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Research Article

POTENTIAL HYPOGLYCEMIC PROPERTY OF *ALBIZIA MYRIOPHYLLA* IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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ABSTRACT

Diabetes is a global health problem. The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Due to the current global interest on natural or traditional remedies, the present work on *Albizzia myriophylla* (ABZ) may provide another alternative to the treatment of diabetes. Initially, oral glucose tolerance test was carried out in normal rats treated with 5 mg/kg, 25 mg/kg and 50 mg/kg of aqueous bark extract of ABZ, respectively. This was then followed by administration of ABZ at doses of 5 mg/kg and 25 mg/kg, respectively, to normal and streptozotocin-nicotinamide induced type 2 diabetic rats for 4 weeks. Subsequently, fasting blood glucose levels, levels of aspartate transaminase, alanine transaminase, urea and creatinine were investigated in normal and diabetic rats. Additional histological findings of the kidney of normal and diabetic rats were also evaluated. Significant reduction of the glucose levels were seen in diabetic rats treated with ABZ at 5 mg/kg and 25 mg/kg, respectively. The result also indicates the safety of consumption of ABZ. This is confirmed from the biochemical analysis and histological findings from the kidney, where insignificant changes were seen in the normal rats treated with ABZ at 5 mg/kg and 25 mg/kg, respectively compared to the normal control rats. Thus, ABZ at 5 mg/kg and 25 mg/kg, respectively show hypoglycemic activity in streptozotocin-nicotinamide induced diabetic rats with no obvious toxicological effects on the liver and kidney. In addition, ABZ at 5 mg/kg was able to ameliorate the liver and kidney damage induced by diabetes.

Keywords*: Albizia myriophylla*, Aqueous bark extract, Streptozotocin-nicotinamide induced diabetes, Anti diabetic effect.

INTRODUCTION

Diabetes mellitus is a metabolic disorder featured by hyperglycemia caused by insulin deficiency, often combined with insulin resistance. Various complications may develop after many years such as retinopathy, microangiopathy and nephropathy. Worldwide, the incidence of diabetes amongst all age groups is increasing. In the year 2000, an average of about 2.8% of the world's population suffer from this disease. By the year 2030, the numbers are expected to increase to 4.4%. In developing countries, the urban population suffering from diabetes is expected to double[1]. The World Health Organization (WHO) estimates that in 2030, Malaysia would have a total number of 2.48 million diabetic cases as compared to 0.94 million in 2000[2].

Present anti diabetic medications have been shown to be effective but with major side effect which includes hypoglycemic coma and involves hepatorenal disturbances. Hence the search for safer and more efficacious hypoglycemic agent continues. Due to the current global interest on natural or traditional remedies, the present study on ABZ provides another alternative to the treatment of diabetes.

ABZ belongs to the subfamily Mimosoideae and genus *Albizia.* This genus consists of about 145 species, distributed in tropical and subtropical regions in the world which includes Thailand, Malaysia, Vietnam and other countries. It is used in traditional medicine as mouth wash in Thailand[3] and for rice beer production[4]. A substantial proportion of Thai people uses traditional medicine to treat diabetes and this includes the use of ABZ as an anti diabetic agent[5]. An *in vitro* study showed that it has a α-glucosidase inhibitory activity[6]. Phenolics have been found to be effective anti hyperglycemic agents[7] and subsequently, 12 phenolic acids were isolated qualitatively[4]. This current information provides integral evidence as to how ABZ may act as an anti diabetic agent. However, its hypoglycemic effect has yet to be proven scientifically *in vivo*.

Scientific evidence found in the previous study stated above, signify the importance of evaluating the anti diabetic effect of ABZ in the animal studies, so as to provide a basis of its efficacy in controlling blood sugar level.

MATERIAL AND METHODS

Collection of plant material

The freshly picked parts of the bark of ABZ were collected in December 2008, from Pasir Mas, Kelantan, Malaysia. The plant was authenticated by Dr. Samsul Kamis, a Botanist at the Institute of Bioscience, Universiti Putra Malaysia. A voucher specimen (ACPO 122) has been deposited in UPM Agriculture Conservatory Park at Institute of Bioscience, Universiti Putra Malaysia. The park is used for the conservation of medicinal plant. The bark collected was then air-dried at room temperature for 2 weeks, with no direct sunlight. The chips of dried bark of the plant ABZ were then stored for further used.

Preparation of aqueous bark extract of ABZ

The chips of dried bark of ABZ were pulverized to a coarse powder in a mechanical grinder. Then 300 grams of dried bark powder of ABZ is then mixed into 1.5 liter of boiling hot distilled water for 2 minutes. The aqueous extract was then filtered, concentrated and lastly freeze dried. The residue was then stored in a refrigerator at 2 - 8° C for use in subsequent experiments.

Animals

Healthy adult male *Sprague-dawley* (SD) rats between 5 – 6 weeks of age and weighing 150 – 250 grams were used for the study. The rats were housed in individual cages. The rats were kept in the departmental animal house at 25±3°C under standard conditions which includes 12 h light and dark cycles with about 44-55% relative humidity. The animals were maintained on a standard laboratory rat pellets and water *ad libitum*. The study was permitted by the Animal Care and Used Committee of Universiti Putra Malaysia (UPM/FPSK/PADS/BR-UUH/00347).

Collection of blood and determination of blood glucose levels

Blood samples were collected from the tail vein and glucose levels were estimated by using a glucose oxidase-peroxidase reactive strips and a glucometer (Accu-Chek, Roche diagnostics, USA).

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT)[8] was performed in overnight fasted (18H) normal rats. Rats were divided into four groups (n=6)

which were administered either drinking water or aqueous bark extract 5, 25 and 50 mg/kg, respectively. Glucose (2g/kg) was fed 30 min after the administration of the extract[9]. Blood samples were collected from the tail of rats at time -30 min (just before the administration of the extract), time 0 min (prior to the glucose load), 30, 60 and 120 min after the glucose load.

Induction of diabetes mellitus

Type 2 diabetes mellitus was induced[10] in overnight fasted *Sprague-dawley* male rats weighing 150 – 250 gram by injecting a single intra peritoneal injection of 50 mg/kg streptozotocin (Sigma Aldrich, Germany). This injection is given 15 minutes after the intra peritoneal administration of 110mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in 0.1 M of cold citrate buffer (pH 4.5) and nicotinamide in normal saline. STZ is capable of causing fatal hypoglycemia as a result of an enormous amount of pancreatic insulin release thus rats were given 10% glucose solution after 6 hour of STZ administration for the next 24 hours[11]. Hyperglycemia was confirmed after a period of 1 week. Rats with a high blood glucose levels (>13.8 mmol/l or > 350 mg/dl) were used for the anti diabetic study[12].

Animal preparation for anti diabetic study

Animals were randomly divided into seven groups of six rats each for the anti diabetic study as stated below.

Group I: normal control rats administered drinking water

Group II: normal rats given ABZ aqueous extract (5 mg/kg)

Group III: normal rats given ABZ aqueous extract (25 mg/kg)

Group IV: diabetic control rats administered drinking water

Group V: diabetic rats given ABZ aqueous extract (5 mg/kg)

Group VI: diabetic rats given ABZ aqueous extract (25 mg/kg)

Group VII: diabetic rats administered the standard drug acarbose (80 mg/kg)

During the experimental period of 28 days, the rats were weighed daily and the mean change in body weight was calculated. The fasting blood glucose was also determined on day 0, week 1, week 2, week 3 and week 4 of the anti diabetic study.

Biochemical Analysis

At the end of the experiment, after a period of 28 days, the rats were anaesthetized under diethyl ether following a 12 hours fast. 3 ml of blood samples were withdrawn via cardiac puncture into plain tubes. For the serum analysis, blood were collected in separate tubes and centrifuged at 2000 rpm for 10 minutes. Total serum aspartate transaminase (AST), alanine aminotransferase (ALT), urea and creatinine level were examined by Hitachi machine at Hematology and Clinical Biochemistry Laboratory in Universiti Putra Malaysia.

Histology of the kidneys

The animals were sacrificed at the end of the experimental period of 28 days via excess diethyl ether. The kidney samples were collected and weighed. The collected organs were fixed and kept in 10% buffered formalin for histology studies. Sections of the kidneys were stained with haematoxylin-eosin. Histological examination and grading of the kidney were done. The quantity of the necrotic glomeruli of the kidney was done under light microscopy (Olympus BX51, Japan) at 100x magnification or more. The comprehensive histological observations were quantified on at least 20 fields per slide in a battlement (zigzag) approach[13].

Statistical Analysis

Data were statistically evaluated using one-way analysis of variance (ANOVA), followed by *post hoc* Tukey's test using SPSS, version 17. The values were considered significant when p value < 0.05.

RESULTS

Oral Glucose Tolerance Test Studies

When ABZ aqueous bark extract at 5 mg/kg and 25 mg/kg was administered to glucose loaded normal rats, hypoglycemia was noted after 60 minutes. The decline in blood sugar further reduced at 120 minutes (Table 1). The results showed that ABZ 5 mg/kg and 25 mg/kg produce significant lowering of serum glucose in normal rats. The group treated with ABZ 50 mg/kg only showed a significant decrease in serum glucose levels after 60 minutes of study compared to normal control rats. Statistically insignificant changes were seen at 120 minutes. Thus, the ABZ 50 mg/kg group was omitted from the planned experimental anti diabetic study.

Table 1: Effect of ABZ aqueous bark extract on OGTT

Group	Blood glucose level				
	Minus 30min	0 min	30 min	60 min	120 min
Control	4.51±0.51	5.0±0.56	7.28±0.82	5.8±0.4	5.0±0.5
ABZ(5mg/kg)	4.13±0.36	4.4±0.39[a]	6.64±0.72	5.11±0.62[a]	4.06±0.26[a]
ABZ(25mg/kg)	4.23±0.33	4.24±0.17[a]	7.33±0.53	4.77±0.29[a]	4.17±0.85[a]
ABZ(50mg/kg)	3.97±0.95	4.49±0.37	6.74±1.6	4.46±0.34[a]	4.24±0.32

ABZ, *albizia myriophylla* extract; values are expressed as mean ± S.D (n=7); [a] represents statistical significance versus normal control (p<0.05).

Effect of ABZ on Glucose Levels

In the diabetic control group, the fasting blood glucose level was consistently high (> 13.8 mmol/L) throughout the study and is significantly increased (p<0.05) when compared to the normal control rats. In the diabetic group treated with ABZ aqueous bark extract at 5mg/kg (DABZ1) and 25 mg/kg (DABZ2), significant hypoglycemic activities was seen. While in the diabetic group treated with the synthetic anti diabetic drug acarbose at 80 mg/kg (DACAR) also showed significant reduction in fasting blood glucose level at week 1, week 2, week 3 and week 4 when compared to the diabetic control group (p<0.05) (Table 2).

Table 2: Effect of aqueous bark extract of ABZ on fasting blood glucose level (mmol/L) in normal and diabetic rats

Group	Fasting Blood Sugar				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal control (NC)	4.82±0.38	5.03±0.41	4.75±0.31	5.03±0.27	5.68±0.24
Normal+ABZ(5mg/kg) (NABZ1)	4.65±0.16	5.82±0.7	5.53±0.18	5.68±0.26	5.8±0.65
Normal+ABZ(25mg/kg) (NABZ2)	5.03±0.47	5.62±0.27	5.38±0.64	5.12±0.67	4.93±0.59
Diabetic control (DC)	20.12±2.97[a]	23.37±3.6[a]	18.6±3.51[a]	22.7±3.7[a]	27.57±3.36[a]
Diabetic+ABZ(5mg/kg) (DABZ1)	16.82±2.27[a]	8.57±1.11[b]	7.53±1.06[b]	7.35±1.18[b]	6.57±0.78[b]
Diabetic+ABZ(25mg/kg) (DABZ2)	17.6±1.94[a]	12.35±7.46[b]	10.18±6.65[b]	10.26±7.7[b]	9.97±7.43[b]
Diabetic+Acarbose(80mg/kg) (DACAR)	21.87±4.75[a]	10.52±5.26[b]	7.6±2.42[b]	6.53±0.84[b]	7.17±1.73[b]

Values are expressed as mean ± S.D (n = 6). [a] represents statistical significance versus normal control (p<0.05). [b] represents statistical significance versus diabetic control (p<0.05).

Effect of ABZ on AST, ALT, Urea and Creatinine

Significant difference was observed in serum AST, ALT, urea and creatinine level in diabetic rats. A significant reduction of AST, ALT and creatinine level was seen in the diabetic rats treated with ABZ 5 mg/kg and 25 mg/kg. There was no statistical significance in parameters estimated in normal animals (Table 3).

Figure 1 shows the normal and abnormal histology of the glomerulus of the kidney.

Significant increase of kidney necrotic cells was seen in the untreated diabetic rats. However, after treatment, a significant reduction of the necrotic cells was observed. (Figure2).

Table 3: Effect of ABZ on ALT, AST, urea and creatinine in normal and diabetic rats after 28 days of treatment

Group	ALT (U/L)	AST (U/L)	Urea (mmol/L)	Creatinine (mmol/L)
NC	52.7±10.1	132.9±14.9	7.3±0.6	61.5±7.7
NABZ1	52.8±12.5**	121.7±26.2	7.1±1.3	56.3±8.5**
NABZ2	57.0±20**	142.1±2.8**	7.4±2.0	70±6.4
DC	106.5±22*	204.5±36.8*	9.7±1.4	78.5±8.7*
DABZ1	50.7±12.5**	121.4±31.8**	7.6±0.5	62.5±3.8**
DABZ2	76.2±21.5	198.9±32.1**	8.2±2.2	61.2±4.4**
DACAR	102.8±31.1*	183.5±16*	7.8±1.6	70.0±5.5

NC, normal control; NABZ1, normal+ABZ(5mg/kg); NABZ2, normal+ABZ(25mg/kg); DC, diabetic control; DABZ1, diabetic+ABZ(5mg/kg); DABZ2, diabetic+ABZ(25mg/kg); DACAR, diabetic+Acarbose(80mg/kg); values are mean ± SEM (n=6). *-represents statistical significance versus normal control (p<0.05).

Effect of ABZ on Kidney Necrotic Cells

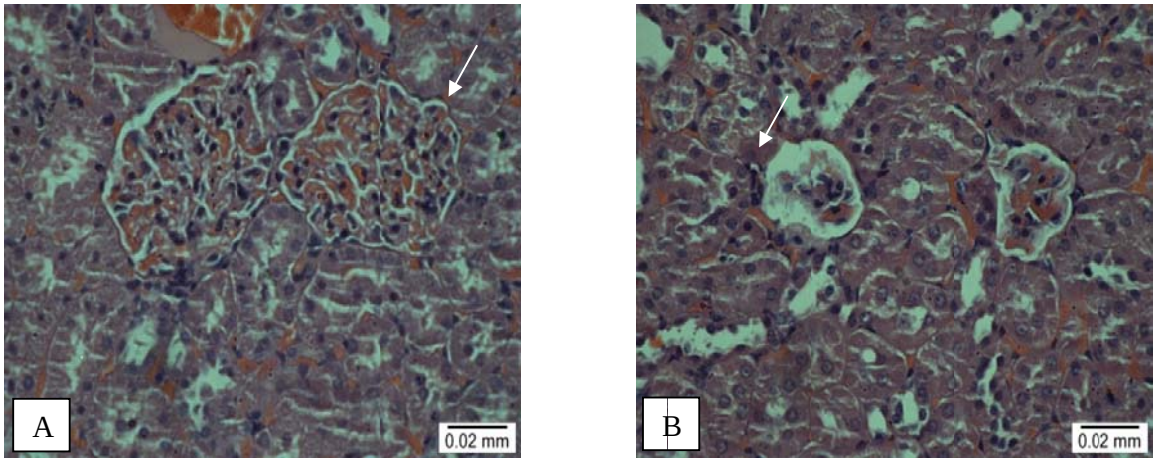

Fig. 1: Histology of kidney in normal control rat and diabetic control rat, depicting the glomerulus, marked by the white arrow. Panel A: Normal glomerulus with thin delicate capillary loops around prominent mesangial regions. Panel B: Necrotic glomerulus with shrunkened glomerulum or glomerular loss with Bowman's space enlargement and collapse capillaries.

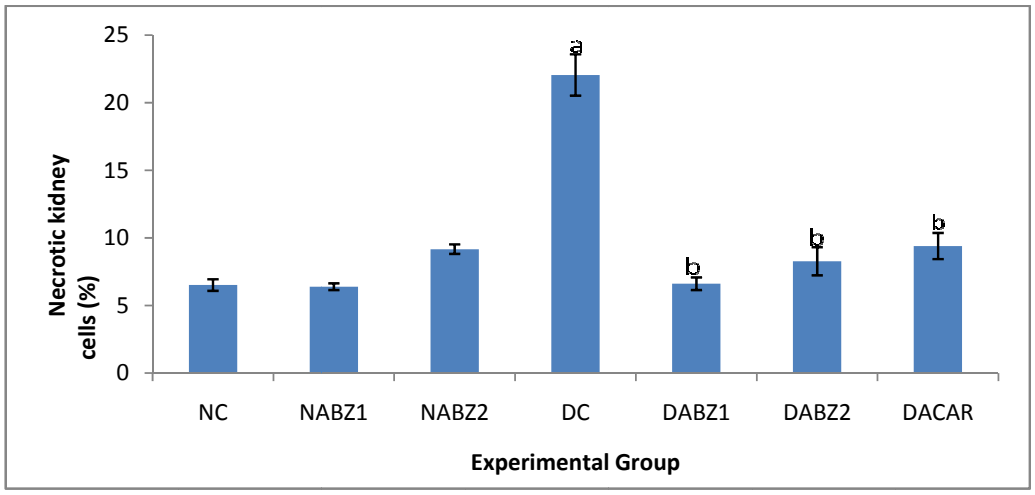

Fig. 2: Necrotic glomerular cells in kidney (%) in normal control and diabetic control rats fed with ABZ 5 mg/kg, ABZ 25 mg/kg and Acarbose 80 mg/kg.

a – represents statistical significance versus normal control (p<0.05). b - represents statistical significance versus diabetic control (p<0.05).

DISCUSSION

Glucose is a form of carbohydrate which constitutes the body's main source of energy. Hyperglycemia occurs when there is uncontrolled hepatic glucose output with reduction of glycogen synthesis and reduction of glucose uptake by the skeletal muscle. OGTT, a test of immense value, in favor of using the fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of hyperglycemia or diabetes[14]. In this study, OGTT was used as a preliminary investigation, to test the hypoglycemic effect of *Albizzia myriophylla* (ABZ) in 3 different doses which were 5 mg/kg, 25 mg/kg and 50 mg/kg, respectively. The test was carried out in normal control rats and glucose induced hyperglycemic normal rats. The reduction in the blood glucose levels could be due to the delayed absorption of the glucose from the gastrointestinal tract caused by the extract[15]. The ABZ at 5 mg/kg and 25 mg/kg was found to further reduce blood glucose level significantly after 120 minutes compared to the normal control group. This effect, could be due to an increase in the uptake of glucose, which may be initiated by the extract given. However, ABZ at 50 mg/kg did not show any significant reduction of blood glucose levels after 120 minutes. Thus, the results indicate, that ABZ at 5 mg/kg and 25 mg/kg, may possess possible hypoglycemic activity. These results identified the concentration of 5 mg/kg and 25 mg/kg as effective and optimum concentration for the study of severely diabetic models in rats.

The pathogenesis of type 2 diabetes is complex involving progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic β-cells leading to hyperglycemia[16]. STZ administration at a dosage of 50 mg/kg body weight, along with nicotinamide intraperitoneal injection at 110 mg/kg significantly elevated the blood glucose level[17] in rats. STZ selectively destroys the pancreatic insulin secreting β-cells while nicotinamide provides partial protection against the toxicity of streptozotocin. Thus, resulting in inducing diabetes mellitus in rats[18]. This effect is being depicted by the high level of glucose detected in the STZ-nicotinamide induced diabetic rats. In all diabetic patients, treatment should aim to lower blood glucose to near normal level. The present investigation fulfills this statement by producing a significant fall in blood glucose levels especially seen in rats treated with ABZ. Previously, a study had shown that the water soluble extract from Thai Mimosaceous Plant which included ABZ, had displayed a α-glucosidase inhibitory activity[19] which was similar to the action of acarbose. Besides this, the possibility of the presence of phenolics in ABZ[4] may have added beneficial effect as phenolics are found to be effective anti hyperglycemic agents[7]. Thus, the anti diabetic effect of ABZ may be due to the presence of more than one anti hyperglycemic principle and their synergistic effects.

Diabetes mellitus is a chronic disease affecting multiple organs. Persistent hyperglycemia can lead to organ damage especially in tissues where there are no insulin receptors preventing the entry of glucose into the cells. The insulin receptors found in the liver, are able to protect the liver from direct damage due to hyperglycemia. Although hyperglycemia does not directly induce damage in the liver, it is found that the incidence of liver disease is higher in patients with diabetes[20]. In this study, the significant increase in the activities of serum AST and ALT in diabetic control group compared to the normal control group indicated that diabetes may induce hepatic dysfunction[21]. Therefore, the increment of the AST and ALT in the serum may be mainly due to the leakage of these enzymes from the liver enzyme into the blood stream[22], which gives the indication on the hepatotoxic effect caused by hyperglycemia or diabetes. On the other hand, treatment of the diabetic rats with ABZ at 5 mg/kg caused significant reduction in the activity of these enzymes compared to the diabetic control group. In short, treatment of diabetic groups with ABZ 5 mg/kg for 28 consecutive days could restore the activities of AST and ALT to their normal levels. Thus, treatment with ABZ at 5 mg/kg may inhibit the liver damage induced by hyperglycemia. In contrast, treatment of diabetic rats with ABZ 25 mg/kg and acarbose 80 mg/kg, showed a significant increase of AST and ALT levels when compared to the normal control group. In other words, no significant reduction in the activity of these enzymes was seen when compared to the diabetic control group. Thus, treatment with ABZ at 25 mg/kg and acarbose at 80 mg/kg, were unable to reduce the liver damage. In addition, one of the side effects detected from the chronic use of acarbose is hepatotoxicity which may lead to an increase in AST and ALT level[23].

Generally, blood creatinine is directly associated with glomerular filtration rate (GFR) and creatinine secretion rate. Blood creatinine tends to increase relative to the extent of fibrosis in renal cortex mesangium[24]. Blood urea nitrogen represents protein uptake and renal excretion capacity because it is a major end product from protein metabolism and synthesized from ammonia in the liver[25]. Therefore, blood creatinine and blood urea nitrogen are often used as markers for renal functions. In this study, the diabetic control group showed a significant increase in serum creatinine and also an increase in serum urea nitrogen level, when compared to the normal control group. This finding suggests that there were abnormalities in renal functions of STZ-nicotinamide induced diabetic rats. The diabetic group treated with ABZ 5 mg/kg and 25 mg/kg showed significantly lower level of creatinine with considerably lower level of serum urea nitrogen than the diabetic control group. Therefore, it is expected that ABZ will be effective in improving renal functions. In addition, the normal rats treated with ABZ 5 mg/kg and 25 mg/kg, respectively, showed insignificant changes when compared to the normal control group. This result signifies that ABZ given at the dose of 5 mg/kg and 25 mg/kg have no adverse effects on renal functions in normal rats.

Diabetic nephropathy has been considered an important cause of mortality and morbidity. STZ-nicotinamide induced diabetic rodents result in development of nephropathy similar to the early stage of human diabetic nephropathy[26].Carbohydrate metabolism impairment in diabetes causes increase free radicals level, leading to necrotic or inactive cells in the kidney. Diabetes eventually caused abnormal glomeruli or glomeruli loss, Bowman's space enlargement and tubular degeneration[13], as seen in the glomerulus of the diabetic rat in this study (Fig.-1, panel B). Admittedly, the research study was carried out in shorter duration and this might be insufficient for significant vascular changes in the kidney of the diabetic rats. The histological study performed on the kidneys of diabetic control group showed significant increase in the percentage of necrotic kidney cells compared to the normal control group. The diabetic control group possessed many abnormal glomeruli signifying glomerular damage, which includes some glomeruli loss and shrunken glomerulus. The free radicals may alter the structure and function of the kidney, especially the glomerulus and cause diverse types of glomerular lesions pathophysiology, ranging from inflammatory to apoptosis[27]. However, when treated with ABZ 5 mg/kg and ABZ 25 mg/kg, the diabetic rats showed a significant reduction in the percentage of the necrotic kidneys cells compared to the diabetic control group (Fig.-2). In short, the treated diabetic rats showed healing features, which resembled that of a normal kidney with a significant reduction of necrotic lesions in the glomeruli of the kidney (Fig.-2). Currently, there is no available synthetic drug which fully relieves the problem of kidney insufficiency. However, indigenous plant such as ABZ may possess tissue rejuvenator activity, thus may have kidney healing property[28].

The normal rats treated with ABZ 5 mg/kg and ABZ 25 mg/kg, did not show any toxic effect on the kidney, as the percentage of the kidney necrotic cells were similar to that of the normal control group with insignificant changes (Fig.-2). All the kidneys of ABZ treated normal rats were normal without any obvious observable injury indicating the non-toxic nature of ABZ.

CONCLUSION

ABZ at 5 mg/kg and 25 mg/kg, respectively show hypoglycemic activity in streptozotocin-nicotinamide induced diabetic rats with no obvious toxicological affect on the liver and kidney. Longer duration studies of *Albizia myriophylla* (ABZ) and its isolated compounds on chronic models are necessary to develop a potent anti diabetic drug. In short, natural products isolated from herbal plants will still remain an essential component in the search for new anti diabetic medication[29].

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