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Extraction, Isolation And Evaluation Of Anti-Diabetic Activity Of Morus Alba

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Abstract:

A member of the Moraceae family is the mulberry, or Morus Alba L. Numerous secondary metabolites have been discovered from Morus Alba Linn, including triterpenoids, flavonol glycosides, anthocyanins, and steroids. The purpose of this study is to evaluate the extract of Morus Alba L. in mice model for potential anti-diabetic effects and preliminary phytochemical screening. Although there is growing evidence regarding the mulberry branch's (MB) ability to prevent diabetes, the main active ingredient in this action is yet unknown. The streptozotocin-induced diabetic ICR mouse was given oral MB ethanol (EtOH) extracts [0.5 or 1 g/ kg body weight (BW)] once a day for 22 days. This drastically decreased the diabetic control mouse's plasma glucose level and fasting blood levels in a dose-dependent manner. Oxyresveratrol [ORT, 0.6 g/kg BW], a primary constituent of MB EtOH extracts, was administered to diabetic ICR mice, which also markedly lowered their fasting plasma glucose level. Moreover, ORT raised the transcription of the hepatic glucose transporter 2 and the amount of glycogen. There was no difference in intestinal disaccharidase activity or plasma insulin levels between the diabetes control and ORT groups. This implies that ORT decreased plasma glucose by promoting the liver's absorption of glucose and storage of glycogen. MB EtOH extracts and ORT may be useful as complementary treatments for the treatment of diabetes.

Keywords: Morus Alba L., Mulberry branch, Antidiabetic, Streptozotocin, Etc.

Introduction

Globally, the prevalence of diabetes mellitus (DM) is rising gradually. 382 million people were diagnosed with DM in 2013, and by 2035, that number is predicted to increase to 592 million [1]. Insulin resistance characterizes the pathogenesis of type 2 diabetes (T2DM), while absolute failure of insulin production resulting from pancreatic damage characterizes type 1 diabetes (T1DM). Serious consequences from chronic diabetes mellitus can include both macro- and microvascular problems, including retinopathy, neuropathy, and nephropathy, as well as atherosclerosis and hypertension in the latter case [2]. Therefore, achieving nearly normal blood glucose levels through effective control is the main goal of diabetes care. Insulin therapy is necessary for T1DM patients, while oral hypoglycemic medications are occasionally used as an adjuvant. As of right now, the Food and Drug Administration has only licensed metformin (Met) as an oral hypoglycemic medication for juvenile type 1 diabetes in the US [3]. Nevertheless, it's unclear if treating Met and insulin together is effective. In addition to food control and exercise, biguanides such metformin, sulfonylureas, α-glucosidase inhibitors, and thiazolidinediones are routinely used in the therapy of type 2 diabetes [4]. Nevertheless, a number of adverse effects, including hypoglycemia, decreased ability to secrete insulin, upset stomach, and damage of the kidneys and liver, have been linked to the currently approved medications [4]. Furthermore, the use of several medications has been restricted because they have been linked to severe hepatotoxicity or adverse cardiovascular consequences. As a result, a great deal of research has been done recently on the potential therapeutic uses of phytochemicals derived from plants that have few adverse effects.

The mulberry, or Morus Alba L., is a member of the Moraceae family and is found throughout East Asia, primarily in China, Korea, and Japan [5]. This plant's entire life has been utilized for thousands of years in traditional Chinese medicine. Its roots, leaves, twigs, and fruits are all listed in the Compendium of Materia Medica, a well-known Chinese medical encyclopedia that was penned by Shizhen Li during the Ming dynasty [6]. Modern experimental technologies have confirmed the pharmacological actions of M. Alba, which include anti-diabetic, anti-oxidative, anti-bacterial, anti-inflammatory, anti-atherogenic, and immunological enhancing capabilities [7].

M. Alba is primarily composed of flavonoids, polyphenols, anthocyanin's, terpenes, carotenoids, and alkaloid-type metabolites such kuwanon G, morusin, and 1-deoxynojirimycin, according to earlier photochemistry investigations [8]. Research is still needed on a large number of unidentified metabolites in addition to these well-known molecules.

Conventional Chinese and Korean medicine has traditionally utilized the fruits, leaves, branches, and root barks of the mulberry (Morus Alba L.) to mitigate diabetes. The main active ingredients and mechanism of action of the sericulture

products have been determined by recent investigations. Mulberry branch (MB) in particular has been shown to have notable hypoglycemic effects both in vivo and in vitro. That's why, in this work, we examined in STZ-induced diabetic mice the anti-diabetic potential of MB EtOH extracts and its main active ingredient, Oxyresveratrol (ORT).

Material and Methods

Plant materials and chemicals
Plant Herbarium was submitted and approved by Botany Department of RTM University, Nagpur. The ethanol has procured by Merck HPLC grade solvents were used for all HPLC analyses. Analytical grade reagents were all that were employed in this investigation.

Preparation of ethanol extracts of MB (Mulberry Branch) and Oxyresveratrol
Two kilograms of dried MB were chopped into tiny pieces and refluxed twice in four liters of 60% EtOH for two hours. At 50 oC, the EtOH solution was filtered, allowed to cool, and then allowed to evaporate under low pressure. After being dissolved in the same EtOH solution, the crude EtOH extract was allowed to stand at room temperature for the entire night. The top layer was evaporated in a vacuum to produce an EtOH extract of MB with a yield of 7.4 ± 0.3%. By using a variety of separation techniques, such as solvent fractionation and the previously described silica-gel, ODS-A, and Sephadex LH-20 column chromatographies, ORT and MSA were extracted from the mulberry (M. Alba L.) twig Ethanolic extract.

Determination of ash value and Total Ash Value
Ash value is important in identification of quality & purity of Morus Alba L. 2 gm of completely dried powder of Mulberry Branch was weighed and added in pre weighed silica crucible and burned at room temperature not more than 450^{o} until it become free from carbon. It was determined by cooling the silica dish in desiccator. This process was performed again and again till it gives fixed weight. Total ash percentage was determined by consideration with weight of initial powder of Mulberry Branch.

% Total ash value = Wt. of total ash ÷ Wt. of crude drug taken × 100
Water soluble Ash
Method
% Water soluble ash value = Wt. of total ash - Wt. of water insoluble ash ÷ Wt. of crude drug taken × 100

Acid-insoluble Ash
Method
% Acid insoluble ash value= Wt. of acid insoluble ash ÷ Wt. of crude drug taken × 100

Determination of Loss on Drying or Moisture content
The shallow glass-stoppered weighing bottle was dried and weighed. 2gm crude drug was added in the bottle and closed, the weight was taken and crude drug was spread evenly to a height not more than 10mm. Then the bottle was kept in the oven for drying keeping open without the stopper. The sample was dried to constant weight. It was cooled to normal temperature in desiccator again weighed loss on drying was calculated in percent w/w (Indian Pharmacopoeia 1996).

$$\% \text{ Loss on Drying} = \text{Loss in weightof the sample} \div \text{weight of the sample} \times 100$$

Preliminary phytochemical screening of different extracts of Mulberry Branch (Morus Alba L.) [9,10]
The qualitative phytochemical evaluation of the chloroform, ethyl acetate and Ethanolic extracts of Morus Alba L were performed to detect different chemical constituents. The different reagents and tests were used to detect various secondary metabolites.

Extraction and isolation
After being air-dried for 750 g, the bark was extracted using 95% MeOH (6 x 500 mL) using cold percolation, and it was then steeped for four days at 30 ± 2°C ambient temperature. The mixture was periodically agitated before being filtered through Whatman No. 1. For total solvent removal, the MeOH extract was filtered and condensed to dryness using a rotary evaporator operating at decreased pressure and 45 oC. Subsequently, the 30.4 g of dried MeOH extract was fractionated using petroleum ether in a straightforward column chromatography filled with column-grade silica gel. 5 percent aqueous hydrochloric acid in 50 milliliters was used to wash the petroleum ether extract. After adding Na2CO3 to the aqueous layer to get it basic to pH 10, chloroform (5 x 100 mL) was used to extract it. The amorphous material (11.50 g) obtained from the chloroform extract was dried in a vacuum and then put through silica gel column

chromatography (230 - 400 mesh, 10 × 40 cm) with 500 mL of ethyl acetate as eluents. This produced (1) (7.15 g, 62 %), which produced a single spot on TLC; Rf 0.85.

Quantification of MSA and ORT by HPLC

Following a dilution and filtration process using a 0.45 lm membrane filter (Whatmann, Maidstone, England), the 1.0 g of MB's EtOH extract was solubilized in 100 mL of EtOH before being put into an analytical HPLC. A 2998 photodiode array detector at 320 nm and an autosampler were features of the Waters e2690/5 HPLC system (Milford, MA, USA) used for the HPLC analysis. Utilizing a Guard-Pak C18 precolumn insert, the HPLC analysis was performed on a YMC Pack Pro C18 column (46 mm i.d. 9 250 mm, YMC Inc., USA). Using a linear gradient, two solvent systems were used for the separation: solvent B was CH3CN at a flow rate of 0.8 mL/min, and solvent A was 0.05% H3PO4 in H2O. The gradient elution program was run as follows: a 10% B (v/v) run for five minutes, then a forty-five-minute linear gradient to 80% B, with a five-minute holding period. Ten uL was the injection volume. By comparing their retention times to those of the two reference compounds that were previously isolated, MSA and ORT were found. For two substances, linear correlation values were better than 0.995. The calibration curves of the two standards (MSA; y = 1.5084 x + 1.43723, ORT; y = 5.7642 x - 1.5011) were used to determine the levels of the two chemicals, which were then expressed as mg per 100 g of dried weight of MB. Two compounds had recovery rates greater than 97%. The two reference chemicals' typical HPLC chromatograms as well as the EtOH extract of MB are shown in Fig.1 [11].

Animals

Male ICR mice six weeks of age were bought. The animals were housed at 25 ± 2 oC, with a 12-hour light/dark cycle, and a humidity of 50 ± 10%. Throughout the trial, the mice were given access to food and drink at will. For the duration of the experiment, their body weight was recorded twice a week.

Experimental procedure

Mice were split into four groups at random for experiment I: diabetic control (DC, n = 10), normal control (NC, n = 10), diabetes + MB EtOH extracts 0.5 g/kg BW (MBE0.5, n = 10), and diabetes + MB EtOH extracts 1 g/kg BW (MBE1, n = 10). Four groups of mice—NC (n = 7), DC (n = 7), DORT (diabetes + ORT, n = 10), and DMet (diabetes + metformin, n = 10—were used in experiment II. In order to cause diabetes, mice were given intraperitoneal injections of STZ (40 mg/kg/day) for five days in a row after it had been dissolved in 0.1 M citrate buffer (pH 4.5). In experiment I, animals were given MB EtOH extracts (0.5 or 1 g/kg BW) orally once a day for 22 days in a row after it was determined that the blood glucose concentration from the tail vein had reached 300 mg/dL using STZ injection. In the second experiment, mice were given either Met (0.6 g/kg BW) or ORT (0.6 g/kg BW) orally for a total of 22 days. Mice in the DC and NC groups received vehicle treatment [12].

Tissue preparations

Mice were fasted for 12 hours, given CO2 anesthesia, and then killed when tests I and II were finished. A heparin-coated tube containing blood was filled with inferior vena cava blood, and the plasma was separated by centrifuging the tube at 3000 g for 15 minutes at 4 oC. Following blood collection, the liver was extracted quickly, cleaned, weighed, frozen in liquid nitrogen, and kept at -70 °C for examination. The small intestine—the portion located 10 cm from the pylorus and just before the appendix—was removed right away, and ice-cold phosphate buffer saline (PBS) was used to gently flush it. The crude enzyme solution for the measurement of disaccharidase activity was prepared using it, and it was separated into three sections: proximal, middle, and distal [13].

Plasma and whole blood biomarkers

Using a blood glucose monitoring kit and a glucose analyzer, whole blood drawn from the tail vein after a 12-hour fast was used to determine the fasting blood glucose concentration. Commercial kits, including the glucose and mouse ultrasensitive insulin ELISA Kit, were utilized to assess the levels of insulin and plasma glucose [13].

Disaccharidase activities

Using glucose release measurements from lactose, sucrose, and maltose, intestinal disaccharidase activity were ascertained. To summarize, each intestinal segment was opened, cleaned, and then mixed with four volumes of distilled water to extract the mucosa. This was done by scraping the tissue with a glass slide. The activity of lactase, sucrose, and maltase was measured by diluting the homogenate supernatant one, eight, and ten times, respectively, following centrifugation at 7000 g for 10 min at 4 oC. Each diluent received an equivalent volume of 0.1 M sodium maleate buffer (pH6.0), which contained 56 mM disaccharide solution. The mixture was then incubated for one hour at 37 oC. After cooling the mixture after two minutes of boiling in water, a glucose oxidase reagent containing o-dianisidine as a chromogen was added. At 420 nm, the absorbance was measured following a 1-hour incubation period at 37 oC. As U/mg protein, disaccharidase activity was expressed [14].

Measurement of hepatic glycogen content

The method of Seifter and Dayton was modified in order to determine the hepatic glycogen content. In short, six liters of 30% (w/v) KOH solution were used to homogenize the liver tissue, which was then dissolved for thirty minutes at 100 oC. O/N was incubated at 4 oC after three liters of 95% EtOH were added. The pellet was recovered and dissolved in distilled water after it was centrifuged at 5000 g for 15 min at 4 oC the next day. Glycogen content was assessed by measuring the absorbance at 620 nm after the dissolved pellet was treated with an anthrone reagent (0.02 g anthrone/L of 95% (v/v) H2SO4) [15].

Statistical analysis

The SPSS software was used for all statistical analyses. After undergoing one-way analysis of variance (ANOVA), the data were displayed as mean ± standard error mean (SEM). When the differences reached $p<0.05$, they were deemed significant.[16]

Molecular docking

Auto Dock 4.2.6 software was used for molecular docking in order to further ascertain OXY's binding position. The grid spacing was 0.375 Å, and the grid box dimensions were 126 Å × 126 Å × 126 Å. One hundred runs of ligand flexible docking simulations were carried out. Additionally, energy calculations and docking results display were performed using Auto Grid 4.0 and VMD software, respectively.

Result and Discussion

Table 1: Physico-chemical parameters of Morus Alba L.

Sr.no.	Standardization parameters	Results
1	% Foreign Organic Matter (w/w)	<2
2	% Total Ash (w/w)	7.64
3	% Acid Insoluble Ash (w/w)	0.25
4	% Water Soluble Ash (w/w)	1.75
5	Sulphated Ash Value (%)	0.884
6	Moisture Content (w/w)	1.412
7	% Extractive Values (w/w)	1.35
8	Alcohol Soluble	26.76 %
9	Water Soluble	43.65 %

Preliminary Phyto-chemical screening

Table 2: Preliminary Phytochemical screening of Morus Alba L. extract

Sr. No	Name of test	Chloroform extract	Ethyl acetate extract	Ethanolic extract
1	Test for Carbohydrates	+	+	-
2	Test for Proteins	-	+	-
3	Test for Phenolic	-	-	+
4	Flavonoids Test	-	+	+
5	Test for Glycosides	-	-	-
5a	Cyanogenic Glycosides	+	+	+
5b	Test for Anthraquinone Glycosides	+	-	-
5c	Saponin Glycosides	+	-	+
5d	Cardiac Glycosides	+	+	+
6	Alkaloids Test	-	-	+
7	Test for Tannins	+	-	-
8	Test for Coumarins	-	+	+
9	Test for Saponin	-	-	+
10	Test for Steroids	+	-	+

In above table + indicates presence & - indicates absence of the phytochemical constituents which were screened using various identification tests.

Quantification of MSA and ORT by HPLC

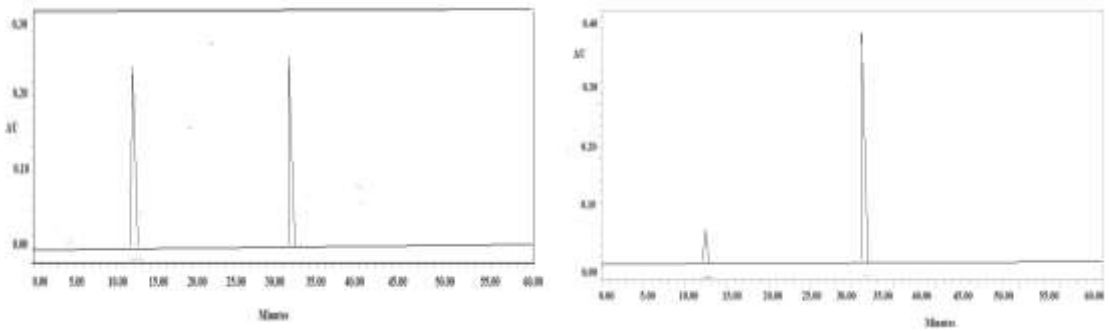


Fig. 1: HPLC chromatograms of the mulberry branch (MB) EtOH extract (B) and the two standards, oxyresveratrol (ORT) and mulberroside A (MSA) (A). 1) Oxyresveratrol (ORT), 2) Mulberroside A (MSA)

Levels of MSA and ORT in mulberry branches

The HPLC was used to assess the ORT and MSA content of mulberry twigs (small, thin branches, < 1.0 cm in diameter) and stems (large, thick branches, 1.0–3.0 cm in diameter) before MB was chosen. In Table 3, it is shown that the percentages of MSA and ORT for mulberry stems and twigs were 2.10 and 2.22 percent, respectively. Mulberry twigs had a greater MSA level than mulberry stems, although the ORT level of mulberry stems was higher than that of twigs. Here, the sample consisted of MB's EtOH extracts, which included the stems and twigs of mulberries.

Table 3: Contents of ORT and MSA of mulberry branches including twigs and stems

Part of mulberry branch	Content (%, EtOH ext.)	
	MSA	ORT
Stem	3.09 ± 0.24	12.96 ± 1.13
Twig	3.22 ± 0.30	4.84 ± 0.32
Average	3.15 ± 0.27	8.90 ± 0.72

MB decreased the fasting blood and plasma glucose in diabetic mice

Products made from mulberry trees, such as their fruits, leaves, and root barks, have a well-established history of causing hypoglycemia. Nevertheless, only a few number of research have documented MB's hypoglycemic effect in animal models of diabetes.

A ten-day period following the oral dose of MB, FBG (fasting blood glucose) was assessed. The FBG level was considerably greater in the DC group's mice (584.4 ± 11.58 mg/dL) than in the NC group's mice (87.3 ± 7.85 mg/dL). But in comparison to the DC group, the FBG level was substantially lower in the MBE 0.5 group (43%) and MBE1 group (81%) [Fig. 2(A)]. After the animals were put to sleep, the plasma glucose concentrations were determined. The mice with NC and DC backgrounds had plasma glucose concentrations of 88.1 ± 3.15 and 226.8 ± 9.72 mg/dL, respectively [Fig. 2(B)]. The plasma glucose levels of the MBE0.5 and MBE1 groups significantly decreased in a dose-dependent manner as compared to the DC group. Interestingly, MBE1 reduced plasma glucose levels in comparison to that in the NC group.

Long used to cause Type 1 Diabetes in experimental animals, STZ is a naturally occurring substance that selectively cytotoxic to insulin-producing beta cells in the pancreatic islets in mammals. Because of its structural similarities to glucose, it has been assumed that the glucose transport protein, GLUT2, is responsible for delivering STZ to cells. However, experiments have demonstrated that other glucose transporters are unable to identify STZ. Because beta cells express GLUT2 at relatively high levels, this finding explains why it is unique to these cells.

Water extracts of MB at dosages of 1.25, 2.5, and 5 g/kg BW were shown to reduce blood glucose concentration in a dose-dependent manner in both normal and alloxan-induced diabetic mice and rats. Guo et al. found that giving oral polysaccharides (PS) extracted from MB to STZ-diabetic mice at a dose of 0.6 g/kg BW dramatically lowered their fasting blood glucose levels. Furthermore, the injection of PS efficiently recovered the severely damaged structures and number of pancreatic beta cell islets. We found that MB EtOH extracts have strong hypoglycemic action in this investigation as well. Notably, there were significant differences between the FBG and plasma glucose levels in this investigation. The plasma glucose level was approximately twice as high as the FBG levels in the diabetic animals. While the precise cause is unknown, we hypothesize that this variation resulted from the transient health or metabolic state of the mice. Within the same species, STZ-induced diabetes mice had reported FBG levels ranging from 150–500 mg/dL. Nevertheless, the injection of MBE markedly and dose-dependently decreased both FBG and plasma glucose.

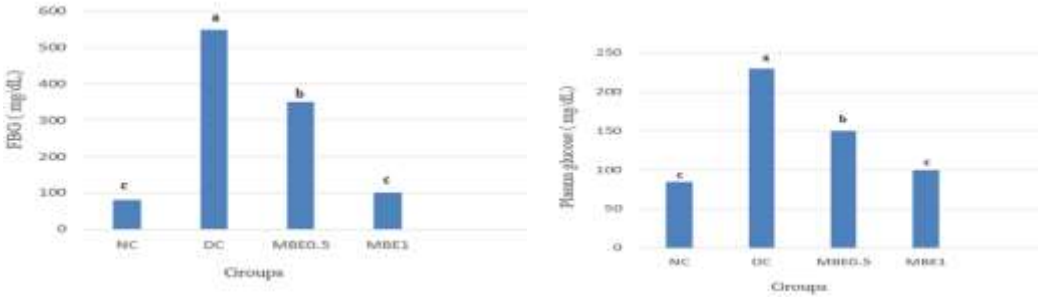
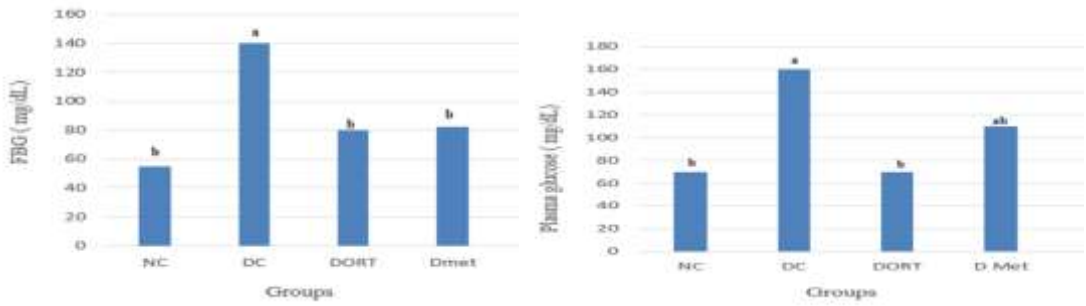


Fig. 2: Effects of MB EtOH extracts on hypoglycemia. EtOH extracts of MB (0.5 or 1 g/kg BW) were given orally to STZ-induced diabetic ICR mice for a period of 22 days. The mouse tail vein was used to assess the fasting blood glucose (FBG) (A) after ten days of oral treatment. Following euthanasia, the plasma glucose (B) concentration was assessed. The mean ± SE is used to present values. Using Duncan's multiple range test, means with different letters are substantially different at p~<0.05. MBE0.5, mulberry branch EtOH extract 0.5 g/kg BW; MBE1, mulberry branch EtOH extract 1 g/kg BW; NS, not significant; NC, normal control; DC, diabetes control. ORT Improved hyperglycemia.

The extracts were discovered to include minor its glycoside, MSA (2.15 ± 0.27 %) and substantial ORT (7.90 ± 0.72 %) in the prior HPLC investigation of the MB EtOH extract (Fig. 1). Thus, we postulated that ORT is the main bioactive ingredient responsible for MB's anti-diabetic effects.

In order to verify our theory, we gave the diabetic rats 19 days of ORT at 0.6 g/kg BW and contrasted its effects with Met's ability to lower blood glucose (0.6 g/kg BW). In documented mice experiments, the dose of Met was adjusted within the range of 0.25–1 g/kg BW. In our investigation, we used 0.6 g/kg BW of ORT since Guo et al. examined the anti-diabetic effects of polysaccharides extracted from mulberry branches against those of Met at 0.6 g/kg BW in STZ-diabetic mice. The FBG level in the DC group was considerably higher than in the DORT and DMet groups [Fig. 3(A)]. As shown in Fig. 3(B), the plasma glucose levels of the DORT and DMet groups were lowered to a level comparable to that of the NC group. In a manner akin to that of MB, the injection of STZ markedly decreased the level of plasma insulin [Fig. 3(C)]. Nonetheless, there was no discernible variation between the groups with diabetes. Once more, these findings imply that insulin secretion was not a prerequisite for ORT's anti-diabetic action.

Mulberry trees naturally contain significant concentrations of ORT, a hydroxylated version of resveratrol. The biological activities of ORT have not been thoroughly investigated, despite its strong solubility in aqueous solution and high bioavailability (around 50%). It is known that ORT inhibits the activities of rat liver mitochondrial ATPase, cyclooxygenase, and tyrosinase. Additionally, ORT has been shown in microglial cells to be a strong antioxidant and free radical scavenger. Its anti-inflammatory and anti-immune properties have also been shown both in vivo and in vitro. According to Mouihate et al., ORT's suppression of the neuroimmune response was primarily caused by its suppression of the generation of tumor necrosis factor-alpha. It has been documented that in alloxan-induced diabetic mice, MSA derived from mulberry root barks significantly reduced blood glucose levels. The plasma glucose level was significantly lowered by MSA (50 mg/kg), and this reduction was similar to gliclazide's. Together, these findings suggest that whereas MSA was shown to have hypoglycemic action in T2DM, ORT's hypoglycemic impact has not yet been proven in T1DM. To the best of our knowledge, this is the first study to describe ORT's anti-diabetic properties and its effectiveness as a powerful blood glucose-lowering drug in an animal model of STZ-induced type 1 diabetes.



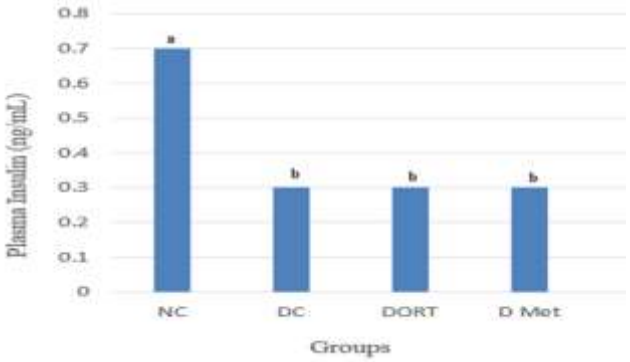


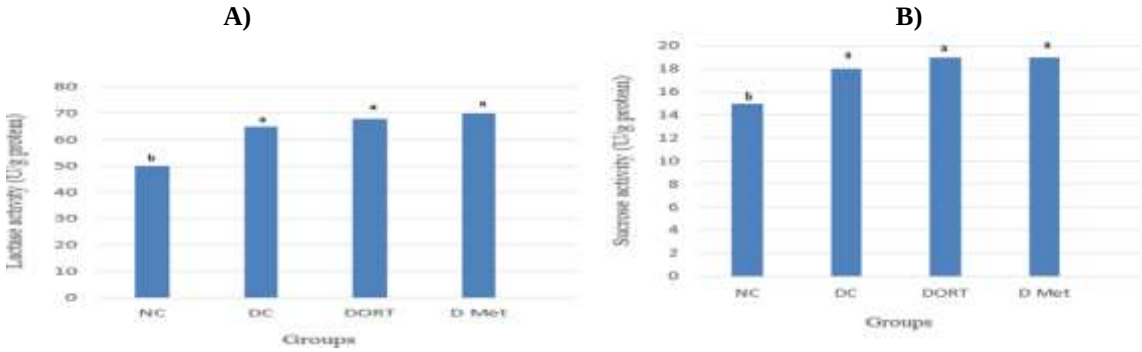
Fig. 3: Impact of Oral Rehabilitation Therapy on Insulin and Plasma Levels. ORT (0.6 g/kg BW) and Met (0.6 g/kg BW) were given orally to STZ-induced diabetic ICR mice for a period of 22 days. Following a 10-day oral regimen, the mouse tail vein was used to test the fasting blood glucose (FBG) (A). When the animal was put to sleep, the concentrations of insulin (C) and plasma glucose (B) were assessed. The values are shown as the mean ± SE. Duncan's multiple range test indicates that means with different letters differ significantly at p~<0.05. Diabetes + oxyresveratrol (DOT), diabetes + metformin (DMet), NC normal control, DC diabetes control, and NS not significant.

Intestinal disaccharidase activity of ORT in diabetic mice

First, the small intestine's disaccharidase activity was assessed in order to learn more about the mechanism underlying ORT's anti-diabetic action. The proximal portion of the small intestine was used to measure the enzyme activities since disaccharidase was mostly active there and monosaccharide's are absorbed there, as well as in the proximal jejunum. Lactase, sucrose, and maltase activity were all greater in diabetic mice than in NC animals, as Fig. 4 illustrates. Nonetheless, there was no discernible variation between the experimental groups.

Digestion enzymes produced into the small intestine break down carbohydrates into oligosaccharides and disaccharides. Disaccharidase, which is found on the brush boundary membranes of the small intestine mucosa, subsequently transforms disaccharides into monosaccharides. Lactase, maltase, and sucrose are components of intestinal disaccharidase. Since they can catalyze the release of a-glucose from the non-reducing end of the substrate, sucrose and maltase are referred to as a-glucosidase. One of the main risk factors for diabetic patients is postprandial hyperglycemia, which would be directly caused by the rise in disaccharidase activity.

In fact, the small intestines of diabetic humans and experimental animals have been found to exhibit a substantial increase in disaccharidase activity. Therefore, it is well recognized that restricting and postponing glucose absorption after meals helps to regulate plasma glucose concentration and the difficulties associated with diabetes. After analyzing hundreds of traditional Chinese medicines, the aqueous extracts of MB were found to be a strong a-glucosidase inhibitor in the small intestine of rats and mice. High doses of MB (2.5 and 5 g/kg body weight) had a hypoglycemic effect similar to that of the a-glucosidase inhibitor acarbose. Diabetes induction was reported to promote enhanced disaccharidase activity; however, enzyme activities were not affected by the administration of ORT or Met.



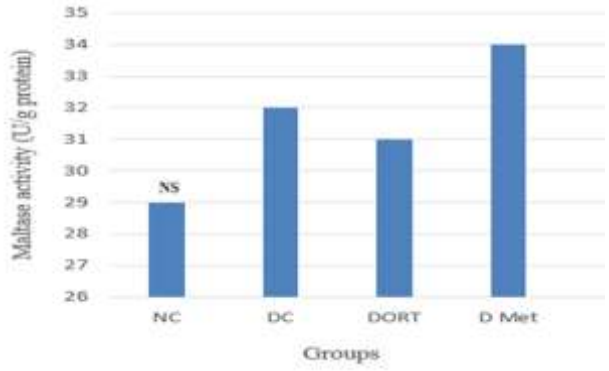


Fig. 4: Impact of ORT on the activity of intestinal disaccharidase. At the proximal section of the small intestine, the activities of lactase (A), sucrose (B), and maltase (C) were assessed. The mean ± SE is used to present values. Using Duncan's multiple range test, means with different letters are substantially different at p~<0.05. DC diabetic control, NS not significant, NC normal control, DORT diabetes + oxyresveratrol, and DMet diabetes + metformin

ORT increased GLUT2 transcription and glycogen contents in the liver

Measurements of the hepatic GLUT2 mRNA level were made to explore whether ORT lowered blood glucose levels via inducing hepatic glucose uptake, as intestinal disaccharidase activity and plasma insulin level did not differ substantially between the diabetes groups. The livers of DC mice had a notably lower amount of GLUT2 mRNA than those of NC animals [Fig.5(A)]. In contrast, the DC group's GLUT2 expression was much lower than that of the DORT and DMet groups.

When hepatic glycogen is broken down, glucose is produced, which enters the bloodstream and adds to hyperglycemia in people with diabetes. Hepatic glycogen levels in DC and NC animals were considerably different, as Fig. 5(B) illustrates. In line with the results of GLUT2 expression, the DORT and DMet groups' hepatic glycogen levels also rose to a degree comparable to the NC groups. When considered collectively, findings indicate that by promoting hepatic glucose absorption and glycogen storage, ORT may be in charge of the anti-diabetic effect of MB. Through its control over glucose uptake and storage, the liver contributes significantly to glucose homeostasis. Liver glucose transport is not insulin-sensitive, in contrast to the glucose transport that is driven by insulin in muscle and fat. The primary isoform of glucose transporters in the liver is called GLUT2, a facilitated-diffusion glucose transporter that is expressed in specific tissues such as the liver, pancreatic b cells, kidney tubular epithelial cells, and intestinal mucosa cells. Numerous investigations have shown that the concentration of glucose controls the expression of GLUT2 in the liver. Research has demonstrated that GLUT2 expression decreased during famine but returned to normal levels upon ingesting meals. On the other hand, liver GLUT2 expression is inconsistent in diabetic animals. Rats with diabetes have been shown to have elevated GLUT2 gene expression. GLUT2 mRNA and protein levels were markedly elevated by glucose stimulation in cultivated rat hepatocytes. GLUT2 expression, however, was demonstrated by Akarte et al. to be lower in the liver of STZ-induced diabetic rats than in normal rats. The hepatocytes from STZ-induced hyperglycemic animals were stained with a dye that revealed a significant decrease in GLUT2 positive hepatocytes in hyperglycemic rats compared to control rats. The current investigation found that the DC group's GLUT2 mRNA expression was considerably lower than that of the NC group.

Diabetes affects the liver's ability to synthesize glycogen, which is why diabetic animals' livers typically have lower glycogen levels. The results of our investigation also shown a substantial difference in glycogen levels between the NC and DC groups in the liver tissues. Insulin resistance or insulin depletion are the main causes of this decrease in glycogen synthase activity. A phosphorylated and inactivated glycogen synthase kinase 3 (GSK3) is the result of the Akt signaling pathway being triggered when insulin attaches to an insulin receptor on the plasma membrane. GSK3 is unable to phosphorylate glycogen synthase when it is inactive. Glycogen synthesis from glucose-6-phosphate is then started by unphosphorylated active glycogen synthase. Diabetic problems arise from any malfunction in the glycogen production pathway. When combined, ORT increased liver glycogen content and GLUT2 expression, which had hypoglycemic effects. Thus, one mechanism of ORT's anti-diabetic impact may be enhanced hepatic glucose absorption and glycogen production.

The study has a number of drawbacks. First, it was not possible to compare the hypoglycemic activity of MB and ORT because their anti-diabetic effect was examined in two separate studies. Second, only oral dosages of ORT were assessed once. Examining whether ORT's anti-diabetic effects show a dose-response connection will be intriguing. Third, an animal model of T1DM was evaluated using a STZ-induced diabetic state; as such, insulin inclusion as a positive control would be intriguing. To the best of our knowledge, however, this work is the first to describe the hypoglycemic and anti-diabetic effects of MB and ORT in animals with type 1 diabetes (T1DM). Given that the only

treatment for lowering blood glucose levels in T1DM patients is insulin injection, the insulin-independent blood glucose-lowering effect of ORT raises the possibility that MB extract and its bioactive component ORT could be created as nutritious functional foods for T1DM patients.

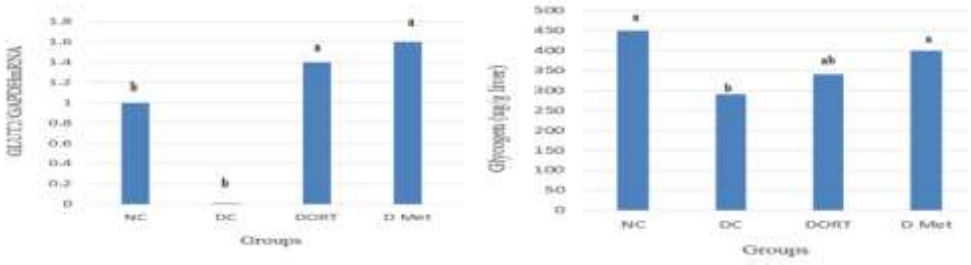


Fig. 5: Effect of ORT on GLUT2 expression and the amount of glycogen in the diabetic mouse liver. ORT (0.6 g/kg BW) and Met (0.6 g/kg BW) were given orally to STZ-induced diabetic ICR mice for a period of 22 days. qRT-PCR was used to assess the expression of glucose transporter 2 (GLUT2) mRNA in the liver (A) following euthanasia. It was measured how much liver glycogen (B) there was. The values are shown as the mean ± SE. Duncan's multiple range test indicates that means with different letters differ significantly at p~<0.05. DORT diabetes + oxyresveratrol, DMet diabetes + metformin, NC normal control, DC diabetes control, and NS not significant

Molecular Docking Study

The compounds LYS195, TRP214, GLN221, PRO339, TYR341, PRO447, GLU450, and ASP451 encircled OXY (Oxyresveratrol). The hydrogen bond lengths between OXY's –OH and GLU450, TYR341, ASP451, and PRO339 were 2.79, 2.83, 2.95, and 3.12 Å, in that order.

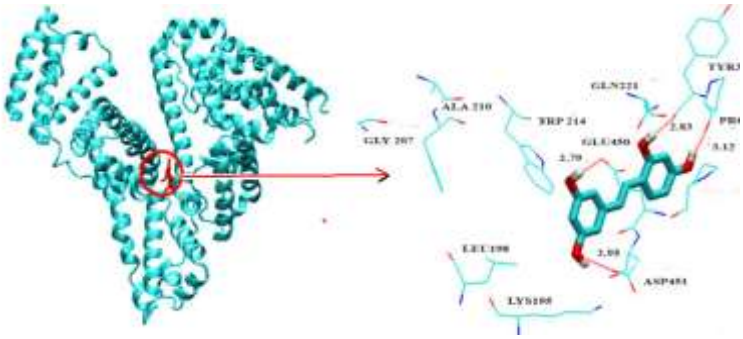


Fig. OXY molecular docking. The drug's interaction with amino acid residues close to the binding position is depicted in the right image, while the left image displays the binding position. The hydrogen bond is indicated by the red line.

Conclusion-

The purpose of this study is to evaluate the extract of Morus Alba L. in mice model for potential anti-diabetic effects and preliminary phytochemical screening. Although there is growing evidence regarding the mulberry branch's (MB) ability to prevent diabetes, the main active ingredient in this action is yet unknown. The streptozotocin-induced diabetic ICR mouse was given oral MB ethanol (EtOH) extracts [0.5 or 1 g/ kg body weight (BW)] once a day for 22 days. This drastically decreased the diabetic control mouse's plasma glucose level and fasting blood levels in a dose-dependent manner. Oxyresveratrol [ORT, 0.6 g/kg BW], a primary constituent of MB EtOH extracts, was administered to diabetic ICR mice, which also markedly lowered their fasting plasma glucose level. Moreover, ORT raised the transcription of the hepatic glucose transporter 2 and the amount of glycogen. There was no difference in intestinal disaccharidase activity or plasma insulin levels between the diabetes control and ORT groups.

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