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Studies on the hypoglycemic effects of *Murraya paniculata* Linn. extract on alloxan-induced oxidative stress in diabetic and non-diabetic models

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ABSTRACT

Objective: To evaluate the effect of extract of *Murraya paniculata* Linn. (Family – Rutaceae) on blood glucose, cholesterol, triglyceride and lipid level and antioxidant status in alloxan induced diabetic and non-diabetic rats. **Methods:** hydro-alcoholic extract of *M. paniculata* leaves (100, 200 and 400 mg/kg) was administered orally for 14 days and its effect on blood glucose, cholesterol, triglycerides and lipid level were estimated in serum. Liver free radical (lipid peroxidation, LPO) and antioxidant (Super oxide dismutase, SOD; catalase, CAT; and reduced glutathione peroxidase, GPx) were also measured after 14 days treatment with extract. Glucose level in non-diabetic rats was estimated after 21 days treatment with *M. paniculata* extract. **Results:** Oral administrations of *M. paniculata* extract (100, 200 and 400 mg/kg) for 14 days significantly reduced the levels of blood glucose, cholesterol, and triglyceride and lipid level. Liver free radical (LPO) significantly reduced and antioxidants (SOD, CAT and GPx) status significantly increase after 14 days treatment of extract in diabetic rats. *M. paniculata* 200 and 400 mg/kg significantly decrease glucose level in non-diabetic rats after 21 day and caused hypoglycemia in normal rats. **Conclusions:** *M. paniculata* leaves extract posses hypoglycemic effect in oxidative stress condition and also in non-diabetic condition. Hypoglycemic action may be by potentiating of the insulin effect by increasing either the pancreatic secretion of insulin from beta cells of islets of langerhans or its release from the bound form. *M. paniculata* could be a potential source of hypoglycemic agent with antioxidant properties.

1. Introduction

Diabetes mellitus is a chronic metabolic disease affecting a large number of people of all ages, races and socio-economic classes throughout the world. It is caused by either a relative or absolute insulin deficiency with possible impaired tissue responsiveness to insulin, which affects numerous organ systems in the body. About 30 to 33 million Indian people suffering from diabetes mellitus and will go up to 74 million by 2025[1]. Diabetes patients have an increased incidence of vascular disease and it has been suggested that free radical activity increased in diabetes. The increased in oxygen free radicals in diabetes could be due to increase in blood glucose levels, which upon autoxidation generate free radicals[2]. Elevated glucose level cause slow but significant non-enzymetic glycosylation of protein in diabetes[3]. Previous studies confirmed the efficacy of several medicinal plants in the modulation of oxidative stress associated with diabetes mellitus[4]. In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defences. Hence, compound with both hypoglycemic and antioxidative properties would be useful anti-diabetic agents[5]. Investigation on hypoglycemic agent from medicinal plants has become more important after recommendation of WHO on diabetes mellitus. Therefore, search for safe and more effective hypoglycemic agent is an important area of research[1].

Murraya paniculata Linn. (Family – Rutaceae) is commonly known as orange jasmine (Kamini in Hindi). It is distributed over the greater part of India, Andaman Islands to an altitude of 1500 m and inhabitant to tropical Asia from India, Srilanka, Myanmar, southern China and

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Taiwan, Thailand, Australia and Caledonia. The leaves are stimulant and astringent and used in the form of an infusion to treat diarrhoea and dysentery. The powder leaves are applies to cuts to promote healing; there decoction is taken internally to treat dropsy. The leaves and root bark are used to treat rheumatism, coughs and hysteria. The warm leaf paste is applied externally to promote the healing of broken bones among the Paudi Bhuinya in northern Orissa. In the Gandhamardn hills of Orissa, the leaves and twigs are boiled to make a bath that is used to relieve stomach-ache in children and rheumatic pains in adults. Myricetin, Coumarins, murralongin, isomurralonginol isovalerate, murrangatin, murpanidin, coumurrayin, toddalenone, aurapten, toddasin gardenin and umhengerin compounds isolated from the leaves of *M. paniculata*. It is reported to have Anti-nociceptive and anti-inflammatory[6], anti-diarrhoeal[7], oxytocic, anti-fertility and In-vitro antioxidant[8] properties.

In our previous study, we have reported *M. paniculat* leaves extract having no toxicity (acute and sub-acute) on mice and rats and no abnormal symptoms either administered orally cause any mortality[9] and posses anti-hyperglycemic activity in streptozotocin induced diabetic rats[10]. Hence, an attempt has been made in scientifically validated alloxan-induced animal models to investigate leaves of *M. paniculata* against oxidative stress in diabetes condition and also the per se (it-self) effect of the extract on non-diabetic rats.

2. Materials and methods

2.1. Collection of Plant and preparation of extract

The whole plant of *Murraya paniculata* (Family -Ruteceae) was collected from Botanical Garden of National Botanical Research Institute, Lucknow, India in month of August. The freshly collected plant materials of *Murraya paniculata* (MP) were washed with distilled water and air-dried at 30 ± 2 ℃ and dried it in tray drier under the control conditions and powdered. The powdered plant materials (500g) was macerated with petroleum ether to remove fatty substances, the marc was further exhaustively extracted with of 50% ethanol (500 ml ethanol + 500 ml distilled water) for 3 days and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. *M. paniculata* extract (MPE) were stored in refrigerator at -20℃ until further use.

2.2. Qualitative phytochemical screening

50% ethanolic extract of *Murraya paniculata* were subjected to qualitative tests for the identification of various active constituents viz. carbohydrate, glycoside, alkaloid, amino acids, flavanoids, fixed oil, tannins, gum and mucilage, phytosterols according to Kokate (2010)[11].

2.3. Animals

Sprague-Dawley rats (170-200g) of either sex were obtained from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at 26 ± 2 ℃ and relative humidity 44 - 56 %, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and water ad libitum. The food was withdrawn 18-24 h before the experiment. 'Principles of laboratory animal care' (NIH publication no. 82-23, revised 1985) guidelines were followed. Approval from the Animal Ethical Committee was taken prior to the experimental work (Reg. No. 222/2000/CPCSEA).

2.4. Drugs and Chemicals

Glibenclamide and alloxan were purchased from Sigma Chemical Company, St Louis, MO, USA. All the other chemicals and reagents were used of analytical grade.

2.5. Induction of diabetes

Either sex of animals were fasted 14 hr prior to induction, diabetes were induced with a single injection of 4 % alloxan prepared freshly at a dose of 200 mg/kg body weight[12]. Alloxan is producing fatal hypoglycemia as a result of massive pancreatic insulin release. Rats were treated with 5% glucose solution to prevent hypoglycemia for 24 hour[13]. Diabetes was confirmed by the determination of tail vein blood glucose levels on the third day after administration of alloxan. Rats having blood glucose levels greater than 240 mg/dL were considered diabetic and selected for the study.

2.6. Treatment protocol

The vehicles (0.5% Carboxymethyl cellulose), *M. paniculata* extarct (100, 200 and 400 mg/kg) and glibenclamide (0.6 mg/kg) were administered orally using intra gastric tube daily for 14 days, vehicle suspension was prepared in 0.5 % carboxymethyl cellulose and in volume of 1 ml/100 g rat after the induction of diabetes with alloxan. *M. paniculata* extarct was studies on blood glucose level, cholesterol and triglyceride and also studied on liver free radicals (lipid peroxidation, LPO) and antioxidants (Superoxide dismutase, SOD; glutathione peroxidase, GPx and catalase, CAT). In other study, four groups of normal animal (either sex) were selected for per se effect. The vehicles (0.5% CMC), *M. paniculata* extarct (100, 200 and 400 mg/kg) were administered orally and daily for 21 days. Blood glucose level was investigated in normal rat after 21 days treatment with *M. paniculata* extarct 100, 200 and 400 mg/kg dose.

2.7. Analytical procedure

Blood glucose was estimated by enzymatic GOD-POD method by using glucose diagnostic kit (Ranbaxy diagnostic kit; DPEC-GOD/POD). The serum cholesterol (CHOD-PAD), serum triglyceride (GPO-ESPAS), and serum lipid level were measured using a commercial kit (Ranbaxy enzokit).

2.8. Estimation of liver antioxidants and free radicals

The liver tissues were excised, rinsed in ice cold saline and then homogenized in Tris-HCl buffer (pH 7.4). The tissue homogenates were used for the following estimation of lipid peroxidation (LPO) by determined thiobarbituric acid reactive substances (TBRAS). The antioxidant such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activity was assayed by the method of Ramachandran (2011)[14].

2.9. Histopathology

Histopathology of the pancreas was done in all the groups on 15th to know the status of beta cells. A piece of pancreas was removed and fixed in 10% buffered formalin and paraffin embedded. 4-6 μm thick sections were stained with Hemotoxylin and Eosin stain for histological evaluation and examined under microscope at 10 X magnification.

2.10. Statistical analysis

Statistical comparison was performed using either unpaired't' test or one way analysis of variance (ANOVA) and for multiple comparisons versus control group was done by Dunnett's test. All statistical analysis was performed using SPSS statistical version 16.0 software package (SPSS® Inc., USA). *P* value <0.05 were considered statistically significant.

3. Results

3.1. Qualitative phytochemical screening

M. paniculata extarct showed the presence of carbohydrate, proteins & amino acids, phenolic compounds, phytosterol, alkaloids and flavonoids while, fixed oil & fats, saponins and gum & mucilage were absent.

3.2. Effect of M. paniculata leaves extract on glucose level in alloxan-induced diabetic rats

Alloxan significant increase in the blood glucose level at 0 day (95.63-253.93, *P*<0.001), after 7 days the *M. paniculata* extarct 100, 200 mg/kg did not showed the significant

Table 1.
Effect of *M. paniculata* extract on lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in alloxan-diabetic rats

Oral treatment(OD x 14 days)	LPO(nm/mg protein)	SOD(U/mg protein)	CAT(U/mg protein)	GPx(mm/g tissue)
Normal control	0.43 ± 0.01	109.45 ± 5.14	61.67 ± 1.43	3.33 ± 0.15
Diabetic control	0.57 ± 0.02***	69.32 ± 4.21***	34.62 ± 1.32***	1.20 ± 0.13***
Diabetic + *M. paniculata* 100 mg/kg	0.53 ± 0.02[a]	80.43 ± 3.82	36.17 ± 1.65	1.51 ± 0.19
Diabetic + *M. paniculata* 200 mg/kg	0.50 ± 0.02[b]	92.38 ± 3.12[a]	51.50 ± 1.52[c]	1.85 ± 0.16[a]
Diabetic + *M. paniculata* 400 mg/kg	0.45 ± 0.01[c]	109.31 ± 2.97[c]	59.18 ± 1.09[c]	2.12 ± 0.19[b]
Diabetic + Glibenclamide 0.6 mg/kg	0.42 ± 0.02[c]	105.25 ± 4.22[c]	60.28 ± 1.18[c]	2.96 ± 0.18[c]

Values are expressed as Mean ± SEM (n=6), *P* value: ***<0.001 compared with respective control group, *P* value: [a]<0.05, [b]<0.01, [c]<0.001 compared with respective diabetic control group

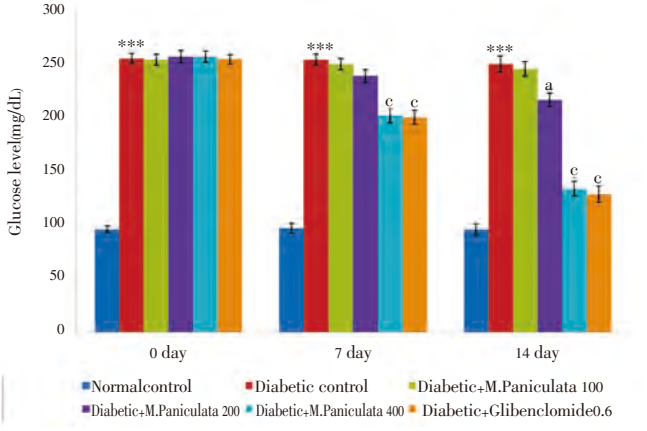

Figure 1. Effect of *M. paniculata* extract on glucose level in alloxan-induced diabetic rats. Values are expressed as Mean ± SEM (n=6), *P* value: ***<0.001 compared with respective normal control group, *P* value: a<0.05, c<0.001 compared with respective diabetic control group

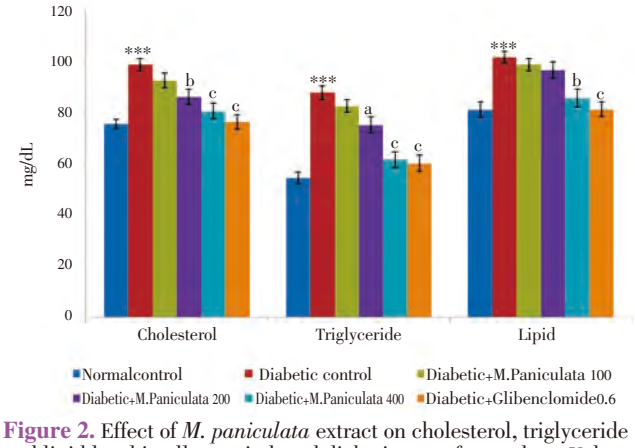

Figure 2. Effect of *M. paniculata* extract on cholesterol, triglyceride and lipid level in alloxan-induced diabetic rats after 14 days. Values are expressed as Mean ± SEM (n=6), *P* value: ***<0.001 compared with respective normal control group (NC), *P* value: a<0.05, b<0.01, c<0.001 compared with respective diabetic control group

reduction in the diabetes but at the dose of 400 mg/kg and glibenclamide showed the significantly decrease with respect to diabetic control group (P<0.001, P<0.001). After 14 days the treatment showed the significant decrease with respect to diabetic control group at a dose of 200 and 400 mg/kg (P<0.05, P<0.001) and glibenclamide also showed the significant decrease in diabetes (Figure 1).

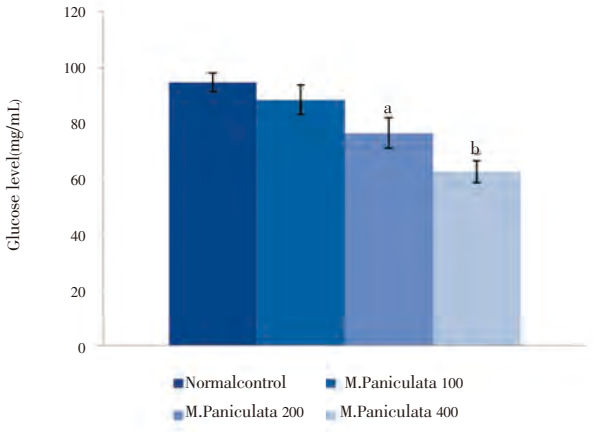

Figure 3. Effect of *M. paniculata* extract on glucose level in normal rats after 21 days treatment. Values are expressed as Mean ± SEM (n=6), P value: a<0.05, b<0.01compared with respective normal control group

3.3. Effect of M. paniculata leaves extract on cholesterol, triglyceride and lipid level in alloxan-induced diabetic rats

Alloxan caused significant elevation of serum markers and alloxan treated group increase in level of cholesterol (P<0.001), triglyceride (P<0.001) and lipid (P<0.001). Treatment with *M. paniculata* extarct 100, 200 and 400 mg/kg once daily for 14 days prevented the diabetes in a dose dependent manner (Figure 2).

3.4. Effect of M. paniculata leaves extract on LPO, CAT, SOD and GPx in alloxan-induced diabetic rats

Alloxan significant elevation in LPO (P<0.001) compared with respective diabetic group, the *M. paniculata* extarct 100, 200 and 400 mg/kg decrease the LPO level (P<0.05, P<0.01, P<0.001), SOD, CAT, GPx significantly decreased by alloxan, *M. paniculata* extarct 100, 200 and 400 mg/kg increased the level of SOD (P<0.01, P<0.001), CAT (P<0.001), GPx (P<0.05, P<0.01, P<0.001). Standard drug glibenclamide decreased significantly SOD (70.24-105.70, P<0.001), CAT (36.63-65.21, P<0.001), GPx (1.24-4.10) compared to respective diabetic group (Table 1).

3.5. Effect of M. paniculata leaves extract on glucose level in non-diabetic rats

M. paniculata extarct 200 and 400 mg/kg was significantly decrease the glucose level (94.78-62.52, P<0.01, P<0.001) compared to respective normal control group (Figure 3) and not significant difference seen on body weight (188.7-214.8).

3.6. Histopathology

Pancreatic section of normal rat showed normal morphology of beta cells with well preserved cytoplasm

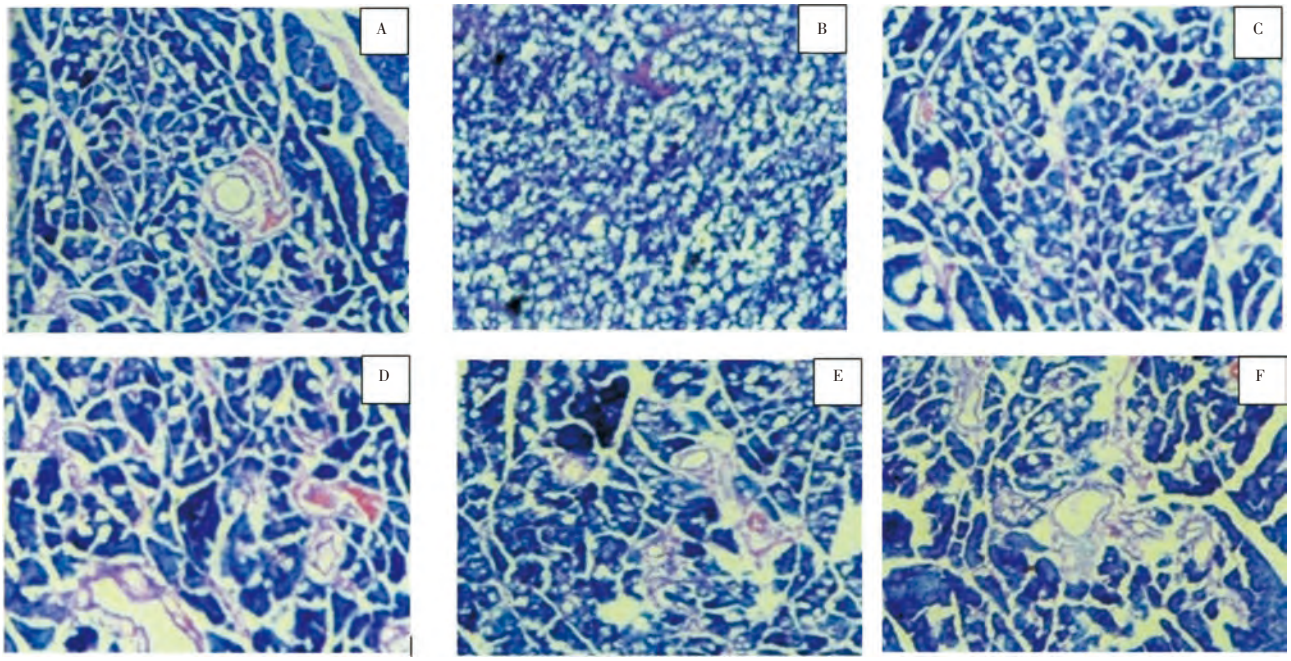

Figure 4. Histological of rat pancreas (10 x magnifications)
A: Pancreatic section of normal rat showing normal morphology of cells with well preserved cytoplasm and nucleus. B: Diabetic control rats pancreas section showing damaged islets cells in irregular form, not well defined and defect in cell membrane and also necrosis of the cells. C, D and E: *M. paniculata* extract 100, 200 and 400 mg/kg respectively showing improvement in cell structure, cell membrane and nucleus. F: Standard drug glibenclamide 0.6 mg/kg treated pancreas showing near to normal islets, cell membrane and nucleus in the pancreas.

and nucleus. Diabetic control rat pancreas section showed damaged islets cells in irregular form, not well defined and defects in cell membrane and also necrosis of the cells. *M. paniculata* extract 100, 200 and 400 mg/kg respectively showed improvement in beta cell structure, cell membrane and nucleus in pancreas. Treatment with standard drug glibenclamide 0.6 mg/kg showed near to normal islets, cell membrane and nucleus in the pancreas.

4. Discussion

Alloxan is one of the standard substances used for the induction of diabetes mellitus and it has a destructive effect on the beta cells of the pancreas[15]. In our study, significant increase in blood glucose level was observed in alloxan induced diabetic rats. Alloxan cause immense reduction in insulin release by the destruction of beta cells of the islets of langerhans and caused hyperglycemia. The adipose tissue and skeletal muscle are unable to uptake glucose from serum in the absence of insulin so glucose conversion to fat and glycogen is blocked in the adipose tissue and skeletal muscles, resulting in increase of blood glucose level[1].

The oral administration of *M. paniculata* extract decreased the glucose level significantly after 14 days. At the end of study the 100, 200 and 400 mg/kg *M. paniculata* extract showed the significant decrease in the glucose level in dose dependent manner. *M. paniculata* extract 200 and 400 mg/kg also decrease the glucose in non-diabetic rats after 21 days treatment. Oral hypoglycemic agents like sulfonylures (glibenclamide) act by stimulating insulin release from pancreatic beta cells and sensitize the target tissue to insulin by increasing number of insulin receptors or improving translation of receptor activation. The possible mechanism of *M. paniculata* extract bring about its hypoglycemic action may be by potentiating of the insulin effect by increasing either the pancreatic secretion of insulin from beta cells of islets of langerhans or its release from the bound form. In this context a number of other plants have also been observed to have hypoglycemic and insulin release stimulatory effects[16].

Alloxan-induced diabetic rats showed significant hypercholesterolemia as compared with control. Treatment with *M. paniculata* extract showed a significant decrease in cholesterol levels. Hypercholesterolemia was associated with hypertriglyceridemia as compared with control animals. Hypertriglyceridemia and lipid level was also significantly prevented by treatment with *M. paniculata*. Insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory process, which in turns leads to accumulation of lipids such as cholesterol and triglyceride in diabetic patients. The levels of serum lipids are frequently elevated in diabetes mellitus and such elevation responsible for diabetes complications. High level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones in adipose tissue mainly due to the action of insulin. Normally insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic condition lipoprotein lipase is not activated due to insulin deficiency consequential in hypertriglyceridaemia. Insulin deficiency may responsible for dyslipidaemia because insulin has an inhibitory action on HMG-CoA reductase. It is key rate limiting enzyme responsible for the metabolism of cholesterol-rich LDL[17].

Persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species (ROS) from glucose auto-oxidation and protein glycosylation. The increase in the level of ROS in diabetes could be due to their decreased production of non-enzymatic and enzymatic catalase (CAT), reduced glutathione (GPx), and superoxide dismutase (SOD) antioxidants and level of these antioxidant enzymes critically influences the inclination of various tissues to oxidative stress in diabetes[5]. Low level of lipoxigenase peroxide stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases it may initiate uncontrolled lipid peroxidation (LPO) leading to cellular infiltration and islet cell damage in diabetes. The activity of GPx was observed to decrease significantly in diabetic rats and the depletion in the activity may result in the evolvement of deleterious oxidative changes due to accumulation of toxic product. SOD and CAT are two major scavenging enzymes that remove the toxic free radical. Reduced activities of SOD and CAT in liver have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals[18]. Administration of *M. paniculata* 100, 200 and 400 mg/kg and glibenclamide decreased LPO that is associated with increased activity of SOD, GPx and CAT in diabetic rats. In our previous study *M. paniculata* having in-vitro antioxidant properties may be due to presence of phytochemical compounds[8].

M. paniculata extract revealed the presence of carbohydrate, proteins & amino acids, phenolic compounds, phytosterol, alkaloids and flavonoids. Alkeloids, flavonoids and phenolic compounds having properties of good antioxidant[19, 20].

Histopathological study of diabetic rats showed degradation of pancreatic islet cells and decrease insulin level. Insulin deficiency causes excessive elevation of blood glucose leading to hyperglycemia[21]. The histopathological study of diabetic treated group *M. paniculata* 100, 200 and 400 mg/kg indicate increased number of islets and increased percentage of beta cells, in the diabetic rats that received the extracts, which may be a sign of regeneration. Signs of regeneration of beta cells and increase of insulin secretion from surviving beta cells of the islets of langerhans and decrease of blood glucose have been reported of some plant extracts[22]. *M. paniculata* leaves extarct may have some chemical components that exert regenerative effects on beta cells, stimulate these cells to produce more insulin or may

have some insulin like substances.

From the present study we concluded that, *M. paniculata* leaves extract posses hypoglycemic effect in oxidative stress condition and also in non-diabetic condition. Hypoglycemic action may be by potentiating of the insulin effect by increasing either the pancreatic secretion of insulin from beta cells of islets of langerhans or its release from the bound form. *M. paniculata* could be a potential source of hypoglycemic agent with antioxidant properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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