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Antidiabetic Activity and Biochemical Parameter Estimation of *Morus nigra* in SD Rat

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Abstract:

The prevalence of diabetes mellitus is rising quickly globally. We picked hydro-alcoholic leaf extract of *Morus nigra* (black mulberry) have antidiabetic action on sprague dawley male rats (SD rats). When used as a positive control, the medication metformin improves glucose tolerance by 14.6% in normal rats when given 300 mg/kg body weight, compared to 8.3% in *M. nigra*. Metformin lowers blood sugar levels by 21.9% when given at 300 mg/kg b. wt to streptozotocin (STZ) induced diabetic rats, *Morus nigra* lowers blood sugar levels by 9.9%. High-density lipoprotein levels increased in diabetic rats when *Morus nigra* hydro-alcoholic leaves extract was administered, while biochemical parameters like total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), creatinine, serum glutamate phosphotransferase (SGPT), urea, and serum glutamate oxaloacetic acid transferase (SGOT) decreased. Overall, this species of Morus has potency to provide antidiabetic compound.

Keyword: *Diabetes, Morus nigra, SD rats, Metformin, STZ, Biochemical parameters*

INTRODUCTION

Diabetes mellitus, which is currently characterised by extreme exhaustion, weight loss, blurred vision, polydipsia, and polyurea, is an endocrine metabolic illness of the pancreas. Diabetes patients' blood glucose levels rise because they are unable to release enough insulin into their bodies[1]. India is a diabetic nation of the world because it has more than 25 million people affected by diabetes mellitus. Several different plant species have been found to have anti-diabetic properties, according to a literature review. Despite the fact that there are many anti-diabetic medications on the market created by the pharmaceutical industry, diabetes and the issues it causes continue to pose serious health risks[2]. A variety of herbal plants can be employed as anti-hyperlipidemic and anti-diabetic medications to treat diabetes mellitus. Ancient Indian texts on the Ayurvedic medical system, including Charak Samhita, Sushrut Samhita, and Astang Hriday, reference numerous medicinal plants for treating diabetes mellitus in either compound or single formulations. *Morus nigra*, is a member of the moraceae family and is used to treat diabetes and its consequences. In sericulture, Bombyx mori, a silkworm, is primarily fed on this plant to produce silk. It can be used to treat vitiated situations involving pitta, vata, and burning[3]. This plant can be utilized as a laxative, brain tonic antibiotic and diuretic. Weight loss results from the breakdown of stored proteins and lipids brought on by insufficient insulin secretion from pancreatic beta cells. Diabetes is characterised by metabolic acidosis, which is brought on by an excessive production of ketone bodies[4].

Plants produce a variety of active phytoconstituents with a range of medicinal benefits. Numerous plants have been used for their antidiabetic properties through a variety of mechanisms, including lowering hepatic gluconeogenesis, inhibiting glucose-6-phosphatase in addition to fructose-1, 6-biphosphatase in the liver, reducing intestinal glucose absorption, raising plasma insulin levels, and enhancing glucose tolerance[5]. Because of an increase in insulin release from pancreatic beta cells, hydro-alcoholic extract of *Morus nigra* leaves demonstrated considerable antidiabetic action in the current study. This implies that the *Morus nigra* leaf extract (hydro-alcoholic) has enormous potency for the development of innovative anti-diabetic medications.

MATERIALS AND METHODS

Collection and Authentication of Plant Material:

Plant material has been collected from IET campus Lucknow, in the month of March and authenticated by CSIR-NBRI Lucknow, Uttar Pradesh as *Morus nigra* (LWG -100983).

Hydro-alcoholic Extract Preparation:

The *Morus nigra* leaves were cleaned with water, dried in the shade, and then ground with a mechanical pulverizer[6]. For the extraction of the crushed material, a hydro-alcoholic solution (60 percent distilled water + 40 percent ethanol) was employed. A thimble of the Soxhlet apparatus was used to pack 50 g of powdered drug, which was extracted at 40ºC in an alcoholic solution. The extraction process was place over the course of 48 hours, or 20 cycles, until the solvent in the soxhlet syphon tube became colorles[7-8]. The filtered extracts were dried using a rotary evaporator, and they were kept chilled[9].

Acute Toxicity Study in SD Rats:

For the evaluation of acute toxicity, normal, healthy SD rats aged 7-8 weeks and weighing 160–20 g was used[10-11]. The medicine was given orally to fasted healthy rats to estimate the toxicity of M. nigra leaf extract (hydro-alcoholic). Three SD male rats were placed in each of the five groups, which were divided into SD rats. Extract was given orally in doses ranging from 2500 mg to 2000 mg

to 1000 mg to 500 mg per kilogramme of body weight[12]. Three other rats were given only distilled water, and this group is regarded as the control. After 24 hours and for a total of 14 days, the SD male rats were regularly watched. No mortality or toxicity was noticed over the course of the 14-day period[13].

In-Vivo Anti-diabetic Activity in SD Rats:

Oral glucose tolerance test (OGTT) in normal SD rats:

SD rats that were between 160 and 20 grams in weight and around two months old were utilised for the research of the oral glucose tolerance test. Experimental animals were housed in polypropylene cages that maintained 50-60% humidity, a temperature of 23–20°C, and a light intensity of 300 lux with a 12h light/dark cycle. Test animals are given unlimited access to drinking water and a standard pellet diet[14-15]. Male SD rats were divided into three groups after a six-hour fast. There are 5 male rats each group. The standard medication and plant sample were dissolved using gum acacia. The standard medicine and plant sample were produced in 1.0% gum acacia at a dose of 300 mg/kg body weight each[16]. Orally ingested suspensions of plant extract and common medication were given to SD male rats. The control group's experimental animals were fed a same amount of gum acacia, which has 1.0% gum content. Each group of rats received a 2 gm/kg vehicle glucose load or 2 gm/kg plant sample orally after 30 minutes. Each animal's blood glucose level was measured before and after glucose delivery at intervals of 0, 0.25, 0.5, 1, 1.5, and 2 hours. Measurements of the blood glucose levels of SD male rats were made using a glucometer. Rats were given only water throughout the experiment[17].

By graphing the relationship between blood glucose level and time (from 0 to 120 minutes), the decrease of blood glucose levels in rats was assessed. The x-axis represents time, while the y-axis represents the blood glucose profile. Every group's area under the curve has been estimated using Prism software. By comparing the percentage decrease in area under the curve of the plant sample and metformin of the controlled group, it was possible to determine the percentage decrease in blood glucose profile[18].

Group design for OGTT in rats:

Group1: Normal control received with 1.0% gum acacia (vehicle)

Group2: Positive control given along with metformin (standard drug)

Group3: *Morus nigra* delivered orally

Study of Anti-diabetic Activity in STZ-Induced Diabetic SD Rats:

For the investigation, two-month-old SD male rats weighing 160-20 grammes were used (figure.1). Polypropylene cages were used to maintain the environmental conditions for the experimental animals, which were kept at 23°C, 50-60% humidity, and 300 lux of light intensity with a 12h light/dark cycle. Ad libitum feedings of drinking water and a typical pellet meal were given to experimental rats[19]. Streptozotocin (STZ) was intraperitoneally injected into overnight fasted rats to induce diabetes. After two days, the blood glucose level was tested using a glucometer to confirm diabetes. Selecting the rat tail and using a needle to obtain a blood sample[20]. Male SD rats are classified as diabetic if their blood glucose level is greater than 280 mg/dl. The four groups each contain four male diabetic SD rats[21-22].

The standard medication and plant sample were prepared in 1.0% gum acacia at a dose of 300 mg/kg body weight. The carrier for dissolving the sample was gum acacia. Each group's SD rats received oral suspensions of metformin and a *Morus nigra* extract[23-24].

Rats in the control group received the same dosage of gum acacia (1.0%). Every male rat's blood glucose level was measured at various time intervals, including 0, 15, 30, 60, 90, 120, 180, 240, and 300 minutes after glucose delivery. Glucostrips from a glucometer were used to measure the blood glucose levels. Rats used in the experiment were only given water during the procedure. With the use of profile between time (0-5 hrs) and glucose level, the improvement in glucose level has been explored. By comparing the percent drop in the area under the curve of the plant sample and the standard medicine in the controlled group, the percent decrease was assessed[25].

Group Design for Streptozotocin (STZ)-Induced Diabetic SD Rats:

Group 0: Normal control group (distilled water)

Group 1: Gum acacia (1.0%) was given to diabetic control group on the second day following their streptozotocin injection.

Group 2: Following the second day of streptozotocin injection, a positive control drug is given.

Group 3: Morus nigra was given orally after receiving streptozotocin on the second day.

Study of Biochemical Parameters:

Up to the conclusion of testing, SD rats were permitted to continue eating what was prescribed for them. After the testing was completed, 2-4 ml of blood was taken from each rat that had been fasting the entire night[26]. Under ether anesthesia, a glass capillary tube was used to draw blood samples from each rat's retro-orbital venous plexus. Blood is allowed to coagulate at room temperature before being centrifuged for 10 minutes at 2,500 rpm to separate the serum. According to established procedures, a number of biochemical parameters were assessed using separated serum [27-29].

The following metabolic traits in male SD rats were examined[30]:

Serum lipid profile:

The research of the blood lipid profile has taken into account the levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol.

Tests of liver function:

Liver function tests are analyzed by SGOT and SGPT tests.

Tests of kidney function:
This section includes the assessment of creatinine and urea.

Statistical Analysis:
All of the data were evaluated using ANOVA, and the outcomes are shown as average standard deviation. P value (0.05) has been used for testing of significance of data.

RESULTS AND DISCUSSION

Acute Toxicity Study of *Morus nigra*:
Normal, healthy SD male rats were treated with plant drugs up to a dose level of 2500 mg/kg body weight without exhibiting any negative side effects. Did not exhibit any signs of death during the 14 days monitoring period (Table 1).

Study of In-vivo Antidiabetic Activity in rats:
Effect of plant extract on OGTT in SD rats:
Every rat had its blood glucose levels checked at intervals of 0.25, 0.5, 1, and 1.5 hours after receiving the test sample. At a dose level of 300 mg/kg b. wt., the test sample (*Morus nigra*) treatment significantly increased the tolerance of glucose in normal rats (Fig. 1A). The area under the curve (0-120 minutes) in SD rats (normal) after glucose treatment shows a considerable improvement in glucose tolerance (Fig. 1B). The common medication metformin, which increases glucose tolerance by 14.6%, was utilised as a positive control. For *Morus nigra*, the percentage improvement in glucose tolerance was estimated to be 8.3% at a treatment level of 300 mg/kg body weight under the same experimental circumstances.

Evaluation of antidiabetic activity in STZ-induced diabetic rats:
When streptozotocin (STZ) is administered to SD male rats, hyperglycemia is induced. At a dose level of 300 mg/kg body weight, a plant sample in hyperglycemic animals caused a time-dependent drop in the blood glucose level of male SD rats (Fig.1C). After test and standard dosing, area under the curve (AUC0-300min) shows a considerable drop in blood glucose levels (Fig. 1D). After 300 minutes of therapy, the percentage drop in glucose levels in the blood of rats for *Morus nigra* was estimated to be around 9.9. When employed as a positive control, metformin reduces the glucose profile by 21.9% in male sprague dawley rats (SD) at a dose of 300 mg/kg b.wt. under the same experimental circumstances. By comparing the data, anti-hyperglycemic activity has been calculated.

Evaluation of biochemical parameters:
Triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), serum glutamate oxaloacetic acid transferase (SGOT), creatinine, urea, and serum glutamate phosphotransferase (SGPT) are biochemical parameters that have significantly changed. These changes were noted and visualised through (Fig. 2 A,B,C,D) & (Fig. 3 A,B,C,D).
Total cholesterol (139.85±2.25), triglycerides (168.10±7.45), serum glutamate phosphotransferase (170.72±9.46), creatinine (0.65±0.04), SGOT (290.1±7.31), , LDL (97.27±3.92), urea (33.2±1.70) have been observed considerably increased in streptozotocin induced diabetic SD male rats as compare to non-diabetic rats, although HDL (15.62±4.49) was considerably reduced in diabetic male rats (streptozotocin induced).
Significant decrease in TC by *Morus nigra* was up to (123.97±2.43) although by standard drug up to (73.25±8.14), reduction in TG by *Morus nigra* was up to (124.07±3.25) although by standard drug up to (102.37±7.58), reduction in LDL by *Morus nigra* was up to (78.32±3.91) although by standard drug up to (61.22±4.80), reduction in SGPT by *Morus nigra* was up to (126.55±3.36) although by standard drug up to (114.07±4.52), reduction in SGOT by *Morus nigra* was up to (127.42±2.28) although by standard drug up to (161.40±3.10), reduction in urea by *Morus nigra* was up to (23.72±2.18) although by standard drug up to (18.10±4.26), reduction in creatinine by *Morus nigra* was up to (0.44±0.01) although by standard drug up to (0.41±0.02). HDL was significantly increased by metformin up to (24.47±3.15) while *by Morus nigra* it was found up to (20.2±1.56).

Table 1: Acute toxicity analysis in SD rats

Groups	Treatment	No. of SD Rats	Doses of drugs	No. of dead animals
Group I	*Morus nigra*	3 rats	300 mg/ kg body weight	Nil
Group II		3 rats	500 mg/ kg body weight	Nil
Group III		3 rats	1000 mg/ kg body weight	Nil
Group IV		3 rats	2000 mg/ kg body weight	Nil
Group V		3 rats	2500 mg/ kg body weight	Nil

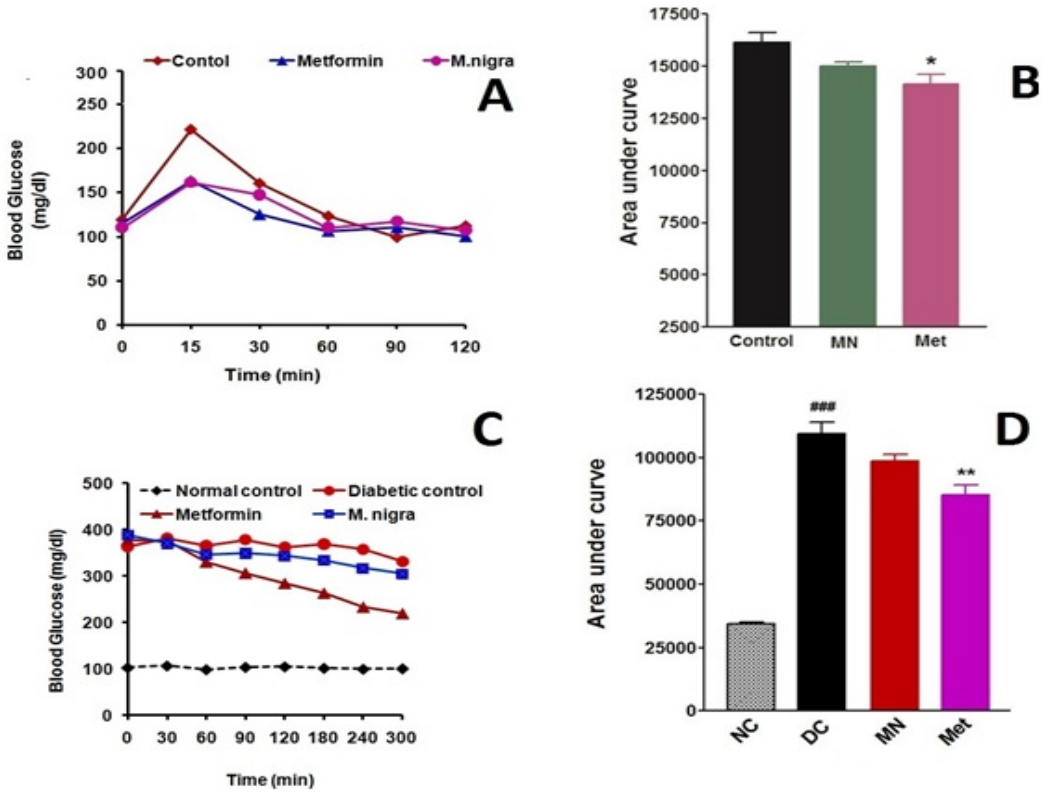

Fig.1: Effect of test sample *Morus nigra* on (A) tolerance level of glucose in normal rats (B) Administration of glucose in normal rats AUC $_{(0\text{-}120\ Min)}$ (C) Glucose lowering efficacy of plant sample in STZ-induced diabetic rats (D) AUC $_{(0\text{-}5\ hrs)}$ in diabetic rats (STZ-induced). Major improvement in glucose level was examined by ANOVA and their related outcome are represented as mean ± standard deviation. ###p<0.001, **p<0.01, *P < 0.05 vs control

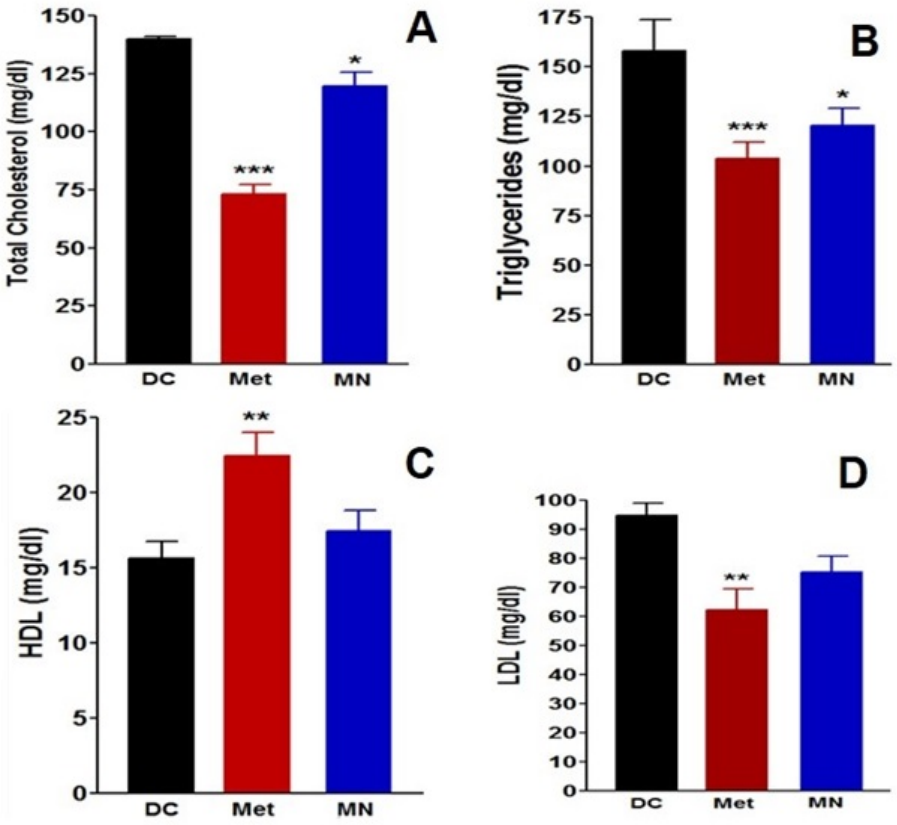

Fig.2: Effect of *Morus nigra* leaves extract (hydro-alcoholic) (A) Total cholesterol (B) Triglycerides (C) High-density lipoprotein (D) low-density lipoprotein in SD rats (diabetic)

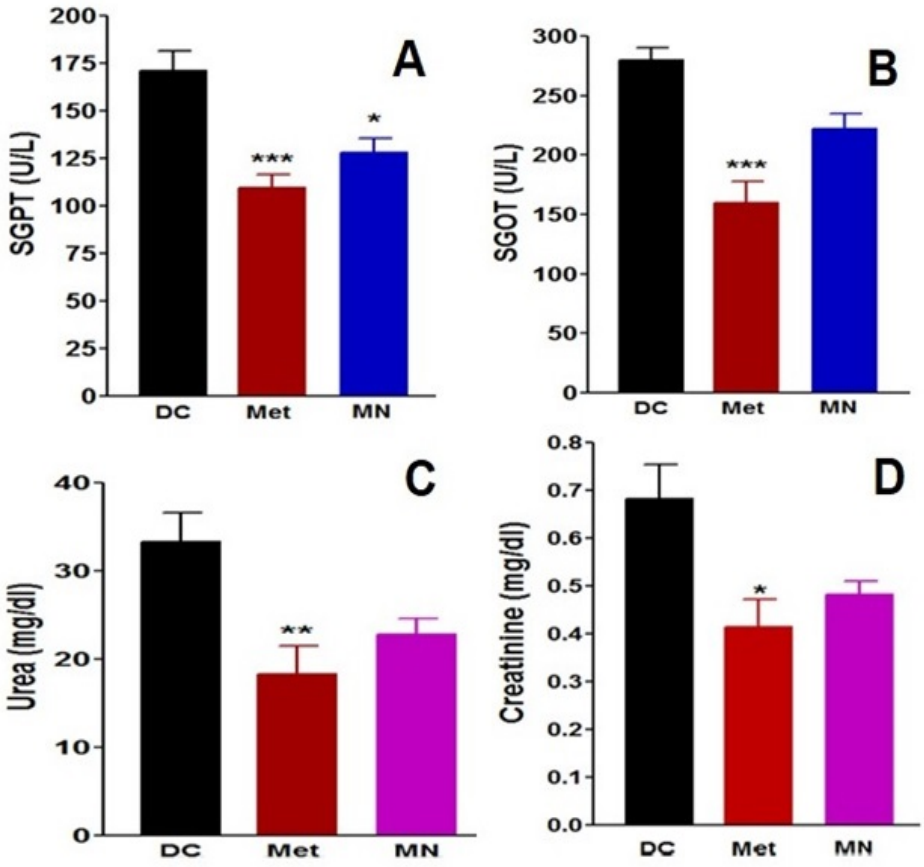

Fig.3: Effect of *Morus nigra* leaves extract (hydro-alcoholic) (A) SGPT (B) SGOT (C) Urea and (D) Creatinine. Results are represented as n = 4, mean (average) ± standard deviation (S.D), these values ***p<0.001, **p<0.01, *p<0.05 have been compared with control group (diabetic).

CONCLUSIONS

In conclusion, this article has described the inquiry into the medicinal qualities of *Morus nigra*. We discovered this plant's high antidiabetic activity with the aid of animal tests. Lever function testing, kidney function testing, and serum lipid profile testing have all led to a modest improvement in biochemical markers. Due to an increase in the insulin release from pancreatic -cells, the *Morus nigra* leaves extract (hydro-alcoholic), used in the current study demonstrated considerable antidiabetic action in SD rat models. This shows that *Morus nigra* hydro-alcoholic leaf extract has a significant deal of potential for the development of innovative anti-diabetic medications to treat this terrible disease.'

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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