

# Leaf Decoction of Mulberry, *Morus alba* (L.) for management of Streptozotocin Induced Diabetes in Brown Rat, *Rattus norvegicus* (L.)

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## ABSTRACT

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. The objectives of the present attempt were to analyze the effect of decoction of leaves of mulberry for treating streptozotocin-induced diabetic brown rat, *Rattus norvegicus* (L.). The physiological, biochemical and histological parameters were used for analysis. Induction of diabetes in experimental animals was carried out through giving the injection of streptozotocin chemical. The dosages of this streptozotocin chemical compound utilized in the attempt was seventy milligram per kilogram weight of the experimental animal. The streptozotocin was well mixed in the buffer of citrate, pH of which was 4.5. The intraperitoneal (IP) injection of streptozotocin was given to the experimental animals, brown rat, *Rattus norvegicus* (L) to one week prior to the treatment of decoction of leaves of mulberry. Streptozotocin exert diabetic influence through distinct characters. These characters include: polydypsia, excessive urination, excessive ingestion, excessive level of glucose in blood, excessive level of triglycerides in blood and histopathological modifications in the pancreatic, hepatic and renal tissues. The water treated individuals were provided 100 ml of water for each day. Experimental animals in treated group were provided with 100 ml of decoction of leaves of mulberry, *Morus alba* (L), strength of which was twenty grams per liter. This schedule of treatment was continued for 14 days. 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 leaf decoction at the rate twenty grams per liter was found to be able to regulate the altered metabolic processes. The demonstrates the medicinal use of mulberry leaf decoction to control or management of diabetes. 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. However, further investigations should be performed to substantiate the potential of mulberry leaves in practical uses.

**Keywords :** Streptozotocin-induced diabetes, Mulberry, *Morus alba* (L.) , Leaf decoction

## I. INTRODUCTION

The credit of identification of pancreas goes to Herophilus, the Greek anatomist and surgeon (335-280 BC) (Howard and John Hess Walter, 2012). There after, name pancreas was suggested by Rufus of Ephesus, another Greek anatomist. Oskar Minkowski in 1889 reported, "Removal of the pancreas from a dog, is responsible for exerting a diabetes". Latter on, Frederick Banting and Charles Herbert Best (1921) reported that, deficiency of insulin (the secretion of pancreas) leads to the diabetic condition. The insulin secretion of is distributed to all the organs through the blood. It is therefore, pancreas is considered as endocrine glands. In the body of human pancreas is situated in the abdominal cavity, in the fold made by stomach and duodenum. The length of pancreas in healthy body measures about fifteen centimeters or six inches in length. Anatomical parts of pancreas include: Head; Neck and Body. Concavity established by duodenum enclose the head part of pancreas. This head part of pancreas is provided with superior mesenteric artery and superior mesenteric vein. In between the head and the main body, there is a neck, measuring about 2.5 cm ( $\pm$  0.003). The body of pancreas is large. Pylorus, the base of stomach is supported by the neck of pancreas. The neck of pancreas is arising from the left upper portion of the proximal region of the head. Direction of the neck of pancreas is firstly to upward and then to forward. From upper side, the neck of pancreas is appearing somewhat flattened or compressed. On the right side, the neck of pancreas is showing groove enclosing gastro-duodenal artery. The body of pancreas is its largest part. The body of pancreas lies posterior to the pylorus. It is at the same level as the transpyloric plane. The spleen is abutted by tail piece of pancreas. The pancreas is deserving both endocrine and exocrine role. The islets of Langerhans are working for endocrine role of the pancreas. They are in the form of discrete units. Islets of Langerhans have a well-established structure and form density routes

through the exocrine tissue. According to Ionescu-Tirgoviste, et al (2015), the exocrine part of pancreas is largest and equipped with secretory acini. Islets of Langerhans are in the form of clusters of the cells in the amongst the acini. There are approximately, about three million cells in a single Islet of Langerhans. There are four different types of cells in a single cluster of islets of Langerhans. These cells in a single cluster of islets of Langerhans are involved in the maintenance of levels of sugar in the form of glucose in the blood. Each type of cell deserve capability of secretion of a different type of hormone. For example,  $\alpha$  alpha cells secrete glucagon. This glucagon is responsible to increase glucose in blood. The  $\beta$  beta cells secrete insulin. The insulin is responsible to decrease glucose in blood. The  $\delta$  delta cells secrete somatostatin. This somatostatin regulates the working of  $\alpha$  and  $\beta$  cells). The gamma or pp-cells, secrete pancreatic polypeptides. All these cells act to control blood glucose through secreting glucagon to increase the levels of glucose, and insulin to decrease it. In the pancreas of rat, there is epsilon, one more type of cell is present in the islet of Langerhans. This epsilon cell secrete ghrelin. This ghrelin is also called as Lenomorelin. In addition to regulate the appetite, this ghrelin or Lenomorelin also have a significant role of energy regulating; energy distribution and rate of use of energy (Burger and Berner, 2014). Histologically, the islets are covered by crisscrossed dense network of capillaries. The capillaries of the islets are lined by layers of islet cells. There is direct contact between the islet cells; blood vessels and the blood. This direct contact between the blood and islet cells is either by cytoplasmic processes or by direct apposition. According to Lakey, et al (2003); the working of islets is independent. They are working as if they are independent endocrine glands. The diabetes mellitus type 1 is recorded as chronic autoimmune disorder. In this diabetes mellitus, the immune system of the body use to attack the beta cells, the insulin-secreting cells of the pancreas. The hormone insulin is essential for keeping level of blood sugar within optimal ranges.

Deficiency of insulin lead to increase the level of blood sugar. The diabetes of type 1 can develop at any age. It is most often diagnosed before adulthood. For people living with diabetes of type 1, injection of insulin is the basic need are their life. Transplantation of cells of islet of pancreas into the body of individual of diabetes of type 1 alone serve the purpose of treatment. This needs the perfect donors of cells of islets of pancreas. This transplantation is generally, in the liver. Such liver become able to produce the insulin.

Diabetes mellitus of type 2 is the most common form of diabetes disease. In this type, there is a combination of insulin resistance and impaired insulin secretion. It is caused through both genetic and environmental factors. The management of diabetes of type 2 relies on a series of changes in diet and physical activity. These changes are for the purpose of reducing blood sugar levels to normal ranges. It also help for increasing insulin sensitivity. According to Longo, et al (2012), the biguanides such as metformin are used as part of the treatment. Insulin therapy should be along with the use of biguanides, such as metformin. Controlling blood sugar (glucose) levels is vitally important. When these level of sugar (glucose) rise sharply (as they do after ingesting foods with a high glycemic index such as potatoes or sweets), the healthy (with reference pancreas) body responds through the production of required amount of insulin. This is to face the situation of increased level of blood sugar (glucose). If the demand of secretion of more insulin occurs too strongly and too often, the pancreatic capability of production of sufficient insulin may become impaired. In this situation, the body cells may become resistant to insulin. Though the insulin is available, the condition of "Insulin Resistance" of the body cell hamper the sugar metabolism. The condition of "Insulin Resistance" may leads to diabetes of type-2. The primary cause of diabetes of type-2 is the obesity. Obesity have had a risk of diabetes of type-2. (Andallu, et al , 2001).

Day by day, the diabetes is become worldwide. It's intensity is increasing. According to the report of WHO (World Health Organization), there are more than 170 million individuals with diabetes as major disease. Further, this WHO (World Health Organization) is postulating that, by 2030, fifty percent of world population will be of diabetic group. Further, the World Health Organization (WHO) is putting the remark of "Greatest increased set of diabetic individuals is going to appear in the developing countries of Africa, Asia and South America (WHO, 2008). In Brazil, status of diabetes for the population of the age group of thirty to sixty nine years is about seven to eight percent. The highest percentage of diabetic individuals in the city São Paulo was about nine to ten percent. The highest percentage of diabetic individuals in the city Porto Alegre was about eight to nine percent (Salgado, 1998). This Diabetes mellitus is thus becoming a chronic metabolic disorder. It is characterized by hyperglycemia (increased level of sugar in the form of glucose). It is also causing the disturbances in metabolism of carbohydrate (sugar), fat (lipids) and protein. The Diabetes is further concerned with creating the condition of "Absolute or Relative Deficiency in the Ability of Secretion of Insulin". This condition is also recognized as Diabetes Mellitus -1 or DM - 1. It is also concerned with resistance for insulin. The condition of resistance for insulin is also recognized as "Diabetes Mellitus - 2 ( or DM-2)" (Savage et al., 2007; Stumvoll et al., 2005). The condition of resistance for insulin or "Diabetes Mellitus - 2 ( or DM-2)" (or Diabetes of DM-2 type) is the most common form of the diabetes. According to Tiwari and Rao (2002), this second type of diabetes is about eighty five to ninety percent in the population. According to Tang, et al (2006), the morbidity and death are through hyperglycemia, hyperlipidemia, and the vascular complications due to diabetes at micro and macro levels vascular complications of diabetes (Tang et al., 2006). According to Tiwari and Rao (2002), mortality through diabetes is rising

unabated. According to Sánchez-Salgado et al. (2007), insulin resistance is responsible for Metabolic Syndrome (MS). In this syndrome, there is a complexity in the metabolism. The characters like increase in blood pressure, disturbances in lipid metabolism, increase in body weight appear in this "Metabolic Syndrome" or "MS". It may also leads into second type of diabetes (DM-2) (Sánchez-Salgado et al., 2007). The modern life style is responsible for diabetes mellitus of second type (DM-2) and Metabolic Syndrome (MS) (Savage et al., 2007).

The control on the diet, regularity in physical exercise and the use of medicines of hypoglycemic or lipid-lowering category are involved in the treatment of diabetes. The commonly used medicines in diabetes treatment include: insulin, sulphonylureas, biguanides and thiazolidinediones (Stumvoll et al., 2005). Most of the oral medicines used for diabetes treatment, are with number of serious adverse effects. According to Revilla-Monsalve et al (2007), less side effects in the management for "Increased blood sugar level" and "Disturbed lipid metabolism" is still a challenge for the system of medical treatment. One more problem facing regarding the cost of the treatment. In developing countries, the cost of medicines for diabetes is high. In future, it is going still higher. The ever increasing cost of medicines for diabetes can not be prohibited (Schoenfelder et al., 2006). Herbal treatment seems to be the best answer to face each and every problem in treatment of diabetes like diseases. Barbosa-Filho et al (2005) reporting two hundred herbal sources popularly utilized for the management of diabetes in Brazil. Brazil is utilizing the herbal sources for the management of diabetes through home-made herbal formulations. The formulations like preparation of tea; preparation of decoction; tincture are some of the home-made medicines in Brazil (Schoenfelder et al., 2006). The major phytochemicals derived from herbal sources for treating the diabetes include: terpenoids, coumarines,

flavonoids, alkaloids, phenolic substances (Barbosa-Filho et al., 2005).

The Chinese people use the mulberry as a medicine. This history of practice of utilization of "mulberry-leaves for treating the diabetes" goes long back. Miyahara, et al (2004), reported the use of "mulberry-leaves are used for the management of diabetes". The chemical contents in the leaves of mulberry are responsible for suppressing high level of sugar in blood (hyperglycemia) of the individual patient of the diabetes. The contents of leaves of white mulberry are with capabilities of inhibition of action of some of the enzymes in human intestine. The contents like 1-deoxynojirimycin (DNJ) and chemical compound: alpha-glucosidase inhibitors in the white mulberry is well established fact (Mudra, et al , 2007; Asai, et al , 2011). This fact may be responsible for Chinese to consider the mulberry leaves for utilization in treating the diabetes. In addition to the digestive enzymes, the human intestine is with the capability of production of "Alpha-glucosidase enzymes". This alpha glucosidase enzyme deserve significant work in the starch-digestion. The alpha glucosidase digest the starch and oligosaccharides into monosaccharides before they are subjected for absorption (Zhong, et al , 2006). If the action of alpha glucosidase like enzymes get suppressed, it may results into delaying the digestion of starch. Simple sugars like glucose also remain being absorbed due to suppression of action of alpha glucosidase. Consequently, there is lagging behind tendency of levels of sugar (glucose) in postprandial condition (Van Der Laar, et al , 2005; Dungan, et al , 2006; Bonora and Muggeo, 2014). The content of DNJ in the mulberry leaf is well established fact. According to Kimura, et al (2007) and Nakamura, et al (2009), 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).

Asano, et al (2001) reported that, sugars with nitrogen contents are present in the extract of leaves of mulberry. One of the mulberry leaf sugar with nitrogen contents is 1-deoxynojirimycin. This 1-deoxynojirimycin deserve activity of strong inhibition of metabolism of disaccharides (especially sucrose). The inhibition of action of 1 – deoxynojirimycin is responsible for restriction of the amount of monosaccharides entered in blood circulation. Asano, et al (2001) also found that, pre-treating 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). Both, leaves and fruits of ficus and mulberry were air dried and processed for extraction through use of ethanol and hexane as solvents. These extractives were evaluated against hyperlipidemia by estimating the rate limiting enzyme for the biosynthesis of cholesterol. The hexane extractives of *Ficus mysorensis* 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). The therapies through “Mulberry” were conducted on type-2 streptozotocin induced diabetic rats. This attempt reported 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).

In one of the attempt, Ahme, et al (2008) collected leaves of mulberry, *Morus nigra* (L) from different locations of Jordan and utilized 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* (L) 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). On this much background, the study has planned.

## II. MATERIAL AND METHODS

The attempt on use of leaf decoction of mulberry, *Morus alba* (L.) to control the streptozotocin induced diabetes in brown rat, *Rattus norvegicus* (L.) was carried through the steps, which include: Preparation

of decoction of leaves of mulberry; Rearing of experimental animals brown rat, *Rattus norvegicus* (L.); Induction of Diabetes among the experimental animals brown rat, *Rattus norvegicus* (L.) ; Grouping the experimental animals brown rat, *Rattus norvegicus* (L.) and treatment; Biochemical analysis; Histological analysis and Statistical analysis of the data.

(A). Preparation of Decoction of Leaves of Mulberry, *Morus alba* (L):

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 were allowed to dry in shade. Such shade dried leaves were processed for preparation of leaf-powder. The mulberry leaf-powder was utilized for the preparation of decoction. Known quantity of mulberry leaf-powder was mixed in known volume of water. This content was kept for boiling . The boiling was carried out for ten minutes. After boiling, the content was allowed to reach the room temperature. It was the filtered through muslin cloth. Filtrate was the utilized for treating the experimental animals in the attempt. The drinkable form of decoction was used in treating the experimental animals. Such decoction was prepared daily fresh before treating the experimental animals. The strength of decoction was twenty grams per liter.

(B). Rearing of Experimental Animals Brown Rat, *Rattus norvegicus* (L):

The male brown rat, *Rattus norvegicus* (L), weighing about  $164.96 \pm 11.72$  g (mean  $\pm$  SEM, n= 71), bred in "Dr. APIS" Laboratory, were used in the present attempt. The rats were kept in the commercially available rat-cages. And such rat cages were kept in

laboratory. The laboratory was made environmentally controlled. Twelve hours of light and twelve hours of dark condition was maintained. The constant temperature of 23°C was maintained in the laboratory of rat cages. The instructions for rearing of rats in laboratory, received from the National Centre for Laboratory Animal Sciences (NCLAS) of National Institute of Nutrition (NIN) Hyderabad India were followed for the purpose to rear the rats in laboratory. The experimental protocol was approved by the Research Ethics Committee of the Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Dist. Pune , India.

(C). Induction of Diabetes Among the Experimental Animals Brown Rat, *Rattus norvegicus* (L):

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. The solution of streptozotocin was prepared. The strength of streptozotocin used for single individual of rat was seventy milligrams per kilogram body weight. 0.1 M citrate buffer of pH 4.5 was used for the preparation of solution for injection of streptozotocin. Before giving the streptozotocin injection, rats were allowed for overnight fasting (Kesari et al., 2007). The intraperitoneal injection of streptozotocin was given to individual rat in the respective group. After this, the rats were allowed for regular feeding. Blood samples were collected one week after the injection of streptozotocin. The orbital sinus of the rats was selected for the collection of blood samples. The blood samples were the processed for assay of sugar level in blood. The individual rat with fasting blood glucose (FBG) levels measuring above 200 mg/dL (11.1 mmol/L) were designated as diabetic individual and selected for the further experimental protocol. Such streptozotocin induced diabetic rats were allowed to drink the decoction of leaves of mulberry, *Morus alba*

(L). The treating the rats with mulberry leaf decoction was carried for twenty one days. During a this twenty one days period of treatment, the rats were fed at the rate of forty grams per day with pellets of food procured from Hindusthan Animal Feeds forty grams per day (**Hindustan Animal Feeds**, Behind Gokulnagar Octroi Check Post, Near Vijaynagar Railway Crossing, Jamnagar – 361004 Gujarat INDIA).

(D). Grouping the Experimental Animals Brown Rat, *Rattus norvegicus* (L) and Treatment:

After diabetic state was confirmed, the rats were placed in four experimental groups (n=15 animals/group), which include:

Group – ( I ) WTND (Water Treated Non-diabetic Control Rats) ( treated with vehicle water): For each day, individual rat was treated with hundred milliliters of water.

Group – (II) WTSID (Water Treated Streptozotocin STZ - Induced Diabetic Rats): For each day, individual rat was treated with hundred milliliters of water.

Group – (III) DTND (Decoction Treated Non-diabetic Control Rats): For each day individual rat was treated with hundred milliliters of mulberry leaf decoction strength of which was twenty grams per litre.

Group – (IV) DTSID (Decoction Treated Streptozotocin STZ - Induced Diabetic Rats): For each day, individual rat was treated with hundred milliliters of mulberry leaf decoction strength of which was twenty grams per litre.

This schedule of treatment for all the groups in the attempt was continued for 14 days.

(E). Biochemical Analysis:

On fifteenth day, the rats were transferred from experimental cages to individual metabolic cages. Here, in metabolic cages, rats were kept for further seven days. In this “metabolic week”, readings

pertaining daily body weight; amount of food and the drink and volume of the total amount of food and drink consumed by the experimental animals in the attempt were noted. This was carried daily. In addition, the readings pertaining amount urine volume released by each individual, in each group, for each day were noted. This was carried daily. Blood sample collection from individual experimental animals was carried out at the end of each week. The eye-venous pool was utilized for blood sample collection. Before blood collection, the individual rat was anaesthetized through use of exposure to ether for thirty seconds. The blood collection was carried through the use of microcentrifuge tube (Eppendorfs-tube). Addition of sodium citrate of 0.1 mL of volume was made in each microcentrifuge tube (Eppendorfs-tube). Use of sodium citrate was to prevent coagulation of collected blood. Sodium Citrate is working as anti-clotting agent. The blood the subjected for centrifugation. Plasma separated through centrifugation was transferred in separate tube and stored in refrigerator. This storage was for the purpose to of analysis of biochemical parameters. After twelve hours of fasting, on the last day of the treatment, the rats were used for dissection. Provision of anesthesia through mild ether was made. Decapitation was used for rat scarification. In the body of rat, liver is the largest and massive gland. It is located below the diaphragm and just above the stomach. Rat liver is with four lobes. Through the falciform ligament, liver is attached to diaphragm. The four liver lobes in rat include: right lobe; left lobe; quadrate lobe and the caudate lobe. The quadrate lobe of liver lies more ventral than other lobes. The quadrate lobe is the largest and caudate lobe is the smallest. All the four lobes of liver were separated. Likewise, the pancreas and pancreas were also separated fro the body. Puncturing the heart was used for blood sample collection. Addition of sodium citrate of 0.1 mL of volume was made. After centrifugation, the plasma was separated and stored in refrigerator. These tissues samples were used for biochemical analysis. The

amount of sugar (in the form of glucose) in the blood was estimated determined through method involving the glucose oxidase. This glucose assay was carried out through method using "Glucose Diagnostic Kits HK-Liquiform (Labtest Diagnostica)". The triglycerides from plasma of the blood was assayed through the use of diagnostic kit "Triglycerides GPO-ANA Cholesterol liquiform (Labtest Diagnostica). For the purpose to determine glycogen, the samples of liver were utilized. The method utilized for glycogen determination was the method of Geary, et al (1981). The method of "Aluminum Tongs and Stored Frozen" was followed for the estimation of glycogen content of liver tissue was estimated (van Handel, 1965). The glycogen content was expressed in the unit: mg/g of tissue.

(F). Analysis of Histological Preparations:

Preservation of tissues, like liver, pancreas and kidneys was carried out through the use of ten percent formaldehyde. Keeping the tissue in ten percent formaldehyde was carried for twenty four hours. Then, the tissues were processed through the method outlined by Tolosa et al. (2003). The tissues were blocked in Histosec. The sections of 7  $\mu$ m-thickness on slide were obtained. Microtome (Microtome RM 2025), stained with hematoxylin-eosin, and analyzed under an optical microscope (Leica CME) were utilized. The samples of the tissues were processed for also staining with Gomori's aldehyde-fuchsin. This stain Gomori's -Aldehyde -Fuchsin is for identification of the effect of procedure for identifying the effects of the treatments on  $\beta$  cells (Tolosa et al., 2003; Diani et al., 2004). The photographs were taken using a digital camera (SONY DSC-W1) attached to the microscope.

(G). Analysis of the data through the use of methods in Statistics:

The whole attempt was revised for three times. This revision was for the purpose to obtain consistency in the results. The collected data was analyzed through

the well known methods of Statistical methods. The mean  $\pm$  S.E.M. (standard error) were used for the expression of the results obtained in the present attempt. The Analysis of Variance (ANOVA) was used for comparison of the results of experimental groups with the control group. It was 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/>).

### III. 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; Biochemical Parameters and Histopathological Parameters. The results are summarized in table - 1; 2 ;3; presented in Figure 1 and Figure 2.

(A). Physiological Parameters:

The weight Gain (g) ( $\Delta W$ ) in the Water Treated Non-diabetic Control Rats (WTNDC) was 157.76 ( $\pm 16.16$ ). The weight Gain (g) ( $\Delta W$ ) in the Decoction Treated Non-diabetic Control Rats (DTNDC) was 177.90 ( $\pm 16.89$ ). The weight Gain (g) ( $\Delta W$ ) in the Water Treated Streptozotocin STZ - Induced Diabetic Rats (WTSID) was 54.108\* ( $\pm 17.66$ ). The weight Gain (g) ( $\Delta W$ ) in the Decoction Treated Streptozotocin STZ - Induced Diabetic Rats (DTSID) was 27.00\* ( $\pm 11.58$ ). The rats of non-diabetic untreated control and water treated control groups were recorded increase in the whole body weight. The rats of diabetic group recorded lower body weight gain. The significance of weight gain in the diabetic rats was  $p > 0.05$ .



The tissue somatic index of liver is also termed as "Hepatosomatic Index (HSI)". The weight of liver is divided by the weight of entire body. The quotient thus obtained was multiplied by hundred. This process yields the "Hepatosomatic Index" for given individual animal. This The index is the indication on status of energy reserve in an animal. In animals like fishes, the hepatosomatic index is lower. That is to say, in fishes, there is less energy reserved in the liver. This index is designating the liver weight with reference to weight of whole body. The present attempt is reporting nil significant difference in the hepatosomatic index among the individuals of experimental groups (brown rat, *Rattus norvegicus* L. Statistically, it was  $p > 0.05$ . This observation was for the group treated with decoction of leaves of mulberry, *Morus alba* L.) (Table – 1). The rats in group of diabetes recorded significant increase ( $p < 0.05$ ) for the the amount of food material ingested. This was during the last week of treatment (Table I). The amount of fluid ingested and the flux of urine release in both diabetic groups was found increased ( $p < 0.05$ ). This was in relation to the two control groups. The rats with diabetes (STZ induced) shown symptoms, which include: Gain in body weight; exceed Hunger; intake of food in large amount; exceed thirst (or polydypsia) and excessive passage of urine as reported by Cavalli *et al.*, 2007 and Dobrzynski *et al.*, 2002. The rats of diabetic untreated group exhibited no diuretic effect. Feature of mulberry leaf decoction used in the study was absence of diuretic effect. This observation seems to the significance of the study. That is to say, the decoction of leaves of mulberry, *Morus alba* (L.) was without diuretic influence. Most of the decoctions including tea and the medicines taken for diabetes management are with diuretic influence as a common side effect. The dosage of final ingestion of the decoction of leaves of mulberry, *Morus alba* (L.) by the rats of control group was 0.738 g/mL . The dosage of final ingestion of the decoction of leaves of mulberry, *Morus alba* (L.) by the rats of diabetic group was 1.67 g/mL.

#### (B). Biochemical Parameter:

The streptozotocin (STZ) destroy all cells of pancreatic islets. Therefore, it is highly probable that one will obtain blood glucose levels over 300 mg/dL or even more if they are fed at libitum. Glucose level in blood plasma of the rats with streptozotocin induced diabetes, one week after the induction of diabetes was observed significantly increased ( $p < 0.05$ ) (Figure 1). This observation was in both the groups: WTSID (Water Treated Streptozotocin STZ - Induced Diabetic Rats) and (DTNDC Decoction Treated Non-diabetic Control Rats) with reference to the group of control individuals. The individual experimental animal with glucose in blood measuring above 13.876 mmol/L (250 mg/dL) was considered as individual of diabetes. Both, Untreated control and water treated control shown no significant variation with reference to the glucose contents in blood sample during the experimental period ( $p > 0.05$ ). At the end of the period of treatment of mulberry leaf decoction, the diabetic group individuals shown twenty six percent reduction in the blood glucose level. This observation is in agreement to that described by Biavatti *et al.* (2004). In 2004, Biavatti *et al* recorded fifteen percent reduction in blood glucose level of glucose in the diabetic rats received herbal decoction of Gabiroba, *Campomanesia xanthocarpa* Berg. According to Salgado *et al.* (2007) and Cavalli *et al.* (2007), the glucose level reduction ranging about 12 – 20 is the expected reading for management of diabetes through herbal formulation. This inference by Salgado *et al.* (2007) and Cavalli *et al.* (2007) was through series of pharmacological investigations involving hypoglycemic plants. The level of blood glucose in rats of the non-diabetic group treated with decoction of leaves of mulberry, *Morus alba* (L.), have shown no significant difference.

The total cholesterol in blood plasma in all groups evaluated in present study, exhibited no significant changes. However, in WTSID (Water Treated

Streptozotocin STZ - Induced Diabetic Rats) and DTNDC (Decoction Treated Non-diabetic Control Rats), levels of triglycerides in blood (Table - 2) had increased significantly ( $p < 0.05$ ) in relation to the WTNDC (Water Treated Non-diabetic Control Rats) by period of finishing the experimental work. The levels of lipids in serum are reported higher in the individuals with Diabetes Mellitus by the researchers like Kesari *et al.*, 2007; Pushparaj *et al.*, 2007 and Savage *et al.*, 2007. The significant risk factor of coronary heart disease is concerned with higher serum lipid level. According to Pushparaj *et al.* (2007) and Savage *et al.* (2007), significantly high serum lipid level is supposed to be the results of action of lipolytic hormones on fat deposits without embarrassment.

The hypercholesterolemia, also called high cholesterol. It is the presence of high levels of cholesterol in the blood. It is a form of high blood lipids and hyperlipoproteinemia (elevated levels of lipoproteins in the blood). High level of triglycerides in the blood leads to Hypertriglyceridemia. The increased levels of triglycerides are associated with atherosclerosis, even in the absence of hypercholesterolemia (high cholesterol levels), and predispose to cardiovascular disease. Very high triglyceride levels in blood also increase the risk of acute pancreatitis (Berglund, *et al.*, 2012). According to Pushparaj *et al.* (2007), the conditions of both, higher cholesterol and higher triglycerides may also occur in streptozotocin or STZ-treated rats. Only triglycerides exhibited a significant increase in the group of rats treated with streptozotocin, whereas the decoction of leaves of mulberry, *Morus alba* (L.) exerted no significant changes the levels of triglyceride in present attempt of study. These observations are parallel with the observations of Biavatti *et al.* (2004). Feeding the rat with hypercaloric was reported for significantly higher level of serum lipids. Biavatti *et al.* (2004) recorded no significant changes due to herbal decoction treatment on the lipid levels in such rats (of rats with

significantly higher level of serum lipids). The very first step on this line is to carry out further pharmacological studies on use of mulberry leaves for diabetes management and then to encourage further pharmacological studies. The decoction of leaves of mulberry, *Morus alba* (L.) should not be encouraged until further pharmacological studies have been undertaken.

Quantity of glycogen in liver cells, among different groups of rats in present attempt is presented in table - 2. The Water Treated Non-diabetic Control Rats (WTNDC) exhibited reduction in the liver cell glycogen, measuring about thirty six percent. The streptozotocin induced diabetic rats treated with mulberry decoction were shown the similar results on liver cell glycogen level to that of the group of animals of untreated control group ( $p > 0.05$ ). The hepatic cells have a crucial working. They are keeping the energy homeostasis during feeding/fasting transitions. Skeletal muscles, generally referred as "the peripheral tissue" is concerned with postprandial (resulted soon after meal), insulin-stimulated disposal of glucose. The cells of liver are concerned with buffering action for ingested carbohydrate. This is achieved through suppressing the output of glucose from the liver cells and followed by conversion of glucose into glycogen for storage in liver cells. In the state of "fasting", "Hepatic-Glycogen-Storage" is instantly changed for the purpose to maintain glucose in circulating blood. glucose concentrations, representing around fifty percent. The endogenous glucose production in the first hours of fasting, while gluconeogenesis accounts for the remaining fifty percent (Savage *et al.*, 2007).

There was thirty six percent reduction in liver cell glycogen reported in present study. This is in comparison with control group. This observation is not surprising. This is distinguishing character of diabetic patients. Deficiency of insulin-hormone is responsible for becoming smaller rate of formation of

glycogen in the liver cells. Of course, the smaller velocity of formation of glycogen in the hepatic cells get reflect into enhanced rate of deposition of glucose in plasma of the blood. In addition, glucagon levels are high in such condition. This condition is further responsible for stimulation of glycogenolysis (Breaking down the glycogen for glucose) and gluconeogenesis (production of glucose from non-carbohydrate source). Both, glycogenolysis and gluconeogenesis are responsible for reduced glycogen in liver cells (Jhiang, Zhang, 2003). It is now possible to explain the reason for "Reduced glycogen levels in diabetic patients". The quantitative contents of glycogen in liver cells (Hepatic Glycogen) in rats of diabetic group treated with the mulberry decoction and in rats of control, in present attempt observe similar. This observation is suggesting that the mulberry leaf decoction treatment is able to restore or preserve liver glycogen. Isn't it? Grover *et al.* (2002) reported the results of similar type effects in one attempt on treating the diabetic mice with extractives of *Eugenia jambolana* (L). The herbal formulation, say for example the decoction of leaves of mulberry, *Morus alba* (L) as in present attempt, possibly deserve stimulatory action and exert a grand secretory influence on pancreatic islet cells. The working of  $\beta$  cells through mulberry leaf decoction may either through so called "the insulinomimetic action" of a contents of herbal source utilized. According to Oliveira *et al.* (2008) and Paula, *et al* (2010) anti-diabetic action of herbal source may occur through the stimulation of insulin signaling pathways in peripheral tissues.

#### (C). Histopathological Parameters:

The results on histomorphological changes through the provision of decoction of leaves of mulberry, *Morus alba* (L) are presented in Figures 2 and 3. The islets of pancreas of the rats of control groups exhibiting features of normal healthy condition. The islets of normal healthy pancreas are observing oval in outline (Figure 2A). further, the islets of normal

healthy pancreas are appearing to spread uniformly through the exocrine part of pancreas. This observation in present attempt is exactly similar to the explanation cited by Tang *et al.* (2006). According to Diani *et al.* (2004), Gomori stain as appearing in Figures 2B and 2C, is demonstrating exact position of different types of islet cells. The  $\beta$  cells of islets are occupying the central region of the islets while some the cells of  $\beta$  type in islets of Langerhans are in the zone of periphery. There appear green colour in the cytoplasm of these cells of  $\beta$  type. This green colour is confirming the presence of  $\beta$  cells. According to Diani, *et al* (2004), the cytoplasm of  $\beta$  cells is granular. These cytoplasmic granules in  $\beta$  cells belong to insulin granules. In the untreated diabetic rats (Figure 2D), The islets of Langerhans are appearing to get smaller or shrunken. The smaller size or shrunken nature of these islets of Langerhans may be due to reduction in their diameter. It may yield into irregular shape of islets of Langerhans. The cell cytoplasm is showing vacuolization. Tang *et al.* (2006) recorded similar type of pattern of response in untreated diabetic group of experimental animals. The islets of Langerhans stained with Gomori-stain (Figure 2E) were without green colour. This is indicating absence of insulin granules. The islets of Langerhans in the diabetic group of animals treated with the decoction of mulberry leaf decoction (Figure 2F) are exhibiting regular shape. Presence of green coloured matter in this figure is indicating the presence of insulin. This observations are self explanatory. The present finding is suggesting that, the treatment of STZ-diabetic rats with leaf decoction of mulberry, *Morus alba* (L) can reduce some of the damage induced by streptozotocin (STZ). The  $\beta$  cells of islet, in diabetic condition, generally get destroyed. The science of diabetes is of the opinion, "It is practically impossible to regenerate  $\beta$  cells once they are destroyed". The herbal extractives obtained from *Pterocarpus marsupium* (L), however, deserve the capability of regenerating the destroyed the  $\beta$  cells of islet (Ahmed *et al.*, 1998). Further studies is needed to confirm such a novel

activity for the decoction of leaves of various herbal sources including mulberry, *Morus alba* (L).

#### IV. CONCLUSION

Phytochemical contents found in leaf decoction of *Morus alba* (L.) indicates its potential as an important source of medicine. It may also be used for the improvement of condition of human health. Mulberry, *Morus alba* (L.) is a significant and potential source for curing debilitating diseases. The aims of the present attempt were to screen the effects of fourteen days treatment of mulberry leaf decoction (at the rate twenty grams per liter) to non-diabetic and streptozotocin-induced diabetic brown rat, *Rattus norvegicus* (L.). The treatment of diabetic rats using the decoction of Mulberry, *Morus alba* (L.) (20 g/L) was found decreasing the blood glucose levels, inhibited hepatic glycogen loss, and prevented potential histopathological alterations in the pancreas and kidneys. Administration of leaf decoction of Mulberry, *Morus alba* (L.) (20 g/L) was found to be able to regulate the altered metabolic processes. The results suggest that Mulberry, *Morus alba* (L.) leaf decoction (20g/L) is useful for diabetes mellitus management. Mulberry, *Morus alba* (L.) should be explored as medicinal plant to be used to control the diabetes. Efficient use of leaf decoction of Mulberry, *Morus alba* (L.) may open a new avenue in the field of treating the diabetes.

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**Table 1.** Physiological parameters of control and diabetic rats treated with the leaf decoction of Mulberry, *Morus alba* (L.). DTSID: Decoction Treated Streptozotocin STZ - Induced Diabetic Rats.

Groups Parameters	WTNDC (Water Treated Non-diabetic Control Rats).	DTNDC (Decoction Treated Non-diabetic Control Rats).	WTSID (Water Treated Streptozotocin STZ - Induced Diabetic Rats).	DTSID (Decoction Treated Streptozotocin STZ - Induced Diabetic Rats).
ΔW(g) (Weight Gain)	157.76 (± 16.16)	177.90 (± 16.89)	54.108* (± 17.66)	27.00* (± 11.58)
Hepatosomatic Index (liver weight as percent of body weight)	3.253 (± 0.17)	3.263 (± 0.18)	3.021 (± 0.15)	3.061 (± 0.15)
Ingested Food (g/day)	21.826 (± 0.47)	25.414* (± 0.26)	30.331*a (± 0.28)	35.107*a (± 0.24)
Ingested Drink	38.411	37.218	85.769*	83.415*

(mL/day)	(± 2.31)	(± 2.011)	(± 4.45)	(± 1.07)
Urine (mL/day)	7.273 (± 2.43)	7.231 (± 2.39)	39.901* (± 1.22)	42.441* (± 1.55)

- Each figure is the mean of the three replications.
- Figure with ± sign in the bracket is standard deviation.
- ΔW: Weight gain, difference between the final and initial weights of the rats.
- The values are expressed as mean ± S.E.M.
- \*: Statistically significant difference compared to water control group (p < 0.05).
- a: Statistically significant difference compared to decoct control group (p < 0.05).

**Table 2.** Biochemical parameters in plasma and liver of control and diabetic rats treated with the leaf decoction of Mulberry, *Morus alba* (L.).

Groups Parameters	WTNDC (Water Treated Non-diabetic Control Rats).	WTSID (Water Treated Streptozotocin STZ - Induced Diabetic Rats).	DTNDC (Decoction Treated Non-diabetic Control Rats).	DTSID (Decoction Treated Streptozotocin STZ - Induced Diabetic Rats).
Total Cholesterol (mmol/ L)	1.027 (± 0.079)	1.237 (± 0.123)	1.084 (± 0.068)	1.085 (± 0.109)
Triglycerides (mmol/ L)	0.446 (± 0.041)	0.581 (± 0.061)	0.691* (± 0.078)	0.697* (± 0.069)
Liver Glycogen (mg/g of tissue)	149.41 (± 20.18)	124.33 (± 20.21)	96.384* (± 15.88)	132.09 (± 27.31)

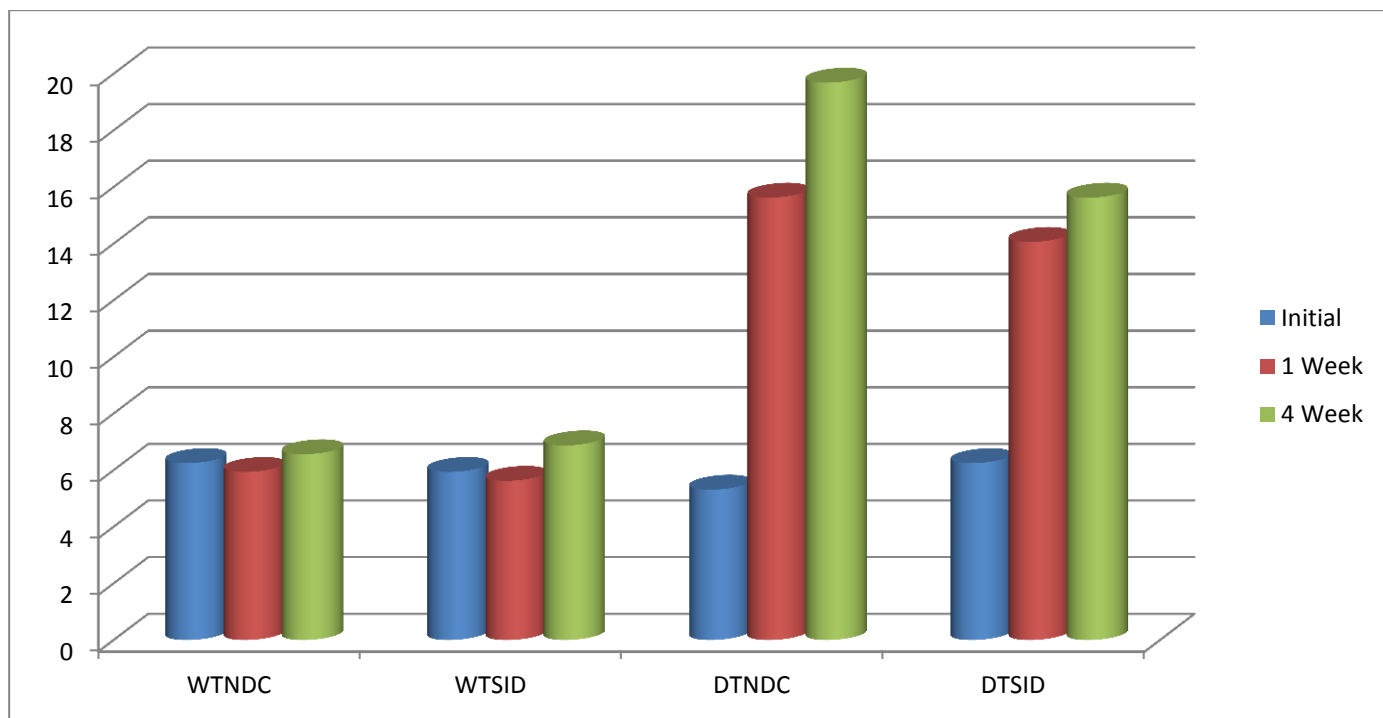
- Each figure is the mean of the three replications.
- Figure with ± sign in the bracket is standard deviation.
- \*: Statistically significant difference compared to water control group (p < 0.05).
- a: Statistically significant difference compared to decoct control group (p < 0.05).
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**Table 3.** Plasma Glucose Levels (mmol/L) in control and diabetic rats treated with the leaf decoction of Mulberry, *Morus alba* (L.).

Interval Group	Initial	1 Week	4 Weeks
WTNDC ( Water Treated Non-diabetic Control Rats).	6.251 (± 0.481)	5.937 (± 0.457)	6.562 (± 0.507)
WTSID (Water Treated Streptozotocin STZ - Induced Diabetic Rats).	5.938 (± 0.477)	5.625 (± 0.449)	6.875 (± 0.553)
DTNDC (Decoction Treated Non-diabetic Control Rats).	5.312 * (± 0.786)	15.625* (± 0.617)	19.687* (± 0.963)
DTSID	6.249*	14..062*	15.625*

(Decoction Treated Streptozotocin STZ Induced Diabetic Rats).	(± 0.849)	(± 0.857)	(± 0.784)
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- Each figure is the mean of the three replications.
- Figure with ± sign in the bracket is standard deviation.
- \*: Statistically significant difference compared to water control group (p < 0.05).
- a: Statistically significant difference compared to decoct control group (p < 0.05).



**Figure 1. Plasma Glucose Levels (mmol/L)** in control and diabetic rats treated with the leaf decoction of Mulberry, *Morus alba* (L.).

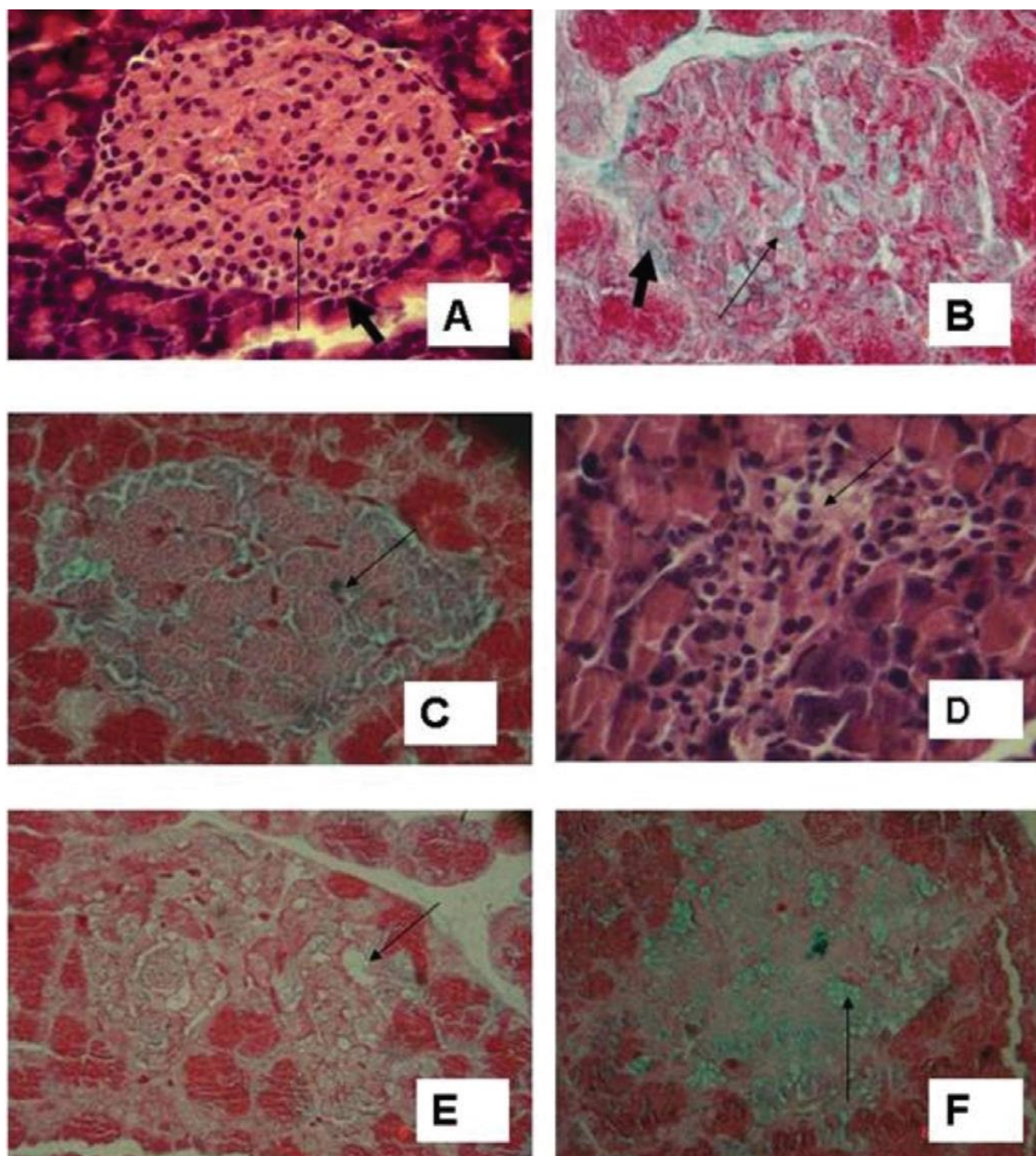
WTNDC : Water Treated Non-diabetic Control Rats.

WTSID: Water Treated Streptozotocin STZ - Induced Diabetic Rats.

DTNDC: Decoction Treated Non-diabetic Control Rats.

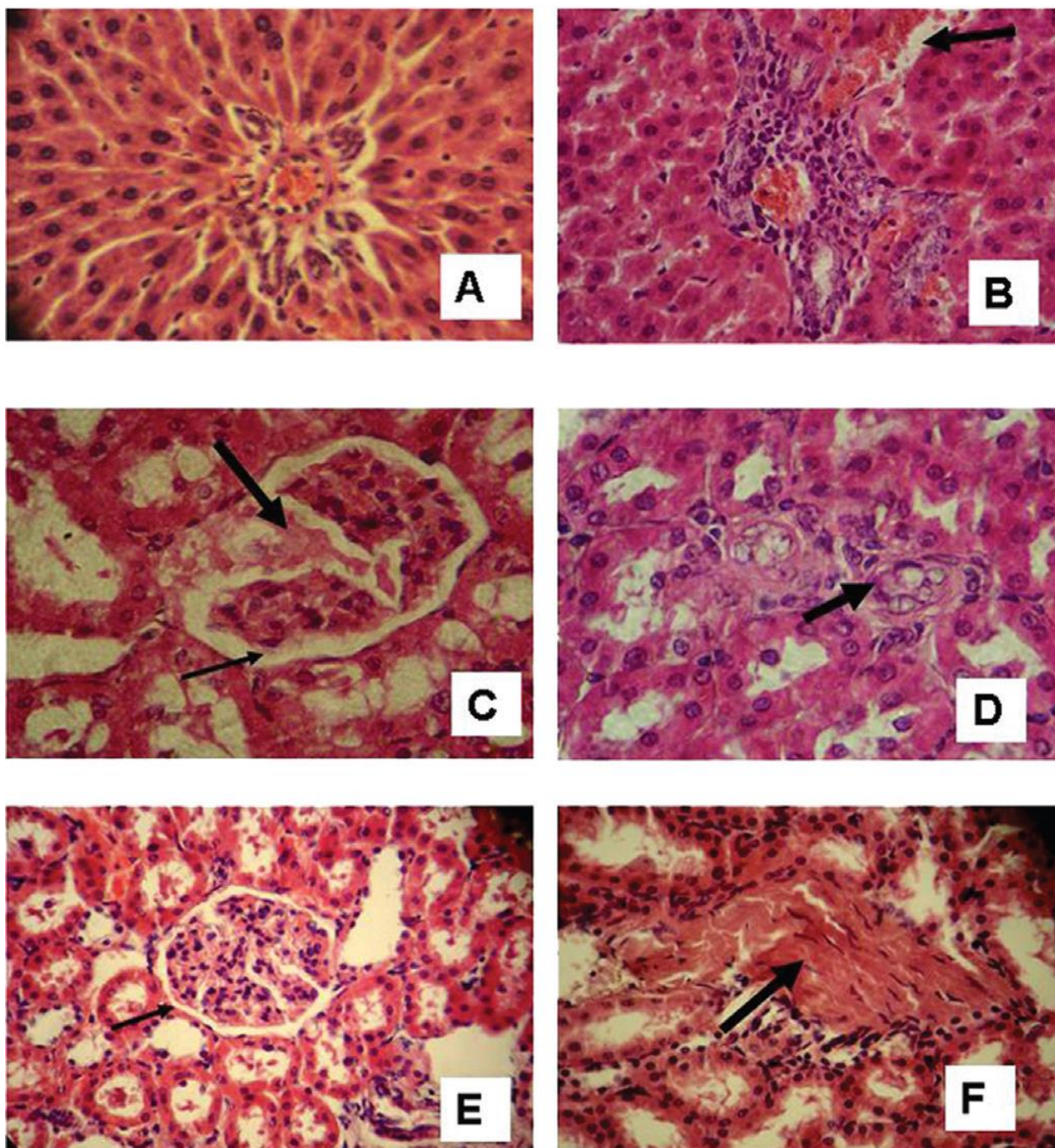
DTSID: Decoction Treated Streptozotocin STZ - Induced Diabetic Rats.





**Figure 2 .** Photomicrographs (400X) showing effects of different treatments on histopathological parameters of pancreatic islets. **A:** Photomicrograph of normal pancreatic islet from water control group, islets cells (thin arrow), conjunctive tissue (large arrow), HE stained. **B:** Photomicrograph of normal pancreatic islet from water control group, Gomori-stained, showing insulin granules (green spots) within  $\beta$  cells (thin arrow), conjunctive tissue (large arrow). **C:** Photomicrograph of normal pancreatic islet from decoct treated control group, Gomori-stained displaying insulin granules within  $\beta$  cells (arrow). **D:** Pancreatic islet from untreated diabetic group displaying altered shape and cytoplasmic vacuolization (arrow), HE stained. **E:** Pancreatic islet from untreated diabetic group stained with Gomori, displaying altered shape, cytoplasmic vacuolization (arrow) and absence of  $\beta$  cells, 400X. **F:** Pancreatic islet from diabetic group treated with decoct, Gomori-stained, displaying less altered shape and presence of  $\beta$  cells with insulin granules (arrow).





**Figure 3.** Effects of the different treatments on histopathological parameters of liver and kidneys: A: Normal liver from water control group, HE-stained, showing portal triad, 250X. B: Liver from diabetic animal with inflammatory process in the portal triad and enlarged stroma (arrow), 250X. C: Kidney from diabetic rat treated with water displaying altered glomerular morphology, inflammatory process in renal papilla (large arrow) and dilatation of tubules (thin arrow), 400X. D: Kidney with concentric fibrosis from diabetic rat treated with water (arrow), 400X. E: Kidney with normal glomerular morphology (arrow) from diabetic rat treated with decoct, 400X. H: Fibrotic scar (arrow) in renal cortex of diabetic rat treated with decoct, 250X.