td>***P&lt;0.001</td>
</tr>
</tbody>
</table>

Differences Mean difference 95% confidence Interval
From To
Diabetic – Aq. Extract 0.6280 0.5172- 0.7388
Diabetic - Ethanol extract 0.6840 0.5732- 0.7948

For present bio-assaying, LD<sub>50</sub> was determined by the fixed dose (OECD/OCDE) method and LD<sub>50</sub> cut off of the different extracts were 1000 mg/kg body weight. All extracts were administered as 1/5<sup>th</sup> the dose of their respective LD<sub>50</sub> values in vivo experiments. During the present investigation both the aqueous and ethanol extracts of Osbeckia nepalensis leaves were subjected for antidiabetic activity in rats where alloxan monohydrate (150 mg/kg body weight) used as the diabetogenic agent. The findings accord a marked rise in blood glucose level upto 445±4.23 mg/dL after 5 days in diabetic control group of rats, which also became hyperlipidine, restless and irritable. Severe thirst and lack of appetite were observed. Among test rats aqueous and ethanol treated groups of rats showed progressively reduction in blood glucose levels upto 232.4±3.05 mg/dL after 5 days and 110.8±2.11 mg/dL after 5 days respectively. All the extracts have produce distinct anti -diabetic activity on 5<sup>th</sup> day as compared to 1<sup>st</sup> day of treatment.

Perusal on table A.1. revealed that the fully matured plant of Osbeckia nepalensis were free sources of therapeutic organic compounds of antioxidant, saponin, flavonoid. The presence of these bioactive compounds has empathetically emphasized the medicinal potentials of the test herbal. Similar result has reported from different plants 29–27.

The present phytochemical test of plant extract depict an antioxidant content upto 23 µg /ml under free radical scavenging activity technique confirmed the unique presence of the compound in Osbeckia nepalensis. Antioxidant and its ability to balance or trap highly reactive free radicals and oxygen species are very essential in biological systems from a wide variety of sources for longer life of cells. These free radicals may oxidised nucleic acids, protein, lipids or DNA and can initiate degenerative diseases. Further antioxidant compounds like phenolic acid, polyphenols and flavonoid scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism which leads to degenerative disease. There are number of clinical studies suggesting that the antioxidant in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancer 24.

Table A.1. delineated the flavonoid compounds content upto 38.28mg/g in the leaves of Osbeckia nepalensis. The value is also represented by fig A.1. showing the status among the 5 phytochemical compounds of the plant. Flavonoids have attracted a great deal of attention in relation to their potential for beneficial effect on health. Over the past few years, several experimental studies have demonstrated biological and pharmacological properties of many flavonoids especially their antimicrobial activity, anti-inflammatory, antioxidant and anti-tumor effects, which are associated with free radical scavenging action.
Flavonoids have also been reported to posses hypoglycaemic and anti-diabetic effect\(^4\).

The present phytochemical test determined the presence of saponin with 20 mg/g (Table A.1.). Saponins are useful in medicine and pharmaceutical industries due to its foaming ability that produces for the effects in the food industries\(^5\). Saponins is also used in the manufacture of sampoos, insecticides, various drug preparation and synthesis of steroidal hormones\(^6\). Saponins have also been reported to have hypoglycaemic and antidiabetic effects which are very useful in the management of diabetes mellitus\(^7\).\(^8\).\(^9\)

Table A.2. revealed that the *Osbeckia nepalensis* accounts distinct mineral content with remarkable amounts viz. potassium 6.72 mg/g, nitrogen 22.1mg/g, phosphorous 0.53 mg/g, cobalt not detected. Minerals are naturally occurring chemical elements found throughout the human body in the bones, muscles, teeth, blood and nerve cells. Minerals help to maintain a normal water balance within the body, Phosphorus is needed for energy production, metabolism, and healthy bone development. Potassium is needed for muscle contractions and nerve function\(^10\).

Preliminary phytochemical screening of the methanolic extract of *Osbeckia nepalensis* reveals the presence of flavonoids, saponin and antioxidant. Different doses of herbal plant extract in aqueous and ethanol was screened for their oral toxicity. No mortality was recorded upto 4000mg/kg with both of the extracts, hence the extract were confirmed to be safe up to the dose levels of 4000mg/kg, consequently the antidiabetic effect of the test herbal extracts in aqueous and ethanol was further experimentally investigated.

Table B.1. reveals the blood glucose levels of the alloxan induced diabetic rat's with 316 ± 3.56mg/dL, 320.6 ± 3.59; 445.6 ± 4.32 at 48, 49 and 120hrs as against the record of 89.40 ± 1.89mg/dL at 48, 49 and 120 hrs after injection in normal control rats. The finding clearly shows that the alloxan induced diabetic rats have remarkably higher level of blood glucose level ranging from 316±3.56 to 445.6 ± 4.23at 48 hrs. Thus, confirmed the induction of diabetic to the test rats for the present experimentation.

Table B.3. depicts the blood glucose level of animal treated with aqueous extract and ethanol extract with 88.20 ±1.88 and 87.40 ± 1.87mg/dL against the 89.20 ± 1.89mg/dL of control. The finding vividly clarify the extract of the test herbal have significant reducing capability of blood glucose level. The finding was in agreement with that of the other workers from other herbal extracts of different parts of the world\(^11\),\(^12\),\(^13\).

Table B.1. displayed the effect of *Osbeckia nepalensis* in aqueous extract, the blood glucose level of alloxan induced diabetic rats, which accord 332.2 ± 3.65 and 232.4 ± 3.05 mg/dL at 1 hr and 120 hrs. However, in ethanol extract, its accord struck 261.0 ± 3.23, 110.8 ± 2.11 mg/dL at 1 hr and 120 hours after treatment as against the corresponding control values of 320.6 ± 3.59, 445.6 ± 4.24 mg/dL at 1 and 120 hrs after treatment. It is evident from the present finding that a significant hypoglycemic activity of aqueous extract has confirmed in alloxan induced diabetic rats. After oral administration of 200 mg/kg, BW/day of aqueous extract of *Osbeckia nepalensis*, a significant reduction has observed in blood glucose level after 1hr of treatment and then the hypoglycemic become more effective after day 5.

Similarly observation of hypoglycemic effect of ethanol extract of *Osbeckia nepalensis* connote in the blood glucose level of alloxan induce diabetic rats, the effect become more conspicuous after day 5 of treatment. Obviously, in conclusion it patented that both the ethanolic and aqueous extract of *Osbeckia nepalensis* produced significant antihyperglycemic activity against the alloxan induced diabetic rats. The finding was in corroborative with the results of other antidiabetic herbal extracts from other plants\(^14\),\(^15\). The chronic treatment with methanol extract of *A.occidentale* stem-bark at 200mg/kg may be a safe agent that has a protective role against the diabetogenic and atherogenic effects of high fructose diet by reducing the hyperglycemic and hyperlipidemia as well as lipid peroxidation\(^16\). Further the α- glucosidase type I recognizes the glucosyl structure of the substrate, while the α- glucosidase type II recognizes the maltosyl structure \(^17\). *Terminalia bellerica* found to be most active to reduce serum glucose level followed by *E. officinalis* and *T. chebula, Triphala* that is a combination of all the three produced a significant action in reducing the alloxan induce diabetic. The result is slightly different with the result reportedearlier\(^18\) where, *T. chebula* has a higher α- glucosidase inhibitor activity compared to glucose tolerance method with amylog, maltose and sucrose loading as acarbose mechanism is to competitively inhibiting an enzyme (α- amyrase) located at the brush border of the small intestine that is responsible for terminal carbohydrate digestion. This inhibition decrease glucose absorption, thereby reducing alimentary hyperglycemia and hyper-insulinemia\(^19\). Choudhary and Bandypadhyay\(^20\) stated that hypokalemia or potassium depletion could cause glucose intolerance. Ca, K, and traces of chromium play an important role in insulin release from the β cells of the Langerhans islet, which finally helps to lower the blood glucose level. The content of the
metals and antioxidant in the extract of Osbeckia nepalensis herb may add the information on additional role in the anti-diabetes activity of the Osbeckia nepalensis herb.

Table B.4. exhibits the effect on the body weight (gm) of alloxan induced diabetic rats of Osbeckia nepalensis hits (198 ± 2.82gm), (191 ± 2.77gm) in aqueous extract and (196 ± 2.86 gm), (187 ± 2.74gm) in ethanol extract at 0 and 7 day. After treatment (200 ± 2.83 gm) and (183 ± 2.71 gm) in diabetic control rats and 202 ± 2.85 gm and 210 ± 2.9 gm in normal control after 0 and 7 days of treatment. The finding revealed that the ethanol extract of Osbeckia nepalensis have lesser degree of reduction in weight in comparision to normal and diabetic control animals. In this connection Fisman and Tenenbawn,(2008) rightfully claimed that five types of oral antihyperglycemic drugs currently approved for the treatment of diabetes i.e biguanides, sulfruglureas, meglitinides, glitazones and alpha-glucosidase inhibitors have limitations to some extend especially in patients with Coronary Artery Disease (CAD). Further, current data indicates that combined glibenclamide/metformin therapy seems to present a special risk and should be avoid in the long-term management of type 2 diabetes with proven CAD.

CONCLUSION

The alloxan induced diabetic rats have remarkably higher level of blood glucose ranges from 316 ± 3.56 to 445.6 ± 4.23 at 48hrs. The extract of the test herbal have significant reduction capability of blood glucose levels. A significant hypoglycemic activity on aqueous extract comply with alloxan induced diabetic rats. After oral administration of 200mg/kg BW/day of aqueous extract of Osbeckia nepalensis, a significant reduction was accommodated in blood glucose level after one hours of treatment and then the hypoglycemic effect become more pronoune and adopted after day 5.

Further, it patented that both the ethanolic and aqueous extract of Osbeckia nepalensis produced significant antihyperglycemic activity against the alloxan induced diabetic rats. Obviously, the present finding incorporated and getting support with antidiabetic activity of the test Osbeckia nepalensis due to presence of saponin, flavonoid, antioxidant, N, K, P etc.

Acknowledgement

The authors wish to thank UGC, New Delhi for financial assistance.

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