

Evaluation of Anti-Diabetic Activity of *Mahonia nepalensis* in STZ Induced Rat Model

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ABSTRACT

Objectives: To evaluate the Anti-diabetic activity of *Mahonia nepalensis* in rats. **Materials and Methods:** The oral glucose tolerance test was performed in normal rats. Rats were divided into five groups ($n = 6$) as Group I control received saline (5 ml/kg p.o.). Group II and III received methanol extract of root (MNR), and Group IV and V received methanol extract of stem bark (MNB) of *Mahonia nepalensis* at doses of 200 and 400 mg/kg p.o., blood was withdrawn from tail vein at 0, 30, 60, and 120 min and glucose levels were measured using glucose oxidase-peroxidase reactive strips and glucometer. The STZ induced antidiabetic activity of rats was performed by dividing normal and diabetic rats into seven groups ($n = 6$). Group I as non diabetic rats received saline (5 ml/kg p.o.). Group II as diabetic control. Groups III and IV were received MNR (200 and 400 mg/kg. p.o.). Groups V and VI were received MNB (200 and 400 mg/kg. p.o.), and Group VII received glibenclamide (0.5 mg/kg. p.o.) for 14 days. Diabetic rats were evaluated for anti-oxidant activity. **Results:** In oral glucose tolerance test MNR and MNB group showed significant decrease in blood glucose level. In STZ induced diabetic rats fasting blood glucose levels of the treatment group significantly reduced by the 14 days treatment with MNR and MNB extract. In antioxidant activity ferric reducing ability of the MNR and MNB showed in a dose depending manner. **Conclusion:** The crude extract containing methanolic extract of *Mahonia nepalensis* (MNR and MNB) have potent antidiabetic activity and antioxidant activity in streptozocin induced diabetic rats.

Keywords: Glibenclamide, Streptozocin (STZ), *Mahonia nepalensis*, Glucometer.

INTRODUCTION

Diabetes is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism which causes hyperglycemia resulting from insufficient insulin secretion, insulin action or both^{1,2}. It is one of the refractory diseases identified by Indian council of medical research for which an alternative medicine is a need for the treatment. Diabetes mellitus has become a growing problem in the contemporary world³. Today India has become the diabetic capital of the world with over 20million diabetic patients and this number is likely to increase to 57million by 2025⁴. This astronomic increase in the prevalence of diabetes has made diabetes a major public health challenge for India and is become important human ailment afflicting many from various walks of life in different countries and once again the whole world being looked upon ayurvedic the oldest healing system of medicine for the treatment of diabetes. Although there are many synthetic medicines developed for patients, but it is the fact that it has never been reported that someone had recovered that totally from diabetes⁵. The modern oral hypoglycemic agents showed undesirable side effects thus in the recent years considerable attention has been directed towards the antidiabetic potential of medicinal plants and their herbal formulation in the management of disease. The concept

of polyherbalism is peculiar to ayurveda although it is difficult to explain in term of modern parameters. It is evident that there are many herbal formulations of varying potency since these preparation act by different mechanism, it is theoretically possible that different combination of these extract will do better job in reducing blood glucose. In the traditional system of plant medicine, it is usual to use plant formulation and combined extract of plant are used as a drug of choice rather than individual ones⁶ to get the benefit of synergism.

MATERIALS AND METHODS

Plant Material

The plant material was collected from Churia Kothi, Darjeeling, West Bengal in the month of September, 2011 and authenticated by Dr. Kanad Das, Scientist-in-Charge, Botanical Survey of India, Sikkim Himalayan Regional Centre, Gangtok, Sikkim. A voucher specimen (No. SHRC-5/2/91-Tech.217) has been deposited at our laboratory Department of Pharmacology, Himalayan Pharmacy Institute, Majhitar, Rangpo, Sikkim-737136.

Acute Toxicity

The acute oral toxicity of methanol extract of *Mahonia nepalensis* stem bark (MNB) and root (MNR) were evaluated in mice according to the OECD guidelines for testing of chemicals- 425 (OECD, 2001). The

Oral glucose tolerance test (OGTT)

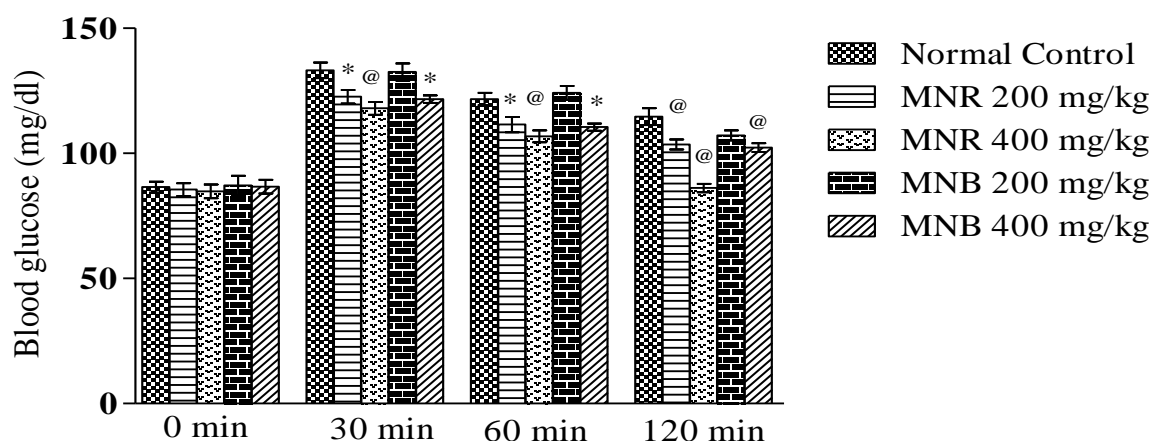


Figure 1: Effect of methanol extract root (MNR) and stem bark (MNB) of *Mahonia nepalensis* on oral glucose tolerance in normal rats.

Blood Glycated Hemoglobin (HbA1c)

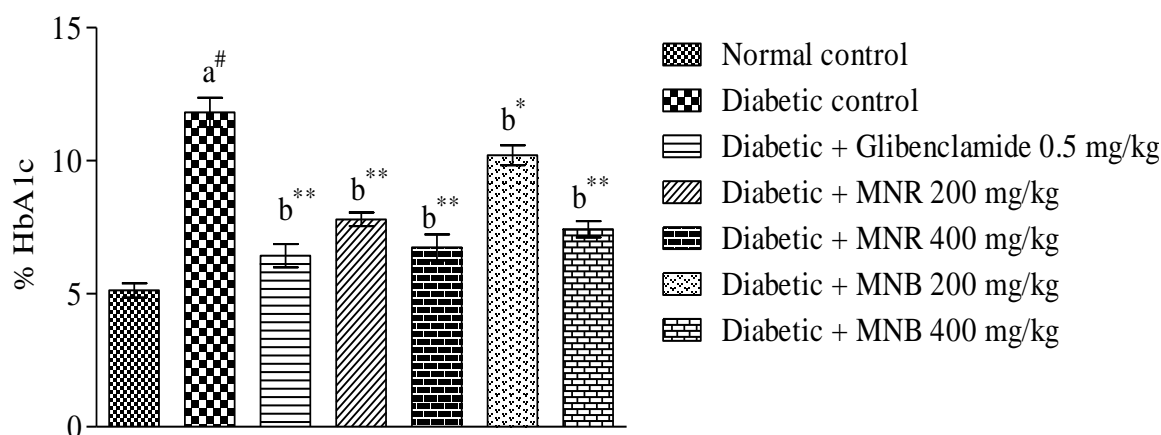


Figure 2: Effect of methanol extract root (MNR) and stem bark (MNB) of *Mahonia nepalensis* on blood glycated hemoglobin (HbA1c) level in rats.

Table 1: Effect of methanol extract root (MNR) and stem bark (MNB) of *Mahonia nepalensis* on fasting blood glucose (FBG) level in streptozotocin (STZ) induced diabetic rats.

Groups	0 th day	5 th day	10 th day	15 th day
I. Normal control	77.90±0.99	80.61±1.23	81.27±2.79	85.12±1.87
II. Diabetic control (STZ 50 mg/kg)	260.20±4.11	313.85±3.21 ^{a,#}	351.90±2.44 ^{a,#}	372.85±2.83 ^{a,#}
III. Diabetic +Glibenclamide 0.5 mg/kg	270.00±2.69	240.69±6.60 ^{b,**}	176.23±1.91 ^{b,**}	125.94±1.71 ^{b,**}
IV. Diabetic + MNR 200 mg/kg	283.10±5.16	245.61±5.88 ^{b,**}	217.33±3.23 ^{b,**}	176.64±1.50 ^{b,**}
V. Diabetic + MNR 400 mg/kg	287.25±2.56	225.10±2.38 ^{b,**}	182.88±1.89 ^{b,**}	144.14±2.12 ^{b,**}
VI. Diabetic + MNB 200 mg/kg	281.75±5.45	267.87±1.79 ^{b,**}	248.61±1.06 ^{b,**}	188.52±1.66 ^{b,**}
VII. Diabetic + MNB 400 mg/kg	267.59±4.34	258.57±1.19 ^{b,**}	188.33±1.29 ^{b,**}	161.07±2.53 ^{b,**}

Values are expressed as mean ± S.E.M. (n = 6). STZ (50 mg/kg b.w.) was injected to control and all other treated groups: ^adiabetic control vs normal group, [#]p < 0.01; ^btreated group vs diabetic control, ^{**}p < 0.01. The MNR, MNB and glibenclamide (0.5 mg/kg b.w.) treatment group significantly (p<0.05, p<0.01) decreased in HbA1c level compared to the diabetic control.

Institutional Animal Ethics Committee (IAEC) approved the protocol (Reg. no. HPI/2011/60/IAEC/0096) Mice were obtained from the animal house, Himalayan Pharmacy Institute. Animals were kept for acclimatization in standard laboratory conditions (temperature 25 ± 3°C) and light and dark cycles of 12h each. A limit test of 2000 mg/kg body weight was performed using five mice. The protocol of up-and-down

method was followed. First one animal was dose at the test dose and observed for 48 hours¹². Then fours animals were subjected to same test dose and mortality was observed for 14 days.

Experimental design

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18 h) normal rats. Rats were divided

Table 2: Effect of methanol extract root (MNR) and stem bark (MNB) of *Mahonia nepalensis* on serum lipid profile in streptozotocin (STZ) induced diabetic rats

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)
I. Normal control	77.32±2.72	70.87±5.97	38.48±0.80	36.95±2.65
II. Diabetic control (STZ 50 mg/kg)	177.03±6.03 ^{a,#}	103.18±4.24 ^{a,#}	28.43±1.12 ^{a,#}	125.90±3.96 ^{a,#}
III. Diabetic + Glibenclamide 0.5 mg/kg	78.25±2.59 ^{b,**}	72.27±4.46 ^{b,*}	55.43±1.02 ^{b,**}	51.92±2.66 ^{b,**}
IV. Diabetic + MNR 200 mg/kg	111.50±5.00 ^{b,**}	62.67±10.61 ^{b,**}	65.2±1.40 ^{b,**}	77.13±4.14 ^{b,**}
V. Diabetic + MNR 400 mg/kg	86.57±1.59 ^{b,**}	67.78±6.31 ^{b,**}	61.90±3.26 ^{b,**}	63.92±5.11 ^{b,**}
VI. Diabetic + MNB 200 mg/kg	119.72±6.73 ^{b,**}	81.85±5.69	34.74±1.23	108.90±4.95 ^{b,*}
VII. Diabetic + MNB 400 mg/kg	94.62±1.39 ^{b,**}	71.27±4.39 ^{b,**}	51.67±1.69 ^{b,**}	72.54±3.81 ^{b,**}

Values are expressed as mean ± S.E.M. ($n = 6$). STZ (50 mg/kg b.w.) was injected to control and all other treated groups: ^a diabetic control vs normal group, [#] $p < 0.01$; ^ball treated group vs diabetic control, ^{*} $p < 0.05$, ^{**} $p < 0.01$. Total protein level and TBARS contents (MDA) was significantly ($P < 0.01$) decreased the total protein and MDA level in liver tissue compared to diabetic control group. GSH, SOD and CAT

Table 3: Effect of methanol extract root (MNR) and stem bark (MNB) of *Mahonia nepalensis* on liver tissue antioxidant defense parameters like total protein count (TP), lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) level in streptozotocin (STZ) induced diabetic rats

Groups	TP (g/dl)	LPO (nM MDA/mg protein)	SOD (Unit/mg protein)	CAT (mM/min/mg protein)	GSH (μ g/ mg protein)
I. Normal control	1.75 ± 0.11	251.67 ± 4.26	2.43 ± 0.13	1.17 ± 0.09	5.78 ± 0.08
II. Diabetic control (STZ 50 mg/kg)	3.34 ± 0.28 ^{a,#}	497.34 ± 16.56 ^{a,#}	0.35 ± 0.02 ^{a,#}	0.073 ± 0.005 ^{a,#}	1.13 ± 0.05 ^{a,#}
III. Diabetic + Glibenclamide 0.5 mg/kg	1.91 ± 0.18 ^{b,**}	272.71 ± 6.15 ^{b,**}	1.12 ± 0.10 ^{b,**}	0.79 ± 0.06	1.65 ± 0.12 ^{b,**}
IV. Diabetic + MNR 200 mg/kg	2.29 ± 0.20 ^{b,*}	405.7 ± 9.85 ^{b,**}	0.67 ± 0.02 ^{b,**}	0.124 ± 0.004	1.68 ± 0.05 ^{b,**}
V. Diabetic + MNR 400 mg/kg	1.96 ± 0.13 ^{b,**}	350.42 ± 3.42 ^{b,**}	0.96 ± 0.04 ^{b,**}	0.33 ± 0.009 ^{b,*}	1.98 ± 0.07 ^{b,**}
VI. Diabetic + MNB 200 mg/kg	2.79 ± 0.34	468.64 ± 3.25	0.48 ± 0.13	0.16 ± 0.03	1.34 ± 0.07
VII. Diabetic + MNB 400 mg/kg	2.36 ± 0.27 ^{b,*}	334.8 ± 4.67 ^{b,**}	0.64 ± 0.03 ^{b,*}	0.248 ± 0.09	3.46 ± 0.10 ^{b,**}

Values are expressed as mean ± S.E.M. ($n = 6$). STZ (50 mg/kg b.w.) was injected to control and all other treated groups: ^a diabetic control vs normal group, [#] $p < 0.01$; ^ball treated group vs diabetic control, ^{*} $p < 0.05$, ^{**} $p < 0.01$.

into five groups (each group consisted of 6 rat; $n = 6$). Group I served as normal control (NC) and received normal saline (0.9% NaCl; 5 ml/kg b.w. p.o.). Group II and III received methanol extract of root (MNR), and Group IV and V received methanol extract of stem bark (MNB) of *Mahonia nepalensis* at the doses of 200 and 400 mg/kg b.w. p.o., respectively. Glucose (2 g/kg b.w. p.o.) was fed to all groups, 30 min after the administration of extracts and normal saline^{13,14}. Blood was withdrawn from tail vein at 0, 30, 60, and 120 min of glucose administration and glucose levels were measured.

Experimental induction of diabetes mellitus

The animals were overnight fasted and diabetes was induced experimentally in rats by a single intraperitoneal

(i.p.) dose of a freshly prepared solution of streptozotocin (STZ) at a dose of 50 mg/kg b.w. in 0.1 M cold citrate buffer (pH 4.5)¹⁵. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. After 72 h of the STZ injection, blood was withdrawn from tail vein of the animals and fasting blood glucose (FBG) level was estimated by glucometer. The rats with a blood glucose level above 250 mg/dl were selected for the experiment as diabetic rats¹⁶.

Treatment

Normal and experimentally diabetic rats were randomly divided into seven groups (each group consisted of 6 rat; $n = 6$). Except Group I, which served as non diabetic and was received normal saline (0.9% NaCl; 5 ml/kg b.w.

Histopathological Study

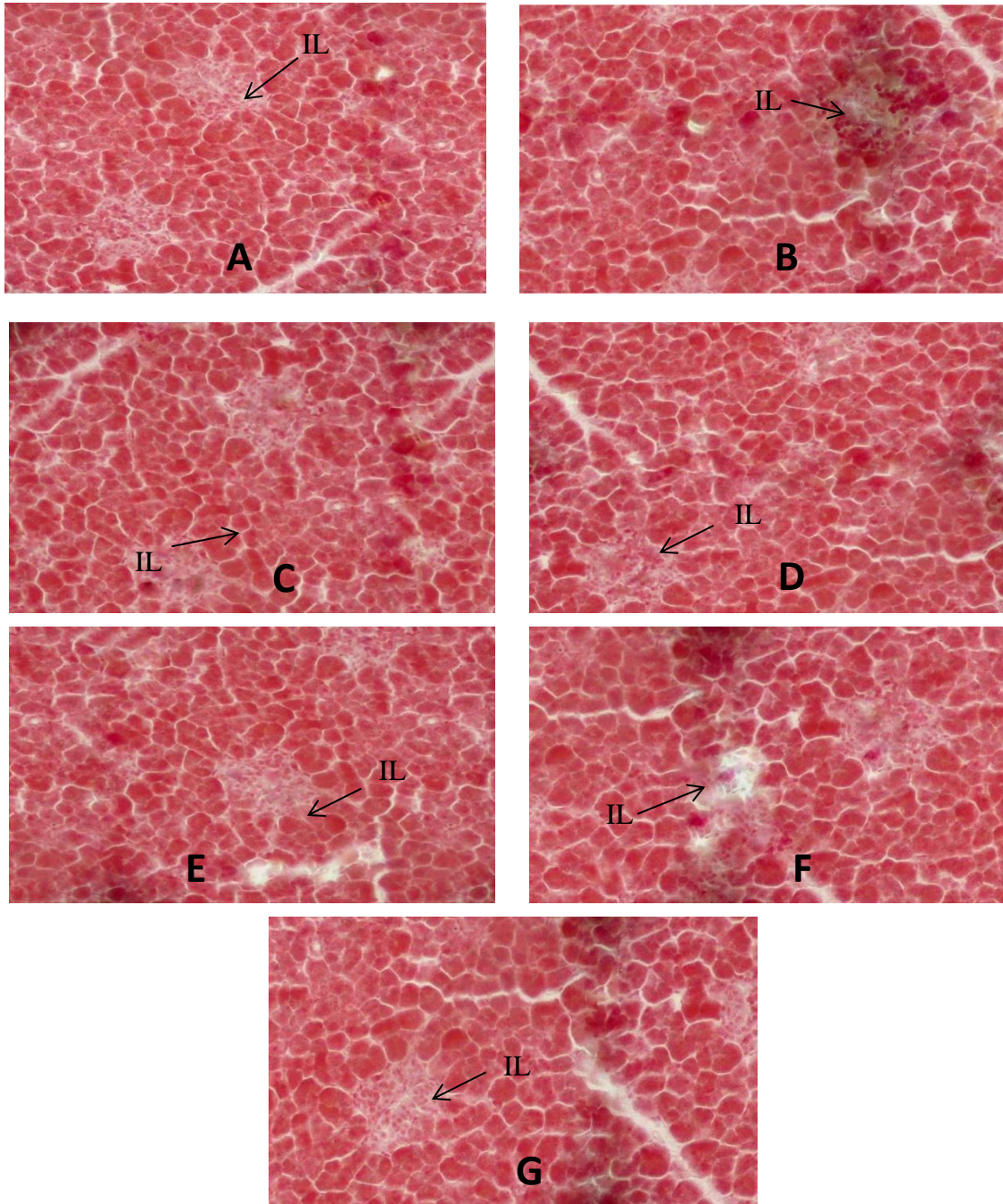


Figure 3: Photomicrographs of pancreas sections stained with hematoxylin and eosin (magnification 100X). (A) Normal control, (B) STZ control, (C) STZ + Glibenclamide (0.5 mg/kg), (D) STZ + MNR (200mg/kg), (E) STZ + MNR (400mg/kg), (F) STZ + MNB (200mg/kg) and (G) STZ + MNB (200mg/kg) - treated group. (IL – Islets of Langerhans)

p.o.), all other group were comprised of diabetic rat. Group II served as diabetic control (STZ). Groups III and IV were received MNR (200 mg/kg and 400 mg/kg b.w. p.o. respectively). Groups V and VI were received MNB (200 mg/kg and 400 mg/kg b.w. p.o. respectively), and Group VII received reference drug glibenclamide (0.5 mg/kg b.w. p.o.) daily using an intragastric tube for 14 days.

Estimation of fasting blood glucose and body weight

Fasting blood glucose of rats was measured on days 0, 5, 10, 15 by using a portable glucometer (Bayer's Contour TS). The day zero was the day on which experimentally diabetic rats selected for the experimental design. During the experimental period, the body weights were monitored daily and change in body weight of the rats was estimated as follows:

$$\text{Change in body weight} = \frac{[\text{final body weight} - \text{initial body weight}]}{(\text{initial body weight})} \times 100 \%$$

..... (1)

Determination of Glycosylated or Glycated Hemoglobin (HbA1c) level

Glycated hemoglobin (HbA1c) expressed as a percentage of total blood hemoglobin concentration and HbA1c % was determined in EDTA-blood samples obtained at the end of experiment by cation-exchange method using a diagnostic kit from Biosystems S.A., Barcelona, Spain.

Estimation of Serum biochemical parameters

Serum biochemical parameters like triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein (TP) were estimated by commercially available kits (Span Diagnostic Pvt. Ltd., Surat, India) with the help of Auto analyzer - Selectra ProXS with software version 1.0.X (Vital Scientific BV, Netherlands)

Estimation of liver biochemical parameters

The tissue antioxidant assay was performed with liver homogenate and evaluation was carried out by measuring the level of total protein (Span Diagnostic Pvt. Ltd., Surat, India), lipid peroxidation (LPO) assay and endogenous antioxidant like superoxide dismutase (SOD), catalase (CAT) assay and non-enzymatic antioxidant i.e. reduced glutathione (GSH) assay was done as per Haldar et al., 2011.

Histopathological examination

The histological changes of pancreas by light microscopy (Olympus CH20i, India) were studied by using paraffin method (Kamble et al., 2011; Ogawa et al., 1993).

Statistical analysis

All results were expressed as the mean \pm standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's *post hoc* test of significance. $P < 0.05$ was considered as statistically significant.

RESULTS

Acute Toxicity Study

During the acute toxicity study, the methanolic extract was administered orally and animals were observed for mortality and behavioral responses. No mortality was observed in rats treated with 2000 mg/kg of methanolic extract. All the mice were normal and no gross behavioral changes were observed till the end of the study period.

Oral glucose tolerance test

The blood glucose levels of the normal control, MNR and MNB extract groups (at dose 200 and 400 mg/kg b.w.) were measured after oral administration of glucose (2 g/kg b.w.). All groups had shown increased blood glucose level was observed after 30 min. Its level remained high over next 60 min. MNR (200 and 400 mg/kg) and MNB (400 mg/kg) group showed significant ($p < 0.05$) decrease in blood glucose level at 60 and 120 min compared to the normal control group shown in figure-1. Significant increases in body weight were observed in treatment groups (MNR and MNB) compared diabetic control group. Values are expressed as mean \pm S.E.M. ($n = 6$). * $p < 0.05$, @ $p < 0.01$ when compared with control group at corresponding time.

Antidiabetic activity

Fasting blood glucose level of the treatment group significantly ($p < 0.05$) reduced by the 14th days treatment with MNR and MNB extract, fasting blood glucose level reduced from 5th day. At the end of the experiment (15th day) Fasting blood glucose level was found to be 176.64 \pm 1.50, 144.14 \pm 2.12, 188.52 \pm 1.66 and 161.07 \pm 2.53 mg/dl for MNR 200 and 400 mg/kg, MNB 200 and 400 mg/kg comparable with Glibenclamide 0.5 mg/kg group shown in Table-1. Values are expressed as mean \pm S.E.M. ($n = 6$). ^adiabetic control vs normal group, # $p < 0.01$; ^btreated group vs diabetic control, * $p < 0.05$, ** $p < 0.01$. Treatment with MNR and MNB extract at dose 200 and 400 mg/kg b.w in diabetic induced rats significantly ($p < 0.05$, $p < 0.01$) decreased the triglycerides, cholesterol, LDL cholesterol level with a significant ($p < 0.05$, $p < 0.01$) increase in HDL-cholesterol level in a dose dependent manner as compared to diabetic control group.

DISCUSSION

DM is a metabolic disorder characterized with developed hyperglycemia due to the insufficiency of insulin release from the pancreas, increased oxidative stress, non-enzymatic glycolization, LPO and changed antioxidative defence system after being exposed to free radicals Jang et al., 2005)¹⁷. The evaluation of antidiabetic potential of the methanol extract of stem barks (MNB) and root (MNR) of *Mahonia nepalensis* performed by using the streptozotocin (STZ; 50mg/kg b.w., i.p.) induced NIDDM model on rats. STZ enters into the pancreatic β -cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore, STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic β -cells are destroyed by necrosis (Mythili et al., 2004)¹⁸. STZ induce diabetes is associated with a characteristic loss of body weight due to excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency results in muscle wasting and weight loss in diabetic untreated rats (Kumar et al., 2011)¹⁹. Diabetic rats treated with the MNR and MNB extract showed significant improvement in body weight as compared to the diabetic control animals. American Diabetes Association (ADA) has been recommended that 6.5% HbA1c as the cut point for diagnosing diabetes. HbA1c is formed throughout the circulatory life of red blood cells (RBCs) by the addition of glucose to the N-terminal of the hemoglobin β chain. This nonenzymatic process reflects the average exposure of hemoglobin to glucose over an extended period (Kumar et al., 2011)¹⁹. HbA1c was found to increase in patients with diabetes mellitus due to glycosylation of a number of proteins, haemoglobin, β -crystalline of the eye lens and increased HbA1c level directly proportional to the fasting blood glucose levels (Parveen et al., 2010)²⁰. In the present study shows the MNR and MNB extract treatment group significantly decline in HbA1c level compared to the diabetic control. In the study it was observed that there was an elevated level of serum TG, cholesterol, LDL

cholesterol and decreased levels of HDL cholesterol in diabetic rats. This might be attributed due to lack of insulin which activates the lipase enzymes, hydrolyzing the stored TG and releasing large amounts of fatty acids and glycerol in the circulating blood (Naskar et al., 2011)²¹. High levels of total cholesterol and LDL cholesterol are major risk factors for cardiovascular diseases, whereas increased HDL cholesterol is associated with a decrease in cardiovascular disease risk (Wang et al., 2010)²². The post treatment results for MNR and MNB extract treated diabetic rats has showed decline in serum TG, cholesterol, LDL cholesterol level with marked increase in HDL levels. These results indicate that the MNR and MNB extract has a lipid-lowering effect on the diabetic rats. The liver protein content significantly decreases in the both MNR and MNB treated groups compared to diabetic control group. The normal liver is able to perform its normal function such as the protein metabolism. STZ-induced hepatotoxicity in diabetic rats (Ohaeri, 2001)²³. cause damage in cell membrane, change enzyme activity and finally induce hepatic injury or necrosis, as a result liver is not capable to perform its normal functions where the protein metabolism is affected. Therefore, deamination of amino acids in order to breakdown the protein fails to arise resulting in the protein accumulation in the liver (Mitchell et al., 1973)²⁴. Streptozotocin selectively destroys pancreatic insulin secreting β -cells and causes enhanced level of ROS in pancreas, liver and related tissues. Increased levels of ROS results in tissue damage and enhanced LPO. GSH antioxidant system plays a fundamental role in first line cellular defense against reactive free radicals and other oxidant species (Gupta et al., 2008)²⁵. Cytosolic free radicals are either removed non-enzymatically or by *in vivo* antioxidant enzymes such as superoxide dismutase SOD and CAT. SOD, a metalloprotein, is involved in the antioxidant defense mechanism as the first enzyme by lowering the amount of superoxide radicals ($O_2^{\bullet-}$), which damages the membrane and biological structures. CAT is a hemoprotein, localized in the peroxisomes and induces the decomposition of H_2O_2 to water and oxygen (Gupta et al., 2008)²⁵. In the present study, MNR and MNB extract not only increases the decreased levels of antioxidant enzymes (CAT, SOD, and GSH) in liver but also protects the tissue damage by inhibiting lipid peroxidation (in the terms of MDA) in stressed conditions. In histopathological examination, pancreatic section of the normal rats showed the clear β cellular architecture in islets of Langerhans which having well preserved cytoplasm and nucleolus. But the pancreatic section of STZ intoxicated rats shows irregular, disarrangement and degradation of cells. Necrosis of the cells was found to be very clear. MNR, MNB and Glibenclamide treatment have showed in restoring the altered histopathological changes (Figure 3).

CONCLUSION

From the overall results, it can be concluded that the methanol extract of *Mahonia nepalensis* root (MNR)

exhibited more potent antidiabetic effect than stem bark (MNB) against STZ-induced diabetic rat in a dose-dependent manner, which may be through increasing insulin expression, beta cell regeneration, reducing the elevated levels of the hepatic enzymes by *in vivo* antioxidant enzyme activity and decreasing lipid peroxidation. The use of this plant for diabetes treatment is promising but the precise active substance(s), site(s) and mechanism(s) of its pharmacological effects are still to be determined.

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