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The levels of plasma glucose and insulin; oxidative stress and body weight in streptozotocin induced diabetic rats treated with aqueous solution of Moracin

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Abstract

The aim of present attempt was to determine possible therapeutic influence of provision of oral Moracin on levels of plasma glucose and insulin; oxidative stress and body weight in the streptozotocin-induced diabetic rats. The well known classical biochemical methods were used for the determination of plasma malondialdehyde (MDA), nitric oxide (NO), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) levels. Enzyme-linked immunosorbent assay (ELISA) was used for the determination of levels of insulin in plasma. The reverse transcriptase-polymerase chain reaction was used for the determination of the levels of brain antioxidant enzymes (SOD, CAT, and GSH-Px). The diabetes-related increase in blood glucose levels was found reduced through the provision of Moracin over the period of eight weeks. The levels of plasma nitric oxide (NO) and glutathione (GSH) of brain tissue were found significantly reduced in the Moracin treated group of streptozotocin-induced diabetic rats. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in the hemolysate samples from Moracin treated streptozotocin-induced diabetic rats were observed significantly increased in comparison with untreated to the group of streptozotocin-induced diabetic rats. The superoxide dismutase (SOD) and catalase (CAT) activities in the homogenates of brain tissue of Moracin treated streptozotocin-induced diabetic rat did not exhibited the significant changes. whereas glutathione (GSH) activity was found increased in the Moracin treated group in comparison with untreated to the group of streptozotocin-induced diabetic rats. The superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) mRNA transcription levels were suppressed in the diabetic group compared to the control. The suppression in superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) mRNA transcription was found stopped through the provision of Moracin. The oxidative damage and low levels of insulin associated with diabetes were ameliorated with the Moracin treatment. The Moracin, the novel compound belong to the mulberry, *Morus alba* (L.) should be introduced in the human diet. The Moracin deserve effectiveness for the diabetic patients.

Keywords: Diabetes induced xidative stress, Moracin, Mulberry, *Morus alba* (L.).

INTRODUCTION

Deficiency of insulin exert chronic metabolic disorder, entitled "Diabetes mellitus". The incidence of this disorder has been increasing all around the world. Many more mechanisms are involved in the pathogenesis of Diabetes mellitus and the complications associated with it. According to Maritim, *et al* (2003), the free radicals are responsible for oxidative damage in the patients of Diabetes mellitus. The living world (plants, animals, and microorganisms) of earth constitute various sources of the natural compounds (Lam, 2007). The medicinal compounds are obtained from the natural bioactive materials. The natural compounds deserve diversity. Therefore, it is possible to synthesize the "Natural Drugs" through the use of diverse natural compounds. The natural, most correctly, the herbal drugs are with complex structures and biological potency (Dias, *et al* , 2012). The extractives of the Plants, like *Bidens pilosa (*L.*); Capsicum annuum (*L.*) ; Carica papaya (*L.*); Gymnema sylvestre (*L.*); Momordica charantia (*L.*); Nymphaea stellata (*L.*) and Panax ginseng; (*L.) are already reported for working in the regulation of cells of islets of Langerhans. The herbal bioactive compounds reported as effective for the regulation of Pancreatic Beta Cell Function include: Berberine, Conophylline, Curcumin, Epigallocatechin-3-gallate, Genistein, Kinsenoside, Quercetin , Resveratrol and Silymarin. (Vitthalrao B. Khyade, 2018 and Vitthalrao B. Khyade, *et al* , 2018).

Provision of leaf decoction of Mulberry, *Morus alba* (L.) (20 g/L) was reported for the regulation of the diabetes - altered - metabolic processes (Vitthalrao B. Khyade, 2018). Mulberry (*Morus alba* L., Moraceae) has been used in traditional Chinese medicine as an anti-headache, anti-hypertensive, anti-diabetic, and diuretic agent (Lee, *et al*, 1981). In particular, mulberry twigs have been widely used for the treatment of aching and numbness of joints in oriental medicine (Zhu, 1998). Several prenylflavonoids, flavonoids, coumarins and stilbenes have been isolated and identified from mulberry twigs (Ko, *et al*, 1997; Oh, *et al*, 2002; Hu, *et al*, 2011; and Chang, *et al*, 2011). Among them, prenylflavonoids and flavonoids have been reported as major principles for anti-obesity, antioxidant, anti-aging, and hepatoprotective activities of mulberry twigs (Ko, *et al*, 1997; Oh, *et al*, 2002 and Hu, *et al*, 2011). In addition, some coumarins and resveratrol derivatives in mulberrytwigs were found to have strong radical scavenging and anti-inflammatory activities (Oh, *et al*, 2002 and Chung, *et al* , 2003). Thus, mulberry twigs are receiving much interest as promising sources of functional foods with health benefits. Mulberry twigs are widely used as a promising source of well-being healthy teas, together with mulberry fruits and leaves. In addition, mulberry soups and wines made with mulberry twigs were known to have potential health benefits in folk medicine against diabetes, stroke, cough, and beriberi, etc. (Lee, *et al*, 1981). Therefore, study on analysis of functional constituents for standardization and quality control of mulberry twig teas, soups, and wines is required.

The biochemical constituents of leaves of mulberry, *Morus alba*(L) serve a lot to orchestrate the progression of life cycle of lepidepteran insects like silkworm, *Bombyx mori*(L). Mulberry leaves are also used for food for livestock (Cattle, goat *etc.*) in the areas where dry seasons restrict the availability of ground vegetation. The traditional Chinese medicine recommend the mulberry fruits to treat the prematurely grey hair, to tonify the blood and to treat constipation and human diabetes. Zhang *et al.*(2009) reported the Moracin –M, Steppogenin–4'– O–beta-D–glucoside and mulberroside- the novel compounds of mulberry, *Morus alba* (L) for hypoglycemic effects. Naowaboot, *et al.*, (2009) studied the effect of Ethanolic extract of leaves of mulberry, *Morus alba* (L) on chronic diabetic rats and observed antihyperglycemic, antioxidant and antiglycationactivity. Cancer induction is distinguished by involvement of oxidative stress in the cells. The cancer induction and its subsequent development, and associated molecular mechanism is becoming increasingly clear (Lahiri. *et al*., 1999 and Ames *et al*.,

1995). There are about fifty percent of the drugs approved by the US Food and Drug Administration that belong to: phytogenic compounds or derivatives thereof. The best examples of natural compound-derived pharmaceuticals are: Aspirin, metformin, morphine, vinblastine, vincristine, quinine, artemisinin, etoposide, teniposide, paclitaxel, and camptothecin (Kingston, 2011). There are about 1200 plants that have been claimed to contain compounds with antidiabetic properties. There are over 400 plants and their bioactive compounds have been scientifically evaluated for type 2 diabetes treatment (Singh, *et al* , 2011).

Moracin C from *Morus alba* or *Artocarpus heterophyllus* is one of the more well-known natural 2-phenyl-benzofuran derivatives (Yao, *et al* , 2016). The moracin C contains three phenolic –OH groups at the 6,3′,5′-positions. Thus, it can also be regarded as a phytophenol (Li, *et al* , 2012). Of course, it is dissimilar to any of the common phytophenols, such as flavonoid (Li, *et al* , 2016), flavonoid glucoside (Li, *et al* , 2014), biflavonoid (Li, *et al* , 2013), volatile phenol (Lin, *et al* , 2014), phenolic cumarin (Li, *et al* , 2017), phenolic alkaloid (Li, *et al* , 2016), phenolic acid (Li, *et al* , 2009 ; Chen, *et al* , 2010), and phenolic acid ester (Li, *et al* , 2015). Like most phytophenols, however, the characteristic phenolic moiety of moracin C makes it of interest to many researchers. Recently, Yao *et al* (2016) used a cellular model to explore its inhibitory effect on the nitric oxide production of RAW264.7 cells (Yao, *et al* , 2016); while Zelová *et al* (2014) reported its anti-inflammatory activity. In addition, moracin C has also been found to inhibit fatty acid synthesis (Kim, *et al* , 2012) and lipoxygenase levels (Lang, *et al* , 2016), both of which are positively correlated with oxidative stress (Li, *et al* , 2016; Tersey, *et al* , 2014). These three inhibitory effects of moracin C are thought to originate from an antioxidant action. However, to the best of our knowledge, there is no relevant study to date on the antioxidant action of moracin C.

Moracin M is a phosphodiesterase-4 inhibitor isolated from *Morus alba* (L.). Moracin -M; Steppogenin – 4' – O – beta- D – glucoside and mulberroside– the novel compounds of mulberry, *Morus alba* (L) were found to produce hypoglycemic effects (Zhang, *et al* , 2009). The ethanolic extract of leaves of mulberry, *Morus alba* (L) had antihyperglycemic, antioxidant and antiglycation effects in chronic diabetic rats (Naowaboot, *et al* , 2009) and therefore, it deserve therapeutic significance and exert a applicable influence. The involvement of oxidative stress in cancer induction and it's subsequent development , and associated molecular mechanism is becoming increasingly clear (Ames, *et al* , 1995 and Lahiri, *et al* , 19993). Recent studies showed an increasing use of medicinal plants or their extracts to ameliorate diseases (Celik, *et al*, 2009 and Mohammadi, *et al* , 2011). Moracin is the novel compound of mulberry, *Morus alba* (L.). In this present attempt, the effects of Moracin for overcoming oxidative damage in diabetes, which plays an important role in the development of diabetes, was investigated. The preventive effects of Moracin were also examined on diabetic disorders such as body weight loss, decreased insulin levels, and decreased plasma glucose levels. Free radicals play an important role in plasma chemistry, biochemistry, and many other chemical processes within the human physiology (Usha Nandhini, *et al* , 2011). Free radical damage could be a reason for many diseases, including diabetes (Agar, *et al* , 2011). When the number of free radicals increases to the point that they outnumber the antioxidants, they can attack the somatic cells and immune system (Hayden, *et al* , 2005). Antioxidants are molecules that neutralize the effects of free radicals by donating an electron to pair with the free radical's unpaired electrons. Healthy people have a balance between free radicals and antioxidants. However, it has been shown that people who have diabetes have higher levels of free radicals, which can cause diabetic complications (Memisogullari, *et al* , 2004). The overall objective of the present attempt was to investigate the possible antidiabetic and therapeutic effects of Moracin on diabetes in streptozotocin (STZ)-induced diabetic rats. The specific aims of the attempt were: 1) to investigate the effect of Moracin supplementation on blood glucose concentration, body weight, lipid peroxidation, and plasma insulin levels; 2) to investigate nitric oxide (NO), glutathione (GSH), and malondialdehyde (MDA) levels and the

antioxidant enzyme [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx)] levels in both blood and brain tissue samples;3) and to investigate the effect of lycopene on the expression of the antioxidant enzyme genes (SOD, CAT, and GSH-Px) that regulate the antioxidative defense mechanisms in brain tissues.

MATERIAL AND METHODS

The study was carried through the steps like: Rearing of experimental animals; Induction of diabetes through streptozotocin; Grouping the experimental animals; Preparation of the Samples For Biochemical Assays; Bioassays of Malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Nitric Oxide (NO); Estimation of plasma insulin level ; Extraction of mRNA extraction and RT-PCR and Statistical analysis.

(A). Rearing of Experimental Animals:

Six week old male rats (*Rattus norvegicus* L.) were obtained from Department of Zoology, University of Pune. The rats were housed in four cages and maintained at 28 degree Celcious and subjected to a 09:15 hours light – dark cycle (Lights on 8.00 a.m. to 5.00 p.m.).The rats in cages were acclimatized for one week before the experimental use. The rats were feed a commercial stock diet and deionized water. The rats were maintained in laboratory through the standard methods. The body weight was measured every week. For the present attempt, permission was issued by the ethical committee of Animal Welfare and Use Committee, Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar (Tal. Baramati Dist. Pune – 413115 India).

(B). Induction of Diabetes Through Streptozotocin:

The experimental animals were allowed for acclimatization for about two weeks. Thereafter, the diabetes was induced in rats with Streptozotocin (STZ, Sigma Chemical Company) through explained by Vitthalrao B. Khyade (2018). After overnight fasting, diabetes was induced
in the rats by a single intraperitoneal injection of Streptozotocin (STZ). The streptozotocin (STZ) was intraperitoneally administered (in a dose of 70 mg/kg/bw) in 0.1 M citrate buffer, (pH 4.5). The control rats received intraperitoneally citrate buffer. A freshly-prepared solution of streptozotocin (STZ, 70 mg/kg/bw) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally to rats that had fasted overnight (Kesari *et al*., 2007). One week later, blood samples were collected from the orbital sinus, and rats with fasting blood glucose (FBG) levels above 200 mg/dL (11.1 mmol/L) were selected for the experimental protocol. During a 21-day period of treatment, normal and STZ-treated rats were fed with 40 g/day of pellet food (**Hindustan Animal Feeds,** Behind Gokulnagar Octroi Check Post, Near Vijaynagar Railway Crossing, Jamnagar – 361004 Gujarat INDIA).

(C). Grouping the Experimental Animals:
Two months of age and weighing between 150 and 250 g, the rats, the experimental animals were allocated randomly and equally. After diabetic state was confirmed, the rats, the experimental animals were used for the studies on Moracin treatment. They were divided into in four experimental groups (n=15 animals/ group):

Group – (I) Control Group. (treated with vehicle water): Received 100 mL of water a day.
Group – (II) Diabetic Group.
Group – (III) Moracin Treated Diabetic Group.

Group – (IV) Moracin Treated Group.
The diabetic group rats were allowed to drink a 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The rats of the control and diabetic groups were fed without any supplements except vehicle water, which was used for dissolving the Moracin. The aqueous Moracin solution at the rate of 4 mg/kg body weight was administrated orally, daily, to the rats of "Moracin Treated Diabetic Group" and to the rats of "Moracin Treated Group" once a day for 8 weeks. The dose of Moracin that was used in the present attempt was selected on the basis of trial and error method.

(D). Preparation of the Samples For Biochemical Assays:

Plasma glucose levels were measured with an Accu-Check Go strip test in a glucometer. Blood sugar levels were measured before and after 48 h of STZ induction. After 48 h of STZ induction, the rats whose blood glucose levels were ≥300 mg/ dL were considered as diabetic. Plasma glucose levels and the body weights of the animals were measured weekly for the duration of the study. The rats were anesthetized by an intramuscular injection of 50 mg/kg of ketamine, and blood was taken by puncturing the heart ventricle at the end of the experiment. Blood samples were centrifuged at 3000 rpm for 20 min and the plasma was separated. Red blood cells that remained on the bottom of the tubes were washed with a phosphate buffer, pH 7.4, and the samples were then kept at –20 °C until they were analyzed. The hemoglobin concentrations of the erythrocytes were measured using the method described by Drabkin (1946).

(E). Bioassays of Malondialdehyde (MDA); Catalase (CAT); Superoxide dismutase (SOD); Glutathione Peroxidase (GSH-Px) and Nitric Oxide (NO):

The brains were removed from each rat, washed with an ice-cold physiological saline (PBS) buffer of pH 7.0 containing protease inhibitor (Pi) mixture (Sigma), and used for biochemical studies. Tissue samples were homogenized in an ice-cold PBS buffer, pH 7.0, containing a complete Pi mixture. The homogenates were centrifuged at 4 °C at 15,000 rpm for 20 min, and the soluble fraction was retained. The protein concentrations of the supernatants were measured using the method described by Bradford (1976), using bovine serum albumin as a standard. The degree of lipid peroxidation was assessed by measuring the MDA levels in the plasma and brain tissue samples (Yoshoiko, *et al* , 1979). CAT enzyme activity in the red blood cells and homogenates was measured using the speed reduction of hydrogen peroxidase (Luck, 1965). Total SOD activity in the homogenates and plasma samples was determined according to the method of Sun *et al*. (1988). The reduced GSH concentrations in the homogenates and plasma samples were determined according to the method of Sedlak and Lindsay (1968). The NO concentration in the plasma and brain tissue samples (homogenates) was determined indirectly by measuring the nitrite levels based on the Griess reaction (Cortas and Wakid, 1990). The GSH-Px activity in the erythrocytes and homogenates was measured according to the method of Paglia and Valentine (1967).

(F). Estimation of plasma insulin level: The concentration of insulin in serum samples was estimated using Enzyme-Linked Immunoabsorbent Assay (ELISA) method using insulin kit from Syntron Bioresearch (USA). The sample used was non-haemolysed serum. Following a standard procedure, a sample of the standard curve was plotted and insulin concentrations in the samples were determined by interpolation from the standard curve (Calabrese, *et al* , 1981; Widajaja, *et al* , 1997).

(G). Extraction of mRNA extraction and RT-PCR:

The mRNA extraction and RT-PCR In the brain tissues, the mRNA expression levels of the antioxidant enzymes (CAT, SOD, and GSH-Px) were determined by reverse transcriptase-polymerase chain reaction (RT-PCR). The RNA was isolated using TRI Reagent (Sigma). The RNA's purity was then checked by spectrophotometer with 260-nm and 280- nm filters. The RNA having a 260/280 measurement of ≥1.8 was studied. Moreover, to ensure the presence of RNA, the RNA structures were observed under an ultraviolet (UV) light that was run in 1% agarose gel (electrophoresis). From each sample, 1 μg of RNA was taken and complementary DNA (cDNA) was produced using reverse transcriptase. Next, Taq polymerase, buffers, and oligonucleotides (primers) were added to 1-μL cDNA samples. Primers were specific for each PCR and 100 ng per sample was used. The base sequences and their product sizes are given for each primer, GSH-Px (Bhor, *et al* , 2004), CAT, and SOD (Zangen, *et al* , 2006) in Table 1. β-Actin was used as a housekeeping (control gene) primer (Kostic, *et al* , 1997). PCR amplification was done using a thermal cycler. Before the PCR products were captured under the UV light, they were run in 1.5% agarose gel. The digital photos were assessed with the DigiDoc-It image analyzer program. The mRNA transcription levels were determined by performing normalization of the control gene.

(H). Statistical Analysis:
For the purpose to get the consistency in the results, the whole experimentation was repeated for three times. The data was collected. The data were analyzed using SPSS 9.05 for Windows for one-way Analysis of variance (ANOVA) and post hoc multiple comparison tests. In a scientific study**,** post hoc analysis (from Latin *post hoc*, "after this") consists of analyses that were not specified before seeing the data. This typically creates a multiple testing problem because each potential analysis is effectively a statistical test. Multiple testing procedures are sometimes used to compensate, but that is often difficult or impossible to do precisely. *Post hoc* analysis that is conducted and interpreted without adequate consideration of this problem is sometimes called *data dredging* by critics, because the more one looks the more likely something will be found.

RESULTS AND DISCUSSION

The results on the attempt of treating the streptozotocin induced diabetic rats with aqueous solution of moracin are summerised in the tables (tables 1 – 7) and presented in the figure (figure – 1). The STZ induced diabetes is characterized by a severe loss in body weight. Due to absolute or relative deficiency of insulin and decreased production of ATP and protein synthesis decreases in all tissues. The measurement of body weight in experimental animals before and after induction of Streptozotocin (STZ) is shown in Table – 2. The average of body weight in the control group was increased. Different results appear in STZ induced diabetic group of rats. The STZ induced diabetic group of rats experienced a sharp decline compared with the control group. The streptozotocin induced diabetes was found exerting influence on body weight. There was gradual increase in the body weight in the individuals of control group. In the first, the diabetic individuals were found with increased body weight in comparison with the control group. From the second to sixth, the diabetic individuals were observed to lose their weight. In the seventh and eighth weeks, the diabetic individuals were observed to increase their weight. Start from the fifth week, a significant ($P < 0.05$) decrease in the body weight in the diabetic group of individuals. The streptozotocin induced diabetes was found exerting influence on body weight. The moracin treatment was found no significant effect on body weight loss. Regarding these variations there was a significant difference between the groups in all periods with reference to the initial weight (Table 2).

The measurement of blood glucose level in experimental group of animals before and after the induction of STZ was shown in Table – 3. The average of blood glucose levels in the control group was in normal

conditions. There was an increased of the MLDSTZ group blood glucose levels at day four after induction.

There was significant increase in the blood glucose levels ($P < 0.001$) as a result of streptozotocin induced diabetes in the experimental animal brown rat, *Rattus norvegicus* (L.). In the moracin treated streptozotocin induced diabetic group of individuals of brown rat, *Rattus norvegicus* (L.), the blood glucose level was observed suppressed during the 8-week period (Table 3). The blood glucose level of diabetic group was significantly higher ($P < 0.001$) than that of control group. The results showed a significant ($P < 0.001$) reduction in blood glucose in moracin treated groups compared with diabetic untreated control group. The blood glucose of moracin treated groups were comparable with that of control group at the end of the experiment

Blood GSH concentrations may serve as an indicator of GSH status and, thus, disease risk in human subjects. The nitric oxide (nitrogen oxide or nitrogen monoxide) is a colorless gas with the formula NO. Nitric oxide is one of the principal oxides of nitrogen. Nitric oxide is a free radical (it has an unpaired electron), which is sometimes denoted by a dot in its chemical formula (·NO). The nitric oxide is also a heteronuclear diatomic molecule, a historic class that drew researches which spawned early modern theories of chemical bonding (Greenwood and *Earnshaw,1997).* The plasma and brain tissue GSH levels and the plasma NO levels in the experimental animal brown rat, *Rattus norvegicus* (L.) of present attempt were found increased due to streptozotocin induced diabetes. The GSH and NO levels in the plasma and the GSH levels in the brain tissue of the moracin treated diabetic group were lower than in the diabetic group (Tables 4 and 5).

The results from this study indicated that the plasma insulin level of diabetic group was significantly ($P < 0.001$) reduced when compared with control group. The results show increase in plasma insulin levels of diabetic rats treated with moracin ($P < 0.001$). The ELISA results indicated that diabetes caused a significant decrease in plasma insulin concentrations (Table 5), and this was significantly ($P < 0.001$) reversed by the oral supplementation of moracin. The lipid peroxidation (MDA) levels were significantly increased in both the brain tissue and the blood samples of the diabetic group compared to the control group. This increase had been significantly ($P < 0.005$) lowered by the moracin supplementation in the brain tissue samples, but there was no significant effect of moracin in the blood samples. The SOD, CAT, and GSH-Px activities in the erythrocytes were increased in the moracin treated diabetic group (Table 6).

The GSH-Px, SOD, and CAT activities were significantly ($P < 0.005$) increased in the brain tissues of the diabetic group compared to the control, but the moracin administration did not have any significant effect in lowering the activities of the enzymes to the levels of the control group. The β-actin gene is a constitutive gene. This gene is used for the normalization (in the basis of the band densities) of mRNA transcription levels. The reverse transcription polymerase chain reaction (RTPCR), a variant of polymerase chain reaction, is a technique commonly used in molecular biology to detect RNA expression. In the RTPCR analyses, in the present attempt, it was found that the CAT gene mRNA transcription levels were suppressed by 7.6% in the diabetic group compared to the control group; they were found stimulated in the moracin treated diabetic group by 187.2% ($P < 0.05$) compared to the diabetic group (Figure - 1). The mRNA transcription levels of the GSH-Px gene were suppressed by 16.8% in the diabetic group compared to the control group, and they were stimulated by 333.5% ($P < 0.05$) in the moracin treated diabetic group of experimental animals in present attempt. Similarly, the SOD mRNA transcription levels were suppressed by 12.4% in the diabetic group compared to the control. Further, the

SOD mRNA transcription levels were reported stimulated by 170.1% ($P < 0.05$) in the moracin treated diabetic group of experimental animals in present attempt (Figure - 1).

The present attempt on moracin treatment indicated that, diabetes caused a significant decrease in body weight; however, there were no significant effects of moracin on body weight loss (Table 2). These observations are consistent with the studies of Mellert *et al* (2002), Sindhu *et al* (2004), and Duzguner *et al* (2008). The lipolysis and gluconeogenesis are the major reasons for weight loss resulted through the attack of diabetes (Quinn, 2002 and Sindhu *et al* , 2004). Diabetes-increased blood glucose levels were suppressed by the lycopene during the 8-week period (Table 3). Similarly, Duzguner *et al* (2008) determined that during 3 weeks of Moracin administration, the blood glucose levels in diabetic rats were decreased. Diabetes caused a significant decrease in the plasma insulin concentration (Table 5) due to the damage caused by the cytotoxic effects of STZ in the pancreatic β cells. This was reversed by the oral administration of moracin. The dysfunction of pancreatic β cells and resulted in the reduction of insulin secretion are further leading to the induction of free radicals. The moracin treatment is serving to decrease the oxidative stress and the damage on essential components of the cells such as lipids, proteins, and DNA by capturing and reducing free radicals (Dixon, *et al* , 1998 and Evans *et al*, 2003).

The lipid peroxidation (MDA) levels were significantly increased by diabetes. This finding is in agreement with the findings of Duzguner *et al* (2008). Dixon *et al* (1998) noted that carotenoids can significantly decrease plasma MDA levels. This finding is not in agreement with the results of the present study. It was determined that lycopene caused a significant decrease of MDA levels in the brain tissues, but not in the plasma (Tables 4 and 5). The increase in NO formation may play a role in the damage of the β cells during the development of diabetes (1994). Seven *et al* (2004) determined that the plasma GSH concentration of STZ-induced diabetic rats increased compared to the control group. Similarly, this study showed that both the NO and GSH levels in the plasma and the GSH levels in brain tissue of the diabetic group were elevated compared to the control group. These increases were lowered in the treatment group compared to the diabetic group (Tables 4 and 5). The SOD activities showed no alterations in the plasma samples or the brain tissue samples among diabetic and treatment groups. This finding is consistent with that of Sindhu *et al* (Sindhu, *et al* , 2004). Additionally, Sindhu *et al* (Sindhu, *et al* , 2004) suggested that hyperglycemia is able to generate ROS, which can either inhibit or have no effect on antioxidant enzyme activities (i.e. SOD). The mRNA transcription levels of the antioxidant enzymes (SOD, CAT, and GSH-Px) were down-regulated by diabetes, and all of them were up-regulated by moracin administration. It was demonstrated that diabetes mellitus caused oxidative damage and lipid peroxidation in the brain tissue and decreased the plasma insulin levels, and these effects were significantly ameliorated by the moracin administrations. The results suggested that moracin has a significant role in silencing diabetic disorders by its antioxidative effects. It is concluded that moracin treatment should be considered in the treatment of diabetic complications and hyperglycemia. Moracin supplementation can be beneficial for humans in order to reduce the harmful effects of diabetes, such as oxidative damage and decreased plasma insulin concentrations and blood glucose levels.

CONCLUSION

The diabetes-related increase in blood glucose levels was found reduced through the provision of Moracin over the period of eight weeks. The levels of plasma nitric oxide (NO) and glutathione (GSH) of brain tissue were found significantly reduced in the Moracin treated group of streptozotocin-induced diabetic rats. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in the hemolysate samples from Moracin treated streptozotocin-induced diabetic rats were observed significantly increased in comparison with untreated to the group of streptozotocin-induced diabetic rats. The superoxide dismutase (SOD) and catalase (CAT) activities in the homogenates of brain tissue of

Moracin treated streptozotocin-induced diabetic rat did not exhibited the significant changes. whereas glutathione (GSH) activity was found increased in the Moracin treated group in comparison with untreated to the group of streptozotocin-induced diabetic rats. The superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) mRNA transcription levels were suppressed in the diabetic group compared to the control. The suppression in superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) mRNA transcription was found stopped through the provision of Moracin. The oxidative damage and low levels of insulin associated with diabetes were ameliorated with the Moracin treatment. The Moracin, the novel compound belong to the mulberry, *Morus alba* (L.) should be introduced in the human diet. The Moracin deserve effectiveness for the diabetic patients.
Moracin, better called mulberry derived medicine, can help manage blood sugar levels in the diabetic individuals. Moracin, incredible variety of herbal formulation lower blood sugar and improve insulin function, but there's a lot to learn to make them work.

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The present attempt is dedicated to the memories of Rolf Luft (29 June, 1914 – 22 May, 2007) Swedish physiologist with endocrinology as special field of research, who like a titan raised the mighty structure of studies in diabetes. Expertise support from Scientific Research Centre through International Journal of Research in Science and Engineering deserve appreciations and exert a grand salutary influence.

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Table – 1: The primers used in the PCR and PCR conditions.

Gene	Primers	Product (bp)	PCR program	Cycles
β-Actin	**F-CATCGTCACCAACTGGGACGA R-CGTGGCCATCTCTTGCTCGAAG**	**466**	**Initial: 95 °C, 10 min / annealing: 95 °C, 1 min – 55°C, 70 s – 72 °C, 100 s / final: 72 °C, 10 min**	**35**
GSH-Px	**F-CTCTCCGCGGTGGCACAGT R-CCACCACCGGGTCGGACATAC**	**290**	**Initial: 94 °C, 5 min / annealing: 94 °C, 30 s – 60°C, 60 s – 72 °C, 30 s / final: 72 °C, 5 min**	**32**
CAT	**F-GGCAGCTATGTGAGAGCC R-CTGACGTCCACCCTGACT**	**116**	**Initial: 94 °C, 5 min / annealing: 94 °C, 30 s – 55°C, 30 s – 72 °C, 15 s / final: 72 °C, 5 min**	**35**
SOD	**F-GTTCCGAGGCCGCCGCGCGT R-GTCCCCATATTGATGGAC**	**192**	**Initial: 94 °C, 5 min / annealing: 94 °C, 30 s – 55°C, 30 s – 72 °C, 20 s / final: 72 °C, 5 min**	**35**

- F: forward primer.
- R: reverse primer.

Table – 2: Effects of Moracin supplementation on the change of body weight (g) in rats.

Week	Control Group	Diabetic Group	Diabetic Group Treated With Moracin	Moracin Group
1	**132.98 (± 2.84)**	**143.16 (± 8.88)**	**138.99 (± 3.02)**	**146.52 (± 3.11)**
2	**143.22 (± 2.62)**	**141.76 (± 7.49)**	**136.42 (± 1.98)**	**158.07 (± 4.82)**
3	**144.86 (± 3.05)**	**138.99 (± 7.98)**	**132.66 (± 2.57)**	**161.86 (± 3.22)**
4	**146.19 (± 3.12)**	**138.21 (± 5.66)**	**133.84 (± 2.51)****	**168.33 (± 2.89)****
5	**152.29 (± 2.75)**	**137.81 (± 6.78)****	**131.86 (± 2.62)****	**168.96 (± 2.77)***
6	**157.73 (± 2.31)**	**128.89 (± 7.24)***	**128.88 (± 2.78)***	**172.75 (± 2.91)****
7	**159.88 (± 3.05)**	**131.68 (± 7.08)***	**130.48 (± 2.78)***	**176.71 (± 2.52)****
8	**168.31 (± 2.96)**	**136.02 (± 6.81)***	**134.24 (± 1.59)***	**182.32 (± 2.57)***

- Each figure is the mean of three replications.
- Figures in parentheses with ± sign are the standard deviations
- *: P< 0.001
- **: P< 0.05 compared to the control.
- *: P < 0.05 compared to the STZ-diabetic group.

Table – 3: Effects of Moracin supplementation on the Plasma glucose concentrations in rats.

Day	Control Group	Diabetic Group	Diabetic Group Treated With Moracin	Moracin Group
1	**130.59 (± 8.61)**	**114.93 (± 4.32)***	**113.32 (± 4.95)**	**134.83 (± 2.77)**
2	**138.57 (± 7.42)**	**452.68 (± 18.51)***	**474.09 (± 11.88)**	**128.57 (± 35.54)**
9	**90.19 (± 8.33)**	**388.64 (± 27.91)***	**386.22 (± 20.31)***	**90.91 (± 2.79)**
16	**108.77 (± 12.47)**	**436.32 (± 46.13)***	**412.48 (± 36.93)***	**115.14 (± 6.63)**
23	**153.72 (± 5.54)**	**411.27 (± 57.91)***	**339.56 (± 67.42)***	**119.18 (± 5.56)**
30	**95.74 (± 4.62)**	**402.18 (± 50.09)***	**253.91 (± 26.73)***	**100.09 (± 5.74)**
37	**104.03 (± 7.12)**	**390.26 (± 46.27)***	**246.64 (± 15.34)***	**105.04 (± 5.23)**
44	**98.27 (± 4.24)**	**412.48 (± 28.11)***	**277.58 (± 23.26)***	**97.97 (± 3.76)**
51	**82.61 (± 16.03)**	**412.28 (± 51.67)***	**252.29 (± 35.4)****	**96.91 (± 5.05)**
58	**104.53 (± 3.26)**	**428.44 (± 37.12)***	**334.51 (± 38.21)***	**95.64 (± 3.46)**

- Unit for Plasma Glucose Concentration: (mg/dL).
- Each figure is the mean of three replications.
- Figures in parentheses with ± sign are the standard deviations
- *: P< 0.001
- **: P< 0.05 compared to the control.
- *: P < 0.05 compared to the STZ-diabetic group.

Table – 4: Effect of moracin administration on the glutathione peroxidase (GPx), glutathione (GSH), malondialdehyde (MDA),superoxide dismutase (SOD), and catalase (CAT) in brain tissue samples.

Group Parameter	Control Group	Diabetic Group	Diabetic Group Treated With Moracin	Moracin Treated Group
GSH-Px (U/mg protein)	**0.94 (± 0.039)**	**1.37 (± 0.045)***	**1.77 (± 0.061)****	**1.18 (± 0.039)****
GSH (nmol/g protein)	**477.85 (± 7.79)**	**496.50 (± 3.15)***	**433.07 (± 4.88)****	**484.08 (± 5.011)***
MDA (nmol/g protein)	**4.90 (± 0.14)**	**6.93 (± 0.21)***	**5.18 (± 0.089)****	**7.02 (± 0.20)*****
SOD (U/mg protein)	**2.41 (± 0.14)**	**2.91 (± 0.008)***	**2.95 (± 0.12)***	**2.53 (± 0.12)*****
CAT (k/g protein)	**0.18 (± 0.013)**	**0.25 (± 0.014)***	**0.27 (± 0.15)****	**0.23 (± 0.012)*****

- Each figure is the mean of three replications.
- Figures in parentheses with ± sign are the standard deviations
- *: P< 0.001
- **: P< 0.05 compared to the control.
- *: P < 0.05 compared to the STZ-diabetic group.

Table – 5: Effect of Moracin administration on plasma MDA, NO, GSH, and insulin in blood/serum samples.

Group Parameter	Control Group	Diabetic Group	Diabetic Group Treated With Moracin	Moracin Treated Group
MDA (µmol/mL)	3.17 (± 0.19)	19.38 (± 0.56)*	20.42 (± 0.43)***	24.02 (± 0.66)**
NO (µmol/L)	37.95 (± 0.79)	61.33 (± 0.91)*	53.98 (± 1.18)***	67.36 (± 1.011)**
GSH (nmol/mL)	659.38 (± 8.58)	664.16 (± 9.37)**	661.01 (± 11.46)**	710.94 (± 5.82)**
Insulin (ng/mL)	4.32 (± 0.82)	0.13 (± 0.01)**	1.92 (± 0.23)**	4.21 (± 0.73)**

- Each figure is the mean of three replications.
- Figures in parentheses with ± sign are the standard deviations
- *: P< 0.001
- **: P< 0.05 compared to the control.
- *: P < 0.05 compared to the STZ-diabetic group.

Table – 6: Effect of Moracin administration on red blood cell GPx, SOD, and CAT.

Group Parameter	Control Group	Diabetic Group	Diabetic Group Treated With Moracin	Moracin Treated Group
CAT (k/g Hb)	4.25 (± 0.62)	8.97 (± 0.63)*	12.05 (± 0.91)**	8.98 (± 0.68)***
GSH-Px (U/g Hb)	0.84 (± 0.016)	0.69 (± 0.022)*	1.04 (± 0.053)**	0.95 (± 0.036)***
SOD (U/g Hb)	119.92 (± 6.82)	217.73 (± 8.19)*	250.91 (± 5.82)**	152.05 (± 4.34)**

- Each figure is the mean of three replications.
- Figures in parentheses with ± sign are the standard deviations
- *: P< 0.001
- **: P< 0.05 compared to the control.
- *: P < 0.05 compared to the STZ-diabetic group.

Table – 7: Proportional gene expression levels of the CAT, GSH-Px, and SOD genes obtained from brain tissues (normalization process based on the average density values of the PCR product bands that were obtained on agarose gel and the density of the target gene, i.e. SOD, CAT, and GSH-Px, proportioned to the density of the control gene, β-actin).

Group Parametere	Control Group	Diabetic Group	Moracin Treated Diabetic Group	Moracin Treated
CAT	2.40 (± 0.83)	2.13 (± 0.91)	7.20 (± 1.07)*	7.46 (± 0.76)
GSH - Px	2.93 (± 0.77)	2.41 (± 0.89)	11.20 (± 1.19)*	9.33 (± 1.01)
SOD	4.26 (± 0.63)	3.46 (± 0.90)	9.86 (± 1.21)*	8.53 (± 0.89)

- Each figure is the mean of three replications.
- Figures in parentheses with ± sign are the standard deviations
- * : P < 0.05 compared with the STZ-induced diabetic group.

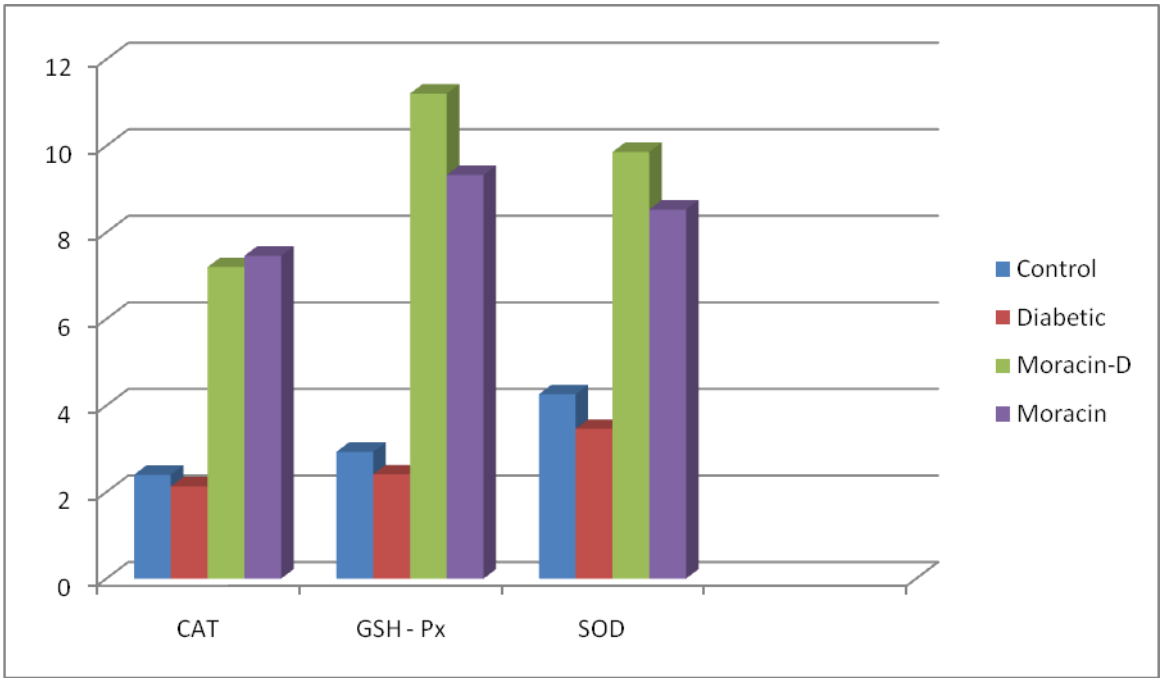

Fig. 1: Proportional gene expression levels of the CAT, GSH-Px, and SOD genes obtained from brain tissues (normalization process based on the average density values of the PCR product bands that were obtained on agarose gel and the density of the target gene, i.e. SOD, CAT, and GSH-Px, proportioned to the density of the control gene, β-actin). *P < 0.05 compared with the STZ-diabetic group. Moracin-D: Moracin Treated Diabetic Group, Moracin: Moracin Treated Group.