EVALUATION OF ANTI-DIABETIC AND ANTI-HYPERLIPIDEMIC POTENTIAL OF METHANOLIC EXTRACT OF *Juniperus communis* (L.) IN STREPTOZOTOXIN-NICOTINAMIDE INDUCED DIABETIC RATS.

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**ABSTRACT**

*Juniperus communis* (Cuppraseae), a coniferous plant widely used as traditional medicine was evaluated for the antidiabetic and antihyperlipidemic activity on Streptozotocin(STZ)-nicotinamide induced diabetic rats. The methanolic extract of *Juniperus communis* (MEJC) (100mg/kg, 200mg/kg, b.w) was administered orally in diabetic group except the standard group (Glibenclamide 10mg/kg, b.w) and the fasting blood glucose levels along with the different biochemical parameters were estimated on 21st day after collecting blood through retro-orbital puncture technique. It was observed that the extract showed significant (P<0.01) reduction in blood glucose levels along with the different lipid profile parameters (with increase in HDL levels) in diabetic rats. This study demonstrated a dose dependent and significant anti-diabetic and anti-hyperlipidemic property of the extract, providing the rationale behind its use as an effective drug against type-2 diabetes.

**KEYWORDS:** Antidiabetic , Antihyperlipidemic , Streptozotocin, *Juniperus communis*.

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INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia with several long term health complications if not checked early due to insulin deficiency. Currently, IDF estimates a total of 371 million cases of diabetes worldwide with 71 million cases of diabetes only in India. An expenditure of 471 billion USD is spent in the year 2012 for treatment of diabetes.\(^1\) WHO estimates an increase of 3.9 billion diabetes cases by 2030.\(^2\) Diabetes increases the risk of heart diseases and stroke due to increased incidence of atherosclerosis along with high blood pressure. Coronary artery diseases (CAD) and coronary heart diseases (CHD) along with diabetes have become one of the leading causes of death in the developing countries. It is estimated that by 2020, 85% of the cardiovascular diseases are expected to be borne by developing nations and the increase in CAD mortality by 2020 is projected to be 120% in women and 137% in men.\(^3,4\) The overall cardiovascular mortality in Indians is predicted to rise by 103% in men and 90% in women between 1985 and 2015.\(^5\) It can also lead to severe complications like diabetic retinopathy, nephropathy, diabetic feet which can be determined by levels of HbA1c in blood.\(^6\) The synthetic antidiabetic agents like sulfonylureas, biguanides, thiazolidinedione and \(\alpha\)-glucosidase inhibitors reduce the blood glucose level but have different adverse effects thus limiting and replacing their use with herbal products. Juniperus communis var. Saxatilis, family Cupressaceae native to Europe and North America is an evergreen dense shrub, having sharply pointed, scented leaves, with a bluish white surface. This is widely distributed across the Himalayas from Kumaon at an altitude of 1700-4200m.\(^7\) Apart from being a good diuretic agent, this species is widely used in folklore medicine system as carminative, in rheumatoid arthritis and as antifungal agent. The dried berries are known to provide good flavoring property and for stimulating appetite.\(^8\) Research work on the anti-inflammatory, anti-pyretic,\(^9\) analgesic\(^10\) and antimicrobial\(^11\) activities has also been successfully conducted to evaluate its effectiveness.

MATERIALS AND METHODS

**Plant materials and extraction**

The leaves of Juniperus communis was collected from Sikkim and was identified and authenticated by the Botanical Survey of India, Botanical Gardens, Howrah, West Bengal. The plant materials (250gm) thus obtained was dried under shade and macerated using Petroleum Ether 40-60. The defatted part was filtered and was further extracted using Methanol (MeOH) for 8-9 hrs using Soxhlet Apparatus. The solvent was finally evaporated using Rotary Vacuum Evaporator (Hahn Vapor HS-2005V, Hahnshin Scientific Co, Korea) and was dried using Freeze drier (IIC Industrial Corp., Kolkata, India).

**Chemicals and Reagents used**

Streptozotocin and Nicotinamide were purchased from SISCO Research Laboratory, Ltd., Mumbai, India. Glibenclamide used was purchased from Aventis Pharma Ltd., Goa, India. The other chemicals and reagents used were of analytical grade.

**Animals**

Healthy adult male Sprague-dawley rats (150-200gms) having age between 2-4months were obtained from Indian Institute of Chemical Biology (IICB, Kolkata, India) were grouped and housed in polypropylene cages under 12hrs light dark cycle at 24±1°C with free access to standard pellet diet and water *ad libitum*. The animals were allowed to acclimatize to the lab condition for 7days prior to the experiment and the procedures followed were in accordance to the Institutional Animal Ethics Committee Recommendations (Approval No: 147/1999/CPCSEA).

**Acute toxicity studies**

The toxicity test was carried out in Swiss Albino mice (20-25gm) in accordance to the OECD
guidelines. No mortality was recorded up to a dose of 5gm/kg p.o. for 24hrs period. So, the effective dose was fixed at 100mg/kg and 200mg/kg body weight for evaluation of antidiabetic activity.

**Oral Glucose Tolerance Test**

Rats were divided into four groups with 6 in each group (n=6) and were administered orally with Normal saline, Methanolic extract of Juniperus communis (MEJC) at doses 100 & 200mg/kg B.W and standard drug Glibenclamide (10mg/kg B.W) respectively. Glucose load of 2gm/kg B.W was administered orally 30mins after administration of the extract. The blood glucose level was estimated at 0, 30, 60, 90, 120 mins after withdrawing blood via retro-orbital plexus of the eye under light ether anesthesia. The glucose level was estimated using the standard GOD-POD kit (Span Diagnostics Ltd, Surat, India).

**Induction of Diabetes**

The animals were fasted overnight and were administered with a single intraperitoneal injection of Streptozotocin (STZ) (55mg/kg,B.W) in 0.1M freshly prepared citrate buffer pH 4.5, 15mins after administration of Nicotinamide (110mg/kg,B.W, i.p.). Normal control group received citrate buffer. As STZ causes severe destruction of pancreatic cells leading to the death of the animal due to hypoglycaemia, so glucose at 10gm/kg B.W dose was provided 6hrs after administration. After 72hrs, the animals were screened for fasting blood glucose (FBG) and the animals with FBG≥250mg/dl of blood were included in the study.

**Experimental design and antidiabetic activity**

In the experiment 30 rats were used which were divided in five groups with six animals in each group (n=6). Group I served as normal control group receiving 0.1ml/kg B.W normal saline solution. Group II as diabetic control rats did not receive any extract. Group III, IV, V were STZ induced diabetic rats treated orally with Glibenclamide (10mg/kg, B.W), MEJC (100mg/kg, B.W) and MEJC (200mg/kg,B.W) respectively for 21 days. Fasting blood glucose levels and body weight was estimated on 0, 7, 14, 21 days regularly. The blood was collected from the retro-orbital plexus and stored using disodium ethylene dinitrilo tetraacetic acid (Titriplex III, Merck Ltd., Mumbai, India) for the estimation of FBG level by GOD-POD kit (Span diagnostics, Surat, India).

**Evaluation of antihyperlipidemic activity**

On 21st day of the study, the animals were sacrificed by cervical dislocation and the blood was collected to estimate the lipid parameters. Blood collected through cardiac puncture technique was used in the study which was allowed to clot and was further centrifuged at 3000rpm for 10mins to separate the serum. The serum samples were analysed spectrophotometrically for total cholesterol (TC) at 560nm, high density lipoprotein cholesterol (HDL-C) at 505nm and triglyceride (TG) at 505nm, by commercially available kit (Span diagnostics, Surat, India) and UV visible spectrophotometer (JASCO, V-650, Japan). VLDL-C and LDL-C were calculated according to Friedwald et al. VLDL=TG/5 LDL= TC-HDL-VLDL

**Measurement of biochemical parameters**

The blood thus collected at the end of the experiment was stored using disodium ethylene diamine tetraacetate for analyzing the biochemical parameters. The analysis of serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvic transaminase (SGPT), total protein and serum urea was done using the standard kits (Span Diagnostics Ltd, Surat, India) using UV visible spectroscopy at 505nm,505nm, 578nm and 525nm respectively.

**Statistical Analysis**

The results obtained are expressed as mean SEM., and were expressed by one-way analysis of variance (ANOVA) followed by Dunnette’s multiple comparison post hoc test using Graph pad Prism 5 software. The values of P<0.05 was considered as statistically significant.
RESULTS

Effect on OGTT

The study shows that the administration of MEJC at 100 and 200 mg/kg, B.W significantly reduced the blood glucose level in normoglycemic group that receives the glucose load of 2gm/kg, B.W after oral administration of extract. Standard drug Glibenclamide (10mg/kg, B.W) also showed significant reduction when compared to control group.

Effect of MEJC on OGTT in normoglycaemic rats.

![Graph showing effect of MEJC on OGTT in normoglycaemic rats.](image)

Effect of MEJC on blood glucose and body weight

The result reveals that the methanolic extract exhibits a significant (P<0.01) and dose dependent reduction in the hyperglycaemic condition of STZ induced diabetic rats. After 21 days of the study, it was found that there was a reduction of 38.58% & 41.03% in the blood glucose levels with administration of MEJC at doses 100 and 200 mg/kg, B.W respectively. Glibenclamide also showed significant (P<0.01) decrease of 60.69% in glucose levels when compared with control group. After the end of the treatment schedule, the normal rats, MEJC and glibenclamide treated rats showed an increase in body weight whereas the diabetic control rats showed a marked fall in body weight.

Effect of MEJC on blood glucose level in STZ induced diabetic rats (n=6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(mg/kg)</th>
<th>Blood Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>Vehicle</td>
<td>91.67 ± 3.12</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>Vehicle</td>
<td>301.50±9.94*</td>
</tr>
<tr>
<td>MEJC</td>
<td>100</td>
<td>263.30±7.12*</td>
</tr>
<tr>
<td>MEJC</td>
<td>200</td>
<td>260.20± 8.51*</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10</td>
<td>277.50±8.50*</td>
</tr>
</tbody>
</table>

*P<0.01, †P<0.05 values represent mean ± SEM when compared with diabetic control group;

4P<0.01 when compared to normal control group by using one-way ANOVA followed by Dunnette’s Multiple comparison test.

This article can be downloaded from www.ijpbs.net
Effect of MEJC on body weight in STZ induced rats.

The animals showed a significant (P<0.01) increase in the CHL (Cholesterol), TG, LDL, VLDL levels in the STZ induced diabetic rats when compared to normal control group. On treatment with MEJC for 21 days, there was a significant (P<0.01, P<0.05) reduction in CHL, LDL, VLDL and TG levels in a dose dependent manner. On the other hand glibenclamide (10mg/kg, B.W) also showed a significant (P<0.01) reduction when compared with diabetic control animals. There is also a significant (P<0.01) increase in HDL level on administration of the extract at a dose of 200mg/kg, B.W and glibenclamide treated group when compared to diabetic control group.

Effect of MEJC on lipid profile in STZ induced diabetic rats (n=6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Serum lipid profile (mg/dL)</th>
<th>Normal Control Vehicle</th>
<th>Diabetic Control Vehicle</th>
<th>MEJC 100</th>
<th>MEJC 200</th>
<th>Glibenclamide 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Cholesterol</td>
<td>Triglyceride</td>
<td>HDL</td>
<td>LDL</td>
<td>VLDL</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>Normal Control</td>
<td>Vehicle</td>
<td>101.20±3.20</td>
<td>70.99±4.38</td>
<td>61.75±2.79</td>
<td>25.21±4.87</td>
<td>14.20±0.87</td>
<td>138.40±4.66</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Vehicle</td>
<td>140.30±5.14</td>
<td>139.04±4.66</td>
<td>25.84±2.15</td>
<td>86.97±4.73</td>
<td>27.69±0.89</td>
<td>117.30±6.67</td>
</tr>
<tr>
<td>MEJC</td>
<td>100</td>
<td>114.20±7.65</td>
<td>117.30±6.67</td>
<td>43.91±2.88</td>
<td>46.79±8.87</td>
<td>23.46±1.33</td>
<td>110.30±5.47</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>110.30±5.47</td>
<td>48.04±3.11</td>
<td>39.83±6.70</td>
<td>22.40±1.05</td>
<td></td>
<td>97.86±3.22</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. *P<0.01 represents values when compared with normal control. †P<0.05 when compared to diabetic control group using one-way ANOVA followed by Dunnette’s Multiple comparison test.

Effect on biochemical parameters

STZ induced diabetic rats showed a marked increase in the levels of serum biomarkers like SGOT, SGPT, total protein and serum urea, when compared to normal control, which was significantly (P<0.01) lowered on administration of the methanolic extract at a dose of 200mg/kg B.W and glibenclamide to a near normal levels.

Effect of MEJC on SGOT, SGPT, total protein, urea in STZ induced diabetic rats (n=6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>Urea (mg/dL)</th>
<th>Total protein (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>Vehicle</td>
<td>42.19±0.89</td>
<td>27.41±0.98</td>
<td>27.83±2.85</td>
<td>7.36±0.18</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Vehicle</td>
<td>120.70±0.60*</td>
<td>55.38±1.04*</td>
<td>75.69±1.75*</td>
<td>5.94±0.29*</td>
</tr>
<tr>
<td>MEJC</td>
<td>100</td>
<td>64.94±2.23*</td>
<td>49.81±1.22</td>
<td>48.90±1.58*</td>
<td>6.29±0.10</td>
</tr>
<tr>
<td>MEJC</td>
<td>200</td>
<td>55.04±2.33*</td>
<td>37.91±2.86*</td>
<td>40.03±3.19*</td>
<td>6.91±0.11*</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10</td>
<td>46.29±2.22*</td>
<td>30.27±1.27*</td>
<td>31.21±0.30*</td>
<td>7.10±0.11*</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. *P<0.01 represents values when compared with normal control. †P<0.01 when compared to diabetic control group using one-way ANOVA followed by Dunnette’s Multiple comparison test.
DISCUSSION

Diabetes mellitus, a metabolic disorder is characterized with increase in blood glucose level. The main objective of the drugs acting as anti-hyperglycaemic is to reduce the blood glucose level to normal so as to reduce the diabetes related complications. In the present study, type 2 diabetes was induced using STZ, after administration of nicotinamide. STZ being a cytotoxic agent causes alkylation of DNA in the beta cells of pancreas resulting in the degranulation and destruction of the beta cells which leads to acute decrease in the level of insulin.\textsuperscript{20, 21} Cellular metabolism of STZ causes release of free radicals like nitric oxide (NO) and lowering of beta cell nicotinamide adenine dinucleotide (NAD\textsuperscript{+}), which further aggravates the DNA strand damage of the pancreatic cells.\textsuperscript{21,22,23} On the other hand nicotinamide acts as an antioxidant preventing the generation of NO as well as causes less damage to beta cells by preventing apoptosis.\textsuperscript{24} Diabetes is normally associated with increased production of oxidative stress which has a key role in the progression of the disorder. The formation of superoxide anion radical from glucose oxidation and if not scavenged by enzymes like catalase(CAT), superoxide dismutase(SOD), or glutathione peroxide (GPx), forms reactive hydroxyl radicals resulting in initiation and propagation of lipid peroxidase (LPO). Again, superoxide anion radical reacts with nitric oxide to form peroxynitrile radicals.\textsuperscript{25,26,27} This study revealed that MEJC has the potential to reduce the elevated glucose level in oral glucose tolerance test to near normal level indicating the ability of the pancreas to revert to its normal condition during the experimental diabetic state. During this condition, the blood glucose level rises due to insulin deficiency but it has been seen that MEJC significantly reduces the FBG in a dose dependent manner probably by increasing the insulin release from the remnant beta cells.\textsuperscript{28} Deficiency of insulin brings about improper metabolism of carbohydrate, lipids and proteins leading to increased gluconeogenesis, glycogenolysis, lipolysis and muscle wasting.

The phenomenon of muscle wasting occurs due to unavailability of carbohydrate for energy metabolism leading to protein breakdown in skeletal muscles and thus causes loss of body weight, most prominent in diabetic animals.\textsuperscript{29,30} However, administration of MEJC at doses 100 and 200mg/kg B.W showed a marked increase in body weight in the animals though it was not significant, providing evidence to its antihyperglycaemic activity. Liver is considered as one of the most vital organ of the body due to its varied functions like metabolism, detoxification, excretion of xenobiotics. SGOT, SGPT are two important markers of liver function. Damage of liver caused due to STZ causes leakage of these enzymes from the cytosol into the blood stream indicating the hepatotoxic effect of STZ.\textsuperscript{31,32} Treatment of the animals with the methanolic extract caused a significant reduction in the levels of these enzymes in the plasma when compared with the diabetic control group providing evidence for their hepatoprotective effect. Standard drug glibenclamide showed a significant and marked decrease in their levels. During the state of diabetes accumulation of urea nitrogen takes place as it is the product of liver and plasma protein catabolism. Decreased serum protein levels were observed due to protein loss in diabetic rats but on administration of MEJC it was found to increase the serum protein levels in a dose dependent manner. Coronary heart disease and cerebrovascular diseases are very common complications associated with atherosclerosis during diabetes. Insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides, but due to deficiency of insulin, hypertriglyceridemia occurs.\textsuperscript{33,34} Also insulin deficiency leads to metabolic abnormalities causing hypercholesterolemia.\textsuperscript{35} The dyslipidemia is associated with elevated levels of TC, TG, LDL, VLDL and a fall in the level of HDL.\textsuperscript{36} This abnormal state can be reversed to normal on administration of the extract which decreases the level of TC, TG, VLDL, LDL and on the other hand increases the level of HDL.
CONCLUSION

The findings in this study reveals the antidiabetic and antihyperlipidemic activity of the methanolic extract of *Juniperus communis* though research work is going on to isolate and characterize the active compound responsible for the activity providing the rationale behind its use as an useful drug for diabetes.

REFERENCES


