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Nephroprotective Influence of Aqueous Decoction of Mulberry Leaves In Hyperglycemia-Induced Oxidative Stress in Brown Rat *Rattus norvegicus* (L).

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Abstract

Streptozotocin induced diabetic male brown rats (*Rattus norvegicus* L.) were divided into five groups, each with 15 individuals. The rats of Group-I served as Normal Healthy group. The Group-II served as streptozotocin induced diabetic rats of solvent treated group. The Group-III served as streptozotocin induced diabetic rats treated with 200 mg/kg decoction of leaves of mulberry, *Morus alba* (L). The Group-IV served as streptozotocin induced diabetic rats treated with 400 mg/kg decoction of leaves of mulberry, *Morus alba* (L). The rats in Group- V were treated with glibenclamide (glyburide) (0.5 mg/kg). All the groups were maintained for three weeks. The blood samples were subjected for bioassays of After 3 weeks, blood samples serum glucose, urea and creatinine. For the purpose to confirm the oxidative damage; attempts on lipid peroxidation and histopathology were conducted. Untreated diabetic rats were found with significant increase in serum glucose, urea and creatinine. Significant rise in lipid peroxidation with a glomerular atrophy and necrotic tubular epithelium in the renal tissue was reported in addition. The rise in serum glucose, urea and creatinine was found ameliorated through the decoction of leaves of mulberry, *Morus alba* (L). The results of the attempt are suggesting that, leaves of mulberry, *Morus alba* (L) are going to serve as effective nutritional supplement to prevent complications of diabetes.

Keywords: Oxidative stress, Kidney, mulberry, *Morus alba* (L), Streptozotocin-induced diabetes, Leaf decoction

INTRODUCTION

The pancreas was first identified by Herophilus (335–280 BC), a Greek anatomist and surgeon *(Howard and John Hess Walter, 2012).* A few hundred years later, Rufus of Ephesus, another Greek anatomist, gave the pancreas its name. It was only in 1889 when Oskar Minkowski discovered that removing the pancreas from a dog caused it to become diabetic (insulin was later discovered by Frederick Banting and Charles Herbert Best in 1921). The pancreas is an endocrine and digestive organ that, in humans, lies in the upper left part of the abdomen. It is found behind the stomach The pancreas is about 15 cm (6 in) long. Anatomically, the pancreas is divided into the head of pancreas, the neck of pancreas, the body of pancreas, and the tail of pancreas. The head is surrounded by the duodenum in its concavity. The head surrounds two blood vessels, the superior mesenteric artery and vein. From the back of the head emerges a small uncinate process which extends to the back of the superior mesenteric vein and ends at the superior mesenteric artery. The neck is about 2.5 cm (1 in) long and lies between the head and the body and in front of the superior mesenteric artery and vein. Its front upper surface supports the pylorus (the base) of the stomach. The neck arises from the left upper part of the front of the head. It is directed first upward and forward, and then upward and to the left to join the body; it is somewhat flattened from above downward and backward. On the right it is grooved by the gastroduodenal artery. The *body* is the largest part of the pancreas and lies behind the pylorus, at the same level as the transpyloric plane. The tail ends by abutting the spleen. The pancreas is a secretory structure with an internal hormonal role (endocrine) and an external digestive role (exocrine). The endocrine part is composed of hormonal tissue distributed along the pancreas in discrete units called islets of Langerhans. Islets of Langerhans have a well-established structure and form density routes through the exocrine tissue. The exocrine part has two main ducts, the main pancreatic duct and the accessory pancreatic duct. These drain enzymes through the ampulla of Vater into the duodenum (*Ionescu-Tirgoviste, et al, 2015).*

Approximately 3 million cell clusters called pancreatic islets are present in the pancreas. Within these islets are four main types of cells which are involved in the regulation of blood glucose levels. Each type of cell secretes a different type of hormone: α alpha cells secrete glucagon (increase glucose in blood), β beta cells secrete insulin (decrease glucose in blood), δ delta cells secrete somatostatin (regulates/stops α and β cells) and PP cells, or γ (gamma) cells, secrete pancreatic polypeptide. These act to control blood glucose through secreting glucagon to increase the levels of glucose, and insulin to decrease it. The islets are crisscrossed by a dense network of capillaries. The capillaries of the islets are lined by layers of islet cells, and most endocrine cells are in direct contact with blood vessels, either by cytoplasmic processes or by direct apposition. The islets function independently from the digestive role played by the majority of pancreatic cells *(Lakey, et al, 2003).*

Diabetes mellitus type 1 is a chronic autoimmune disorder in which the immune system attacks the insulin-secreting cells of the pancreas. Insulin is needed to keep blood sugar levels within optimal ranges, and its lack can lead to high blood sugar. As an untreated chronic condition, diabetic neuropathy can result. Type 1 diabetes can develop at any age but is most often diagnosed before adulthood. For people living with type 1 diabetes, insulin injections are critical for survival. An experimental procedure to treat type 1 diabetes is the transplantation of pancreatic islet cells from a donor into the patient's liver so that the cells can produce the deficient insulin. Diabetes mellitus type 2 is the most common form of diabetes. The causes for high blood sugar in this form of diabetes usually are a combination of insulin resistance and impaired insulin secretion, with both genetic and environmental factors playing an important role in the development of the disease. The management of type 2 diabetes relies on a series of changes in diet and physical activity with the purpose of reducing blood sugar levels to normal ranges and increasing

insulin sensitivity. Biguanides such as metformin are also used as part of the treatment along with insulin therapy *(Longo, et al, 2012).*

Controlling blood sugar (glucose) levels is vitally important. When these levels rise sharply, as they do after ingesting foods with a high glycemic index such as potatoes or sweets, body responds by producing more insulin to deal with the overload. But if this demand for more insulin occurs too strongly too often, the ability of the pancreas to produce enough insulin may become impaired and our cells may become resistant to insulin as it tries to do its job of facilitating glucose transport through the cell walls. The result is insulin resistance a dangerous condition that, if unchecked, leads to type-2 diabetes. Its primary cause is obesity. Generally speaking, if you are obese, your risk for diabetes is high; if not, it‟s low (unless you happen to have a genetic predisposition for diabetes) (Andallu, *et al*, 2001).

More than 170 million people are affected by diabetes worldwide, an incidence estimated to rise 50% by 2030, with the greatest increases set to occur in the developing countries of Africa, Asia and South America (WHO, 2008). In Brazil, 7.6 % of the population aged between 30 and 69 years has diabetes, with the cities of São Paulo and Porto Alegre registering the highest percentages: 9.7 and 8.9 %, respectively (Salgado, 1998). Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. It is associated with an absolute or relative deficiency in the secretion of insulin (Diabetes Mellitus 1, DM1) or with insulin resistance (Diabetes Mellitus 2, DM2) (Savage *et al*., 2007; Stumvoll *et al*., 2005). DM2 is the most common form of the disease, accounting for 85 to 90% of all recorded cases (Tiwari, Rao, 2002). Besides hyperglycemia, hyperlipidemia is involved in the development of the micro and macro vascular complications of diabetes, which are the major causes of morbidity and death (Tang *et al*., 2006). Despite the great efforts made to better understand and manage this disease, serious problems such as retinopathy, nephropathy and lower extremity amputation continue to affect patients, while diabetes-related mortality is rising unabated (Tiwari, Rao, 2002). Insulin resistance can also lead to Metabolic Syndrome (MS), a complex metabolic disease characterized by obesity, hypertension, dyslipidemia and DM2 (Sanchéz-Salgado *et al*., 2007). The current rise in DM2 and MS is believed to be a result of an increase in the sedentary life styles combined with ready access to energy-rich food sources in genetically susceptible individuals (Savage *et al*., 2007).

Treatment of diabetes involves diet control, exercise and the use of hypoglycemic or lipid-lowering drugs such as insulin, sulphonylureas, biguanides and thiazolinediones (Stumvoll *et al*., 2005). However, many oral medicines have a number of serious adverse effects, and the management of hyperglycemia and hyperlipidemia with low side effects is still a challenge for the medical system (Revilla-Monsalve *et al*., 2007). Another problem faced is the cost of the treatment, which is often prohibitively high in developing countries (Schoenfelder *et al*., 2006). In Brazil, some 200 plants are popularly used for the management of diabetes (Barbosa-Filho *et al*., 2005). Such plants are used in formulations of home-made medicines such as teas, decoctions and tinctures (Schoenfelder *et al*., 2006). The main phytochemicals described as useful for treating diabetes are terpenoids, coumarines, flavonoids, alkaloids, phenolic substances and lecitines (Barbosa- Filho *et al*., 2005).

The leaves of Mulberry, *Morus alba* (L.) leaves have long been used in Chinese medicine for the prevention and treatment of diabetes because as we now know it contains chemical compounds that suppress high blood sugar levels (hyperglycemia) following a carbohydrate-rich meal (Miyahara, *et al*, 2004). A research group in Japan has found that, white mulberry leaves contain compounds that inhibit these intestinal enzymes. Mulberry contains 1-deoxynojirimycin (DNJ) and some of its derivatives like alpha-glucosidase inhibitors that have been used as medicines to treat diabetes mellitus (Mudra, *et al*, 2007; Asai, *et al*, 2011). Alpha-glucosidase enzymes in the intestinal lumen and in the brush border

membrane have a main role in carbohydrate digestion of starch and oligosaccharides to monosaccharides before they are absorbed (Zhong, *et al*, 2006). Suppressing the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, this would in turn decrease the absorption of glucose and consequently suppress postprandial blood glucose level elevation (Van Der Laar, *et al*, 2005; Dungan, *et al*, 2006; Bonora and Muggeo, 2014). Recently, several studies in animals and humans have reported that mulberry or sericulture products containing DNJ suppress postprandial increases of glucose (Kimura, *et al*, 2007; Nakamura, *et al*, 2009).

It was reported that, certain nitrogen containing sugars were present in mulberry-leaf extract, notably one called 1-deoxynojirimycin, strongly inhibited the intestinal metabolism of disaccharides (especially sucrose), thereby restricting the amount of monosaccharides entered circulation. They also found that, pretreating the rats with mulberry extract before feeding them carbohydrates significantly suppressed normal postprandial (after-meal) rise in blood glucose levels. This beneficial effect occurred in a dose-dependent manner. Doses were however very large: 0.1-0.5g/kg of body weight for a 70-kg (154-lb) human would be 7-35g. (A lower dose 0.02g/kg, corresponding to 1.4g for a human was ineffective). Nonetheless, researchers suggested that mulberry extract might be beneficial in preventing human diabetes by suppressing intestinal alpha-glucosidase activities (Asano, *et al*, 2001). The air dried leaves and fruits of ficus and mulberry were examined in ethanol and hexane extract and evaluated against hyperlipidemia by estimating the rate limiting enzyme of cholesterol biosynthesis. *Ficus mysorensis* (hexane extract) was evaluated in vivo by lipid profile estimation in hypercholesterodemic rats. Hexane fraction was chromatographed and six isolated compounds were identified. *Ficus mysorensis* recorded hypolipidemic activity (Lee, *et al*, 2007; Awad, *et al*, 2012). Effect of *Morus nigra* (Aqueous extract) on artificially induced diabetic and non-diabetic rats and recorded the lipid profile. In diabetic rats, plant treatment caused reduced MDA, cholesterol, triglycerides and VLDL levels. *Morus nigra* treatment reduced the incidence of internal anomalies in offspring from diabetic rats (Volpato, *et al*, 2011).

Mulberry therapies were conducted on type-2 streptozoticin induced diabetic rats showed improvement in fasting blood glucose levels. Quercetin, the quantitatively major flavonoids glycoside in mulberry leaves effectively suppressed the blood glucose levels (Katsube, *et al*, 2010). Daily consumption of mulberry leaves improved hyperglycemia in diabetic rats and reduced oxidative stress in liver (Kim, *et al*, 2011). Beverages containing mulberry leaf (*Morus alba*) are believed to promote good health, especially people with diabetes in Thailand and the effect of long term administration of an ethanolic extracts of mulberry leaf was studied in blood glucose. Daily administration of 1g/kg of MA for six weeks decreased blood glucose by 22% which was comparable to the effect of 4v/kg insulin. Findings indicated that long term supplement of *Morus alba* has anti-hyperglycemic effects in chronic diabetic rats (Naowaboot, *et al*, 2009; Sun, *et al*, 2011).

Morus nigra leaves were collected from different locations of Jordan and used for the treatment of diabetic symptoms. It was determined by DPPH and ABTS assays in relation to the total phenolic contents of fruit, roots and shoots of mulberry. *Morus nigra* extract and its potential use in radical scavenging made Jordanian population to extensively use the plants as a traditional anti-diabetic agent (Ahme, *et al*, 2008). Antioxidant role of mulberry (*Morus indica*) on the various antioxidant enzymes in rat erythrocytes like glutathione per-oxidase, glutathione reductase, glutathione-S-transferase and super oxide dismutase observed in uncontrolled diabetes were improved by treating with mulberry very efficiently (Andallu, et al, 2003; Fang, *et al*, 2005).
The mulberry is often tried in order to help treat diabetes. It is also tried for treating high cholesterol levels, high blood pressure, the common cold and its symptoms, muscle and joint pain such as from arthritis, constipation, dizziness, ringing in the ears, hair loss, and premature graying. The leaves; bark

and the fruits of Mulberry, *Morus alba* (L.) deserve appreciable medicinal potential. Therefore, tree of mulberry exert a therapeutic influence. In one of the recent attempt in laboratory of author, treating the diabetic experimental animals with mulberry leaf decoction for continuous 14 days was found resulted into lowering the glucose levels in the blood. It was also resulted into restraint glycogen loss in liver, and alteration in histological structure of pancreatic tissues and renal tissues. The Administration of mulberry leaf decoction at the rate twenty grams per liter was found to be able to regulate the altered metabolic processes. Mulberry, *Morus alba* (L.) should be explored as medicinal plant to be used to control the diabetes. Mulberry leaves could be a promising therapeutic option for modulating diabetic risks (Vitthalrao Bhimasha Khyade, 2018). However, further investigations should be performed to substantiate the potential of mulberry leaves in practical uses. Diabetogenic hyperglycemia leads to increased production of reactive oxygen species (ROS). These reactive oxygen species in the form of free radicals are implicated in the complications of diabetes. Various animal studies have proved that hyperglycemia-induced oxidative stress causes a reduction in beta cell mass and impairment in its function (Tokuyama, *et al*, 1995 and Ihara, *et al*, 1999). Ethanolic leaf extract of mulberry, *Morus alba* (L) on 7,12-dimethylbenz(a)anthracene (DMBA)- induced buccal pouch carcinoma in Syrian hamster, *Mesocricetus auratus* (L). Oral squamous cell carcinoma was developed in the buccal pouch of Syrian golden hamsters, by painting with 0.5%
DMBA in liquid paraffin, thrice a week, for 14 weeks. The tumor incidence, volume and burden were determined. Oral administration of ethanol extractives of leaves of mulberry, *Morus alba* (L) at a dose of 300 mg/kg, body weight, to DMBA (on alternate days for 14 weeks)- painted animals significantly prevented the incidence, volume and burden of the tumor. The ethanol extractives of leaves of mulberry, *Morus alba* (L) showed potent antilipidperoxidative effect, as well as enhanced the antioxidant status in DMBA painted animals (Vitthalrao B. Khyade and Sadhana D. Deshpande, 2015).

Epidemiological studies, clinical trials and animal experimental models have proved that dietary supplementation of antioxidants like vitamin E, vitamin C, etc., has reduced the incidence of oxidative damage related disorders such as ageing, cardiovascular diseases, diabetes, inflammation, and neurodegenerative disorder (Stahl, 1997). Ahmadvand (2012) reported that the coenzyme Q10 a natural antioxidant showed significant nephroprotective effect in diabetic rats compared with untreated diabetic animals. Flavonoids (more than 8000) constitute the largest and most important groups of polyphenolic compounds in fruits, vegetables, wine, tea and cocoa (Ross and Kasum, 2002). Recent attention has been focused on the potential use of flavonoids-based drugs for the prevention and treatment of oxidative stress-mediated diseases. Flavonoids can exert their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation. Hence, the present study was done to explore the nephroprotective effect of mulberry decoction against hyperglycemia-induced oxidative stress in streptozotocin induced diabetic brown Rat, *Rattus norvegicus* (L.).

MATERIAL AND METHOD

The study was carried through the steps, which include: Preparation of leaf decoction; Rearing of experimental animals; Induction of Diabetes; Grouping the experimental animals; Mulberry Decoction Treatment; Serum preparation; Tissue sampling; Light microscopic study; Biochemical analysis and Statistical analysis.

(A). Preparation of Leaf Decoction: The eaves of mulberry, *Morus alba* (L,) were collected from mulberry garden of Sericulture Unit, Malegaon Sheti Farm, Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd) Post Box No - 35, Baramati, Pune 413 115, Maharashtra, India and were identified, confirmed in the “Dr. APIS” Laboratory (Shrikrupa Residence, Teachers Society, Malegaon

Colony, Baramati Dist. Pune – 413115 Maharashtra, India) The leaves of mulberry, *Morus alba* (L,) were kept in a dry and ventilated place, protected from light. The leaves were shade dried and powdered. The decoct (20 g/L) was prepared daily, boiled for 10 minutes and when reaching room temperature, the solution was filtered and used for administration to the rats as a drinkable solution.

(B). Rearing of Experimental Animals: Male rat of the species *Rattus norvegicus* (L.), weighing 164.97 ± 11.69 g (mean ± SEM, n= 70), bred in "Dr. APIS" Laboratory, were used in the present attempt. The rats were housed in an environmentally-controlled room with a 12 h light: 12 h dark cycle (lights on at 7:00 am) at a constant temperature of 23 ℃. All the experimental procedures were carried out in accordance with the guidelines of the National Centre for Laboratory Animal Sciences (NCLAS) of National Institute of Nutrition (NIN) Hyderabad India. The experimental protocol was approved by the Research Ethics Committee of the National Centre for Laboratory Animal Sciences (NCLAS).

(C). Induction of Diabetes:
After two weeks of acclimatization, the diabetes was induced in rats with Streptozotocin (STZ, Sigma Chemical Company). 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).
(D). Grouping the Animals (Design of the Attempt):
The design of the experimentation as per the guidelines explained by Lal, *et al* (2012). After diabetic state was confirmed, the rats were placed in one untreated control group and four into the four experimental groups (n=15 animals/ group). Thus total five groups of animals were made.
The rats of Group-I served as Normal Healthy group.
The Group-II served as streptozotocin induced diabetic rats of solvent treated group.
The Group-III served as streptozotocin induced diabetic rats treated with 200 mg/kg decoction of leaves of mulberry, *Morus alba* (L).
The Group-IV served as streptozotocin induced diabetic rats treated with 400 mg/kg decoction of leaves of mulberry, *Morus alba* (L).
The rats in Group- V were treated with glibenclamide (glyburide) (0.5 mg/kg).

(E). Mulberry Decoction Treatment:
The mulberry decoction treatment was carried out everyday morning using intragastric tube for 3 weeks after induction of diabetes. The intragastric tube is a feeding tube. It is a medical device used to provide nutrition to experimental animals (and also to the patients that cannot obtain nutrition through mouth) The state of being fed by a feeding tube is called gavage, enteral feeding or tube feeding. The feeding tube used in the attempt were usually made of polyurethane.

(F). **Serum preparation**

The whole blood was collected from rats of each group in sterile, covered test tubes and labeled. After collection of the whole blood, it was allowed to clot undisturbed for 15-30 mins. The clot was removed

by centrifuging at 1000-2000 ×*g* for 10 mins in a centrifuge. The supernatant serum was obtained for biochemical analysis.

(G). Tissue sampling

The animals were sacrificed by cervical dislocation and the kidneys from all the groups of rats were dissected out. They were processed for histopathological examination (HPE) and biochemical analysis.

(H). Light microscopic study

For histopathological study the rat tissues were perfused with 10% formalin. The kidneys of the rats were excised immediately from the abdominal cavity and fixed in 10% neutral formalin, dehydrated in graded alcohol (80-100%), cleaned in xylene and embedded in paraffin. Then renal tissues were sliced into 3-5 μm pieces with a rotary microtone, deparaffinized in xylene, passed through varying grades of alcohol and finally stained with hematoxylin and eosin for histopathological assessment. The specimens were evaluated with a light microscope. All histopathological changes were examined by pathologist.

(I). Biochemical analysis

Blood urea was estimated by urease method and serum creatinine was estimated by modified Jaffe's method (Allain, *et al*, 1974). Concentration of thiobarbituric acid reactive substances in the tissues was estimated by the method of Niehaus and Samuelsson (Niehaus and Samuelsson, 1968). Reduced glutathione in the tissues were estimated by the method of Ellman (Ellman. 1959). Superoxide dismutase (SOD) in the tissues was assayed by the method of Kakkar *et al*, and the activity of catalase (CAT) in the tissues were determined by the method of Sinha (Kakkar, *et al*, 1984; Sinha, 1972).

(J).Statistical Analysis:

The results were expressed as mean ± S.E.M. (standard error). Comparisons were made using one-way ANOVA followed by Duncan's and Bonferroni's *post-hoc* tests. In all comparisons, values of $p < 0.05$ were considered statistically significant. Statistical tests were performed using the SPSS program (Statistical Package for Social Sciences, version 10.0, for Windows https://spss.en.softonic.com/).

RESULTS AND DISCUSSION

The results on Influence of Leaf Decoction of Mulberry, *Morus alba* (L.) on Streptozotocin Induced Diabetes in Brown Rat, *Rattus norvegicus* (L.) are explained parameter-wise. The parameters include: Physiological Parameters; Glucose level; Lipid Peroxides and Antioxidants; Serum urea and creatinine and Histopathology

Effect of Leaf Decoction of Mulberry, *Morus alba* (L.) on blood glucose levels in Streptozotocin Induced Diabetes in Brown Rat, *Rattus norvegicus* (L.):

The glucose readings were compared to readings obtained for diabetic control rats and mulberry decoction treated rats. In streptozotocin induced diabetic rats, the blood glucose levels remained significantly high for all the three weeks of study. The mulberry decoction treated rats showed a significant reduction in blood glucose levels from the second week after induction. The mulberry decoction treated rats showed maximum reduction in blood glucose level on twenty first day at 400 mg/kg. In glibenclamide treated rats, blood glucose levels were significantly lower for all the three weeks of the study (Table 1).

Effect of Leaf Decoction of Mulberry, *Morus alba* (L.) on Lipid Peroxides and Antioxidatants in Kidneys in Streptozotocin Induced Diabetes in Brown Rat, *Rattus norvegicus* (L.):

Significantly higher level of Lipid Peroxides was reported in the diabetic rats in comparison with normal healthy individuals. The diabetic rats treated with mulberry decoction showed significant reduction in the level of Lipid Peroxides. The antioxidant levels were significantly ($p<0.05$) higher in diabetic rats treated with mulberry decoction in a dose-dependent manner in comparison with untreated diabetic rats. The Lipid Peroxides level in glibenclamide treated rats was significantly lower than the diabetic and mulberry decoction treated rats in the study. The glibenclamide treated rats also showed significant increase in antioxidant levels compared with diabetic rats (Table 2).

Effect of Leaf Decoction of Mulberry, *Morus alba* (L.) on Serum Urea and Creatinine in Streptozotocin Induced Diabetes in Brown Rat, *Rattus norvegicus* (L.):

The serum urea and creatinine levels were increased significantly in streptozotocin induced diabetic rats, compared to normal healthy control rats at the end of third week in the study. The rats treated with mulberry decoction after induction of diabetes showed significant ($p<0.05$) reduction in serum urea and creatinine in a dose-dependent manner compared to untreated diabetic rats. Glibenclamide treated rats also showed significant ($p<0.05$) reduction in serum urea and creatinine compared with diabetic rats (Table 3).

Hitopathological Observation:

Compared to the photomicrograph of renal cortex of normal healthy rats (Figure 1), the diabetic rats showed renal cellular injury, necrotic tubular epithelium with widening of Bowman's capsule and the glomerular atrophy (Figure 2). Diabetic rats treated with mulberry decoction at 200 mg/kg showed severe glomerular atrophy and necrosis of tubular epithelial cells (Figure 3). However at mulberry decoction 400 mg/kg, the renal cortex showed only mild swelling and congestion in the renal epithelium. There was no necrosis and the glomerular atrophy (Figure 4). In diabetic rats treated with glibenclamide, the renal cortex showed moderate degeneration of renal epithelium, atrophied renal corpuscle and mild glomerular capillaries congestion (Figure 5).

The findings of the Diabetic Complications and Control Trial (DCCT) and The United Kingdom Prospective Diabetic Study support the concept that chronic hyperglycemia plays a causative role in the pathogenesis of diabetic micro and macrovascular complications (Triplitt, *et al*, 2011). Diabetic nephropathy is the leading contributor to end-stage renal disease. Hyperglycemia leads to increased production of ROS or superoxide in the mitochondria from glucose auto-oxidation, protein glycosylation and glucose metabolism via sorbital pathway. The ROS and glycosylation lead to disruption of cellular function, oxidative damage to the membrane and other structures, susceptibility to LPO and exhaustion of antioxidant defences. Al-Enazi had reported rise in LPO in hepatic cells due to hyperglycemia induced by streptozotozin in rats. (Al-Enazi, 2014). In the present study also the diabetic rats showed an increase in LPO and decreased in antioxidants in the renal tissue. The oxidative damage to renal cell was further confirmed by increase in serum urea and creatinine in diabetic rats.

There are reports that natural antioxidants such as vitamin E, caffeic acid, lipoic acid, quercetin, melatonin and natural phenolic compounds have protective effects against hyperglycemia-induced oxidative stress (Balkis, *et al*, 2009; Garfinkel, *et al*, 2011). Treatment of diabetic animals with silymarin significantly reduced LPO and increased the levels of SOD, glutathione reductase and CAT. SOD protects tissues against oxygen free radicals by scavenging oxygen. Thus, SOD can act as a primary defense against oxygen and prevents further generation of free radicals (Arivazhagan *et al*., 2000). CAT

protects tissues from highly reactive OH radicals (Sozmen *et al*., 2001). Mulberry decoction by scavenging the free radicals and by raising the antioxidant activities prevented the renal damage and maintained the cell integrity. The Diabetic Complications and Control Trial (DCCT demonstrated that the improvement of glycemic control reduced microalbuminuria (39%) and clinical nephropathy (54%) (Powers, 2005). Varzi *et al*. (2007) had reported that silymarin improved alteration in serum creatinine concentration in gentamicin treated dogs (Varzi *et al*., 2007). In the present attempt also mulberry decoction treated diabetic animals showed a decrease in serum urea and creatinine, which confirms its nephroprotective effect. Next step on this line to plan to study the cytoprotective action of mulberry decoction against hyperglycemia-induced oxidative stress. The histopathological study of renal tissue of diabetic rats showed the glomerular atrophy with necrosis of tubular epithelium. This damage was due to hyperglycemia. In mulberry decoction treated diabetic rats there was restoration of glomerular structure, with only mild congestion in tubular epithelium.

CONCLUSION

This attempt proved that the leaf decoction of mulberry, *Morus alba* (L.) had significant nephroprotective effect against the hyperglycemia-induced oxidative stress in Streptozotocin induced diabetes Brown Rat, *Rattus norvegicus* (L.). The excellent safety; ease in it's availability and low cost of leaf decoction of mulberry, *Morus alba* (L.) are the adavantages. Hence, leaf decoction of mulberry, *Morus alba* (L.) may be added as an adjuvant therapy for preventing or slowing the progression of diabetic nephropathy.

Table 1: Influence of mulberry leaf decoction on blood glucose levels in streptozotocin induced diabetic Brown Rat, *Rattus norvegicus* (L.).

Day after Treatment Group	Day 1 (mg/dl)	Day 17 (mg/dl)	Day 14 (mg/dl)	Day 21 (mg/dl)
Normal Healthy	78.01±4.45^{d}	78.01±4.45^{e}	78.01±4.45^{e}	78.01±4.45^{e}
Diabetic Solvent treated	206.33±2.58^{b}	188.83±4.83^{a}	205.17±9.52^{a}	206.00±10.95^{a}
Diabetic Treated With Mulberry Decoction (200 mg/kg)	202.83±8.73^{b}	175.68± 5.15^{b}	158.50±9.39^{b}	126.34±1.64^{b}
Diabetic Treated With Mulberry Decoction (400 mg/kg)	209.43±11.4^{a}	172.18±2.98^{c}	144.84±6.22^{c}	119.68±3.11^{c}
Diabetes Treated With	198.68±5.14^{c}	154.34±3.45^{d}	132.18±6.16^{d}	107.86±2.16^{d}

Glibenclamide (0.5 mg/kg)				

Values are expressed as means±SD for six rates in each group. Values not sharing a common superscript differ significantly at p≤0.05 (Dunken's test). SD: Standard deviation

Table 2: Influence of mulberry leaf decoction on Lipid Peroxidation and Antioxidants in kidneys in streptozotocin induced diabetic Brown Rat, *Rattus norvegicus* (L.).

Parameter Group	SOD (unit/mg protein)	GSH (µg/mg protein)	CAT (µmol/mg protein)	Lipid Peroxidation (mmole/100 g tissue)
Normal Healthy	16.01 ± 0.36^{a}	32.73 ± 0.87^{b}	20.52 ± 0.98^{a}	3.56 ± 0.37^{d}
Diabetic Solvent treated	5.12 ± 0.59^{e}	8.11 ± 0.22^{e}	3.39 ± 0.47^{e}	16.58 ± 0.38^{a}
Diabetic Treated With Mulberry Decoction (200 mg/kg)	11.13 ± 0.72^{d}	20.38 ± 0.44^{d}	17.93 ± 0.73^{c}	8.74 ± 0.91^{b}
Diabetic Treated With Mulberry Decoction (400 mg/kg)	15.32 ± 0.47^{b}	30.58 ± 0.79^{c}	17.74 ± 0.68^{a}	8.18 ± 0.46^{c}
Diabetes Treated With Glibenclamide (0.5 mg/kg	13.87 ± 0.13^{c}	32.08 ± 1.19^{a}	18.26 ± 0.65^{b}	3.43 ± 0.73^{e}

Values are expressed as mean±SD for six rates in each group. Values not sharing a common superscript differ significantly at p≤0.05 (Dunken's test). SD: Standard deviation, LPO: Lipid peroxides, SOD: Superoxide dismutase, GSH: Glutathione, CAT: Catalase

Table 3: Influence of mulberry leaf decoction on Serum Urea and Serum Creatinine in streptozotocin induced diabetic Brown Rat, *Rattus norvegicus* (L.).

Parameter Group	Urea(mg/dl)	Creatnine (mg/dl)
Normal Healthy	27.13 ± 1.78^{e}	0.59 ± 0.06^{e}
Diabetic Solvent treated	75.16 ± 4.31^{a}	1.78 ± 0.11^{a}
Diabetic Treated With Mulberry Decoction (200 mg/kg)	49.46 ± 0.98^{b}	1.66 ± 0.13^{b}
Diabetic Treated With Mulberry Decoction (400 mg/kg)	31.31 ± 0.57^{d}	0.64 ± 0.05^{d}
Diabetes Treated With Glibenclamide (0.5 mg/kg	31.42 ± 0.78^{c}	0.73 ± 0.03^{c}

Values are expressed as mean±SD for six rates in each group. Values not sharing a common superscript differ significantly at $p\leq0.05$ (Dunken's test). SD: Standard deviation

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