Glucose lowering effect of aqueous extract of
*Bauhinia tomentosa* L. on alloxan induced type 2 diabetes mellitus in wistar albino rats

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**ABSTRACT**

The study was designed to evaluate the anti-diabetic effect of aqueous extract of *Bauhinia tomentosa* L. leaf on alloxan induced Wistar albino rats. Diabetes was induced in albino rat models with alloxan monohydrate (150mg/kg body weight). Aqueous leaf extract of *Bauhinia tomentosa* at the dose of 300 mg/kg was orally administered once a day for 30 days to the diabetic animals. In this study, glyceremic parameters, lipid parameters and serum enzymes were reduced whereas the level of high-density lipoprotein-cholesterol was elevated. The extract significantly increased the total protein and glycogen level in the liver of diabetic rats. Furthermore, the liver carbohydrate metabolizing enzymes were normalized by the administration of the extracts. Histopatholgical examination results of liver, pancreas and kidney were normal in general. The above results indicated the anti-diabetic efficacy of the *B.tomentosa* leaf extract.

**INTRODUCTION**

Diabetes mellitus is a common metabolic and endocrine disorder, characterized by chronic hyperglycemia and disturbance of carbohydrate, fat and protein metabolism associated with absolute or relative deficient of insulin secretion or insulin action [1]. It is a metabolic disorder principally characterized by chronic and persistent hyperglycemia [2]. It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. According to the World Health Organization (WHO) projections in 2005, the 30 million to 33 million diabetes in India will go up to 40 million by 2010 and 74 million by 2025. WHO has issued a warning that India will be the diabetic capital of the world [3].

Different types of oral hypoglycemic agents such as biguanides and sulphonyl urea are available along with insulin for the treatment of diabetes mellitus [4]. But side effects were associated with their uses [5, 6]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even WHO approves the use of plant drugs for different diseases, including diabetes mellitus. Ethno botanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [7].

*Bauhinia tomentosa* L. is a well known, traditional plant used in folklore medicine in tropical regions around the world and especially in greater parts of India. There are about 600 species of *Bauhinia* L. in the tropical regions [8]. *Bauhinia tomentosa* is a fast-growing plant that grows up to 4m in height. Leaves are divided into two lobes, light green in colour, with a leathery texture. Infusion of fresh flowers and barks were used for dysentery. Decotion of root bark was used for liver problems and leaves were externally applied to the forehead for fevers. Large number of *Bauhinia* species were analysed for their antidiabetic effect but the antidiabetic effect of *B.tomentosa* is scarce. So this study aimed in finding the antidiabetic potential of *B.tomentosa*.

**MATERIALS AND METHODS**

**Plant material and extraction**

*Bauhinia tomentosa* leaves were collected from a local farm and authenticated by Dr.G.V.S.Moorthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore. The Voucher No is BSI/SRC/73/5/23/09-10/Tech-723 and the specimen was deposited in the department herbarium. After washing with water the leaves were dried at 25°C for 10 days in the absence of sunlight and powdered well using a mixer. Then they were weighed and kept in an airtight container and stored in refrigerator until future use.

**Animals**

Healthy adult albino rats of both sex, weighing approximately 150-180g were procured from animal house of Karpagam University, Coimbatore and used for the study. Rats were housed at constant temperature of 22±5°C with a 12-hour light, 12-hour dark cycle. Before and during the experiment, rats were fed with standard diet. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

**Induction of diabetes**

Diabetes induction was done by single intra peritoneal injection of alloxan monohydrate (150 mg/kg) in saline. The hyperglycemia was confirmed after 72 hrs by the elevation of blood glucose and the behavioral changes (Excess thirst and frequent urination).The rats with blood glucose level more than 250 mg/dl were considered as diabetics and used for the study.

**Experimental design**

A total of 25 rats (15 diabetic surviving rats, 10 normal rats) were used. Rats were divided into five groups containing five animals in each group. Group I served as untreated control. Group II was diabetic control. Group III and Group IV were diabetic rats received orally administration of standard drug glibenclamide (5mg/kg) and aqueous leaf extract of *B.tomentosa* (300mg/kg) respectively. Group V was normal rats treated with *B.tomentosa* (300mg/kg) alone. The treatment groups were given the extract through the oral gastric tube for a period of 30 days.
**Blood Collection**
After the study period, the animals were kept overnight fast and sacrificed under light chloroform anesthesia. Blood was drawn from the ventricles and centrifuged. Serum was separated and analyzed for various biochemical estimations. Liver, pancreas and kidney were excised immediately, washed with ice cold saline stored in 10% formalin and 0.9% saline, for histopathological and biochemical studies respectively.

**Biochemical Estimations**
Commercial diagnostic kits were used to estimate blood glucose level colorimetrically. Sigma Diagnostics Pvt Ltd, Baroda, India. Lowry et al (1951) method was used for estimation of protein in serum and liver [10]. Serum lipid profile, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), serum urea, creatinine, proteins and albumin content were determined by standard procedures in an auto analyzer using Ecoline kits(E.Merck,Mumbai,India). Liver glycogen was estimated [11] and carbohydrate metabolic enzymes of liver like glycogen phosphorylase [12], Glucose-6-phosphatase [13] and Hexokinase [14] were also analysed. From the lipid profile, atherogenic index (AI) and coronary risk index (CRI) were calculated.

Atherogenic index (AI) = \( \frac{LDL \text{ cholesterol}}{HDL \text{ cholesterol}} \)
Coronary risk index (CRI) = \( \frac{Total \text{ cholesterol}}{HDL \text{ cholesterol}} \)

**Statistical analysis**
All the values were expressed as Mean ± SD of five individual experiment and the statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version (10.0) and the individual comparisons were obtained by the Duncan multiple range test (DMRT) [15]. A value of p<0.05 was considered to indicate a significant difference between the groups.

**RESULTS AND DISCUSSION**

### Changes in body weight
As shown in Figure 1, control rats were found to be stable in their body weight whereas in diabetic rats there was a significant decrease in the body weight. The reduction of body weight in diabetes may be attributed due to insulin insufficiency. This results in starvation of cell for glucose which leads to the utilization of structural proteins [16, 17]. Diabetic treated rats brought about improvement in body weights in group III and IV, indicating the beneficial effect of glibenclamide and B.tomentosa respectively in preventing loss of body weight in diabetic rats due to its ability to prevent the destruction of structural proteins.

### Change in glucose, insulin, hemoglobin and glycosylated hemoglobin (HbA1c)
Table 1 shows significant increase in glucose, glycosylated hemoglobin and significantly reduction in insulin and hemoglobin (p<0.05) in alloxan treated diabetic rats. T he standard antidiabetic drug glibenclamide and aqueous extract of P.edulis (200mg/kg) significantly reduced (p<0.05) the blood glucose level in diabetic rats, but not to the level of control rats. Alloxan induce diabetes mellitus with a single dose administration which cause selective necrosis of pancreatic β-cells of islets of langerhans that initiate insulin deficiency which end up with increase in the blood sugar level. In vitro studies proved that alloxan mediate cytotoxic action by producing ROS that caused destruction of β-cells [18]. Glibenclamide, the oral hypoglycemic agent is known to mediate its hypoglycemic effect by stimulating insulin release from the pancreatic β-cells. It also stimulates the release of somatostatin and suppresses the secretion of glucagons [19].

Excess glucose present in the blood reacts with hemoglobin to form HbA1c so the level of hemoglobin decreased and the glycosylated hemoglobin level increased in alloxan induced diabetic rats. HbA1c is an important parameter of chronic glycemic control in patients with diabetes mellitus and an elevated HbA1c always indicates the uncontrolled diabetes mellitus [20, 21]. Treatment of diabetic rats with B.tomentosa significantly reduced the above parameters and this is due to the improved glycemic control exerted by the B.tomentosa extract. The serum hemoglobin concentration is an important predictor of both macrovascular and microvascular complications of diabetes mellitus that might cause coronary mortality and lower extremity amputations [22]. Since the aqueous extract of B.tomentosa effectively prevents the HbA1c elevations, it prevents complications such as macrovascular and microvascular complications including coronary mortality. B.tomentosa alone treated rats did not show any significant difference with control rats. Numerous studies have demonstrated that a variety of plant contain the secondary metabolites like glycosides alkaloids, terpenoids, flavonoids and tannin, etc. They have been proved to be antidiabetic by different mechanism of action [23].

### Change in renal and hepatic markers
Concentration of renal markers urea, uric acid and creatinine and the liver marker enzymes SGOT, SGPT and ALP were found to be increased in diabetic rats (Table 2). In diabetic nephropathy, there will be a renal vein thrombosis and fibrin

**Figure 1:** Changes in body weight in control and experimental animals in 30 days treatment of B.tomentosa. Values are given as Mean ± S.D for 5 rats in each group. * - Significant at 5% level (t=0.05), compared between initial and final body weight. Diab - Diabetic control; D+Gliben - Diabetes +Glibencclamide treated; D+BT - Diabetes+Bauhinia tomentosa treated; BT - Bauhinia tomentosa alone treated.
degradation that causes significant decrease in glomerular filtration rate (GFR), and increase in blood urea and creatinine. The changes in serum enzyme level in diabetic animals are directly related to changes in the metabolism in which the enzymes are involved; hence the SGOT and SGPT activities have been used as a sensitive indicator of liver function [24, 25]. The urea, uric acid, creatinine level and the liver marker enzymes levels of the diabetic rats administered with \textit{B.tomentosa} reduced to almost normal level. The reversal of renal and hepatic markers to their normalcy is the evidence of the prevention of cellular and tissue damage under diabetic conditions and the revival of insulin secretion to normal levels. The Group V control rats showed no significant change in all the above parameters indicate the safety nature of the extract on these vital organs.

### Change in lipid profile

Hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [26]. Table 3 shows significant elevation of TC, TG, LDL, and VLDL along with a decrease in HDL cholesterol in diabetic rats and its reversal by the administration of aqueous extract of \textit{B.tomentosa}. In diabetic treated rats, significant increase in HDL and decrease in the level of TC, TG, LDL, VLDL was noticed. The lipid profile demonstrated no significant change in Group V when compared with control rats. The results of this study revealed that a continuous administration of aqueous leaf extract of \textit{B.tomentosa} for 30 days prevented the elevation of the level of serum lipids, secondary to the diabetic state. The hypolipidemic effect of

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**Table 1:** Effect of \textit{B.tomentosa} leaf extract on the concentration of glucose, insulin, hemoglobin and glycosylated hemoglobin of control and experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group I)</th>
<th>Diabetic control (Group II)</th>
<th>Diabetic + Glibenclamide treated (Group III)</th>
<th>Diabetic + \textit{B.tomentosa} treated (Group IV)</th>
<th>\textit{B.tomentosa} treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>106.00±1.82(^a)</td>
<td>240.25±1.01(^b)</td>
<td>104.25±0.82(^a)</td>
<td>113.00±1.78(^c)</td>
<td>108.33±1.86(^d)</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>0.42±0.14(^a)</td>
<td>0.25±0.16(^b)</td>
<td>0.49±0.57(^a)</td>
<td>0.40±0.01(^a)</td>
<td>0.41±0.01(^a)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>16.2±1.18(^b)</td>
<td>14.23±0.13(^b)</td>
<td>15.73±0.14(^a)</td>
<td>15.1±0.27(^d)</td>
<td>15.4±0.31(^a)</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>9.16±0.02(^a)</td>
<td>15.7±0.09(^b)</td>
<td>9.07±0.05(^a)</td>
<td>10.4±0.09(^d)</td>
<td>9.12±0.02(^a)</td>
</tr>
</tbody>
</table>

Values are given as Mean ± S.D for five individual experiments.

Values not sharing a common superscript differ significantly (DMRT)

**Table 2:** Effect of \textit{B.tomentosa} leaf extract on the concentration of urea, uric acid, creatinine, SGOT, SGPT and ALP in serum of control and experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group I)</th>
<th>Diabetic control (Group II)</th>
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<th>\textit{B.tomentosa} Treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>35.22±0.52(^a)</td>
<td>43.50±4.43(^b)</td>
<td>31.58±0.55(^c)</td>
<td>33.47±0.22(^d)</td>
<td>35.5±2.08(^d)</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.92±0.12(^a)</td>
<td>3.52±0.10(^b)</td>
<td>2.47±0.09(^c)</td>
<td>2.29±0.15(^d)</td>
<td>1.91±0.06(^a)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.82±0.02(^a)</td>
<td>1.04±0.07(^b)</td>
<td>0.74±0.13(^c)</td>
<td>0.69±0.15(^d)</td>
<td>0.81±0.03(^a)</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>27.25±0.95(^a)</td>
<td>50.75±2.21(^b)</td>
<td>37.75±1.71(^c)</td>
<td>30.50±2.64(^d)</td>
<td>26.00±1.41(^a)</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>44.5±1.29(^a)</td>
<td>53.00±3.16(^b)</td>
<td>39.12±0.85(^c)</td>
<td>37.50±1.90(^d)</td>
<td>42.25±0.95(^a)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>96.16±2.62(^a)</td>
<td>250.51 + 6.31(^b)</td>
<td>130.17 + 2.81(^c)</td>
<td>140.82 + 2.89(^d)</td>
<td>100.31 + 1.86(^a)</td>
</tr>
</tbody>
</table>

Values are given as Mean ± S.D for five individual experiments.

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Table 3: Effect of *B. tomentosa* leaf extract on the concentration of cholesterol, triglycerides, HDL, LDL and VLDL in serum of control and experimental groups.

<table>
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<tr>
<th>Parameters</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>121.03±1.47a</td>
<td>266.33±1.36b</td>
<td>117.73±1.72c</td>
<td>115.2±1.17c</td>
<td>118.0±1.79ac</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>80.67±1.05a</td>
<td>218.8±1.17b</td>
<td>87.47±1.22c</td>
<td>90.93±1.58d</td>
<td>79.73±1.14a</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42.07±1.35a</td>
<td>29.73±1.02b</td>
<td>40.13±1.61c</td>
<td>39.73±1.70c</td>
<td>41.73±1.54a</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>58.73±1.50a</td>
<td>191.33±2.07b</td>
<td>58.53±1.27a</td>
<td>56.00±0.99a</td>
<td>57.73±1.26a</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>20.5±1.16a</td>
<td>45.53±1.52b</td>
<td>18.8±1.25c</td>
<td>17.27±1.15c</td>
<td>18.53±1.74c</td>
</tr>
<tr>
<td>AI</td>
<td>10.95±0.08</td>
<td>2.25±0.05</td>
<td>3.85±0.06</td>
<td>1.45±0.03</td>
<td>1.75±0.02</td>
</tr>
<tr>
<td>CRI</td>
<td>2.02±0.03</td>
<td>12.01±0.04</td>
<td>3.70±0.02</td>
<td>2.98±0.02</td>
<td>1.15±0.04</td>
</tr>
</tbody>
</table>

Values are given as Mean ± S.D for five individual experiments
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Table 4: Effect of *B. tomentosa* leaf extract on the concentration of total protein, albumin, globulin and A/G ratio in serum of control and experimental groups.

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<th><em>B. tomentosa</em> treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>8.08±0.07a</td>
<td>4.47±0.03b</td>
<td>8.03±0.01a</td>
<td>7.77±0.06a</td>
<td>8.06±0.03a</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>5.07±0.02a</td>
<td>2.30±0.09b</td>
<td>4.75±0.03a</td>
<td>4.55±0.03d</td>
<td>5.01±0.03a</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>3.17±0.14ac</td>
<td>2.11±0.08b</td>
<td>3.28±0.09cd</td>
<td>3.35±0.06d</td>
<td>3.06±0.11a</td>
</tr>
<tr>
<td>A/G Ratio</td>
<td>1.59±0.03c</td>
<td>1.09±0.07b</td>
<td>1.45±0.03c</td>
<td>1.41±0.05c</td>
<td>1.66±0.04a</td>
</tr>
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*B. tomentosa* can be explained as a direct result of the reduction in the blood glucose concentration which reduced the formation of triacyl glycerol. The observed result may be primarily because of the hypolipidemic effect of the extract by normalizing the insulin level in the serum of treated animals. Literatures also reported that the flavonoids, alkaloids and tannins are responsible for hypoglycemic and hypolipidemic effect [27]. The atherogenic and cardiac risk index of diabetic rats treated with *B. tomentosa* significantly reduced. This supported that *B. tomentosa* will prevent cardiovascular complications secondary to diabetes.

**Change in total protein, albumin and globulin**

A sharp fall in total protein and a slight decline in albumin, globulin and a significant change in A/G ratio were observed in diabetic rats [28]. In table 4, the total protein and albumin were decreased in diabetic rats but increased with *B. tomentosa* treatment in Group III and Group IV. No significant change was found between control and treatment groups in globulin and A/G ratio. In treated groups, *B. tomentosa* improve the insulin secretion and reverse the altered protein profile by exerting the protein sparing effect. Significant increase in urinary excretion of
protein, albumin, glucose and urea levels indicated the impaired renal function in diabetes, and the treatment of herbal remedies prevented such diabetic nephropathic complications was already established [29].

Changes in liver glycogen and protein
Liver plays a role in buffering the post prandial hyperglycemia and is involved in the synthesis of glycogen. Insulin deficiency in diabetes mellitus is known to impair the normal capacity of the liver to synthesis glycogen. The decrease in glycogen synthase and simultaneous increase in glycogen phosphorylase results in an increased glycogenolysis hence increases the blood glucose. This further worsens the diabetic condition.

In Table 5 significant decrease in liver glycogen and protein level was observed in diabetic rats. The observed depletion of glycogen level in diabetic rats was consistent with the earlier reports [30], indicating that it was possibly due to loss of glycogen synthetase activating system or increased activity of glycogen phosphorylase. It was pertinent to believe that the liver glycogen level of alloxan treated animals would decrease, as the synthesis of glycogen depended on insulin for the influx of glucose. Furthermore, earlier studies also showed that the reduced hepatic glycogen content was normalized by insulin treatment [31]. In diabetic treated groups, increase in glycogen was the direct effect of *B.tomentosa* on insulin secretion [32].

In *B.tomentosa* and glibenclamide treated rats, there was an improvement in protein profile. It may be due to marked change in circulating amino acid levels, hepatic amino acid uptake, and muscle output of amino acid concentrations [33]. It was found that the administration of *B.tomentosa* improved the protein profile in diabetic rats. This indicated that the plant extract have the potency of reversing the altered protein profile similar to that of the drug glibenclamide.

Changes in hepatic enzymes
Significant increase in glycogen phosphorylase, glucose-6-phosphatase and lowered level of glucokinase were observed in diabetic rats (Fig 2). Glucokinase is present in the cells of liver that catalyze the conversion of glucose to glucose-6-

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</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (mg/g of fresh tissue)</td>
<td>4.33±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.24±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.11±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.14±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (mg/g of fresh tissue)</td>
<td>13.70±0.75&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>9.66±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.16±0.36&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>12.90±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.20±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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phosphate and plays a central role in the maintenance of glucose homeostasis [34]. Glucose-6-phosphatase is one of the key enzymes in the homeostatic regulation of blood glucose level whose action is controlled by insulin. In the absence of insulin, increased glycogen phosphorylase activity was observed that lead to decrease in glycogenesis or increase in glycogenolysis in liver [35]. The altered activities of the enzymes were reversed by the administration of B.tomentosa and glibenclamide. The observed result may be due to the direct effect of B.tomentosa on insulin secretion. Insulin, increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase and inhibiting the enzymes like glucose- 6-phosphatase and glycogen phosphorylase.

Histopathology
Figure 3-5 illustrate the effect of B.tomentosa leaf extract on the liver, kidney and pancreas of control and experimental rats. The degenerative changes associated with alloxan administration were reversed by the extract in all the three organs taken for the study.

The result of the study demonstrated that the aqueous leaf extract of B.tomentosa (300mg/kg) by oral administration to diabetic rats contain novel bioactive principles with antihyperglycaemic properties. Although promising results have been obtained, more efforts are still needed for the isolation, characterization and biological evaluation of the active principle(s) of the extracts.

ACKNOWLEDGEMENT
The authors express their sincere gratitude to the Chairman and Chancellor, Advisor, Vice Chancellor and Registrar of Karpagam University, Coimbatore, India for providing facilities to carry out the study.

REFERENCES
Figure 4: Histopathology of kidney.

a. Normal kidney section showing normal nephrocytes.
b. Diabetic control rats showing severe tubular epithelial atrophy.
c. Glibenclamide treated rats with normal hepatocytes.
d. *B. tomentosa* treated diabetic rat kidney section showing mild tubular atrophy.
e. *B. tomentosa* alone treated showing normal histological structure.
31. El-Shenaway NS and Abdel-Nabi Im Hypoglycemic effect of Clove droserifolia leaf extract in experimental diabetes and on non – enzymatic antioxidant, glycogen, and thyroid hormone and insulin levels. Diabetologia Croatica. 2006; 35: 15.