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Full Length Research Paper

Antihyperglycemic and antihyperlipidemic activities of ethanol extract of *Ajuga remota* Benth (Harmegusa) leaves in streptozotocin induced diabetic rats

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The leaves of *Ajuga remota* benth have been utilized traditionally for the treatment of patients with diabetes mellitus. However, its use has not been scientifically validated. The present study was therefore, aimed to assess the antihyperglycemic and antihyperlipidemic activities of ethanol extract of *A. remota* leaves in streptozotocin (STZ) induced diabetic rats. Antihyperglycemic and antihyperlipidemic activities of ethanol extract of *A. remota* leaves (AREt) were studied in streptozotocin induced diabetic rats. The effect of extract on fasting blood glucose, body weight, lipid profile, serum, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, creatinine and total protein were analyzed. Glibenclamide was used as standard drug. Ethanol extract of *A. remota* leaves has showed significant blood glucose lowering effect as compared to the diabetic control group. After diabetic rats were treated with 200 and 400 mg/kg ethanol extract of *A. remota* leaves for 28 days, there were a significant decrease in fasting blood glucose, total cholesterol, triacylglycerol, low density lipoprotein cholesterol, serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, and significant increase in body weight, serum total protein, high density lipoprotein cholesterol levels as compared to untreated control diabetic rats. The results of the present study showed that ethanol extract of *A. remota* leaves might be useful for management of diabetes mellitus and other associated abnormalities. The present study might support the traditional use of *A. remota* for diabetes mellitus treatment.

Key words: *Ajuga remota* benth, diabetes mellitus, streptozotocin.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with deranged carbohydrates, fats and proteins metabolism resulting from absolute or relative lack of insulin secretion or insulin resistance by peripheral tissues mainly the liver, skeletal muscle and adipose tissues. It is also characterized by hyperlipidemia and hyperaminoacidemia (Rao et al., 2010; Sudha et al., 2011). The long-term effects of diabetes mellitus include

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progressive development of retinopathy with potential blindness, nephropathy which may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation and features of autonomic dysfunction. People with diabetes mellitus are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease (Karimulla et al., 2011).

The global prevalence of diabetes mellitus was estimated to be 9% among adults aged 18 and above in 2014. In 2012, an estimated 1.5 million deaths were directly caused by diabetes. Currently, more than 80% of diabetes deaths occur in low- and middle-income countries. In Ethiopia, the prevalence of diabetes mellitus was 800,000 in the year 2000 and expected to reach 1.8 million by 2030 (Feleke and Enquselassie, 2005).

Modern antidiabetic drugs have their own limitations like high cost and effects such as hypoglycemia, weight gain, gastrointestinal disturbances, and liver toxicity (Dey et al. 2002). The use of plants as medicine dates back to early man. It is estimated that 70 to 80% of people worldwide rely on traditional herbal medicine to meet primary health care needs (Seifu et al., 2012.). To date, ethnobotanical information indicates that over 1200 plants are used as traditional remedies for treatment of diabetes mellitus (Fraser et al., 2007) and more than 200 pure compounds have shown blood glucose lowering activities. WHO has encouraged and recommended the use of herbs as an alternative therapy for diabetes mellitus since medicinal plants are often less expensive, easily accessible, less toxic and suitable (Ansarullah et al., 2011).

A. remota (Figure 1) is a herb that belongs to Lamiaceae family, and is locally known as 'harmegusa' or 'etse- medihanit'. It often lies on the ground and its stems grow to 40 cm high. It grows in different regions of Ethiopia at an altitude of 1600 to 2200 m, flowering from September to October (Dagne, 2009).

Phytochemical investigations of *A. remota* showed the presence of neo-Clerodane diterpenoids (Coll and Tandrón 2005), phytoecdysteroids (Kubo et al., 1983), phenolics, flavonoids, glycosides and sugars are major constituents (Debella et al., 2005). Its leaves are known to relieve stomachache, cold, fever and gonorrhea (Githinji and Kokwaro, 1993). It has been reported that *A. remota* is antimalarial (Gitua et al., 2012). Its aerial parts had some potent antimycobacterial (Cantrell et al., 1999), analgesic and antipyretic activity (Debella et al., 2005). In Ethiopia, the leaves of *A. remota* benth are used for diabetes mellitus treatment by traditional healers. The present study aimed to determine the antihyeprglycemic and antihyperlipidemic activities of 70% ethanolic leaves extract of *A. remota* benth in diabetic rats.

METHODS

Chemicals and equipment

In this study, the following drugs, reagents and instruments were used: streptozotocin (Sigma Aldrich, Germany), glibenclamide (Sanofi Winthrop Industrie, France), SensoCard glucometer and strip (77 Electronike Kft, Hungary) and 902 automated chemistry analyzer (Hitachi, Japan). All other chemicals used were analytical grade.

Collection and preparation of plant material

Fresh leaves of *A. remota* were collected from Deneba town, North Shoa about 175 km North-East of Addis Ababa in late September, 2012. The leaves were identified and authenticated by the National Herbarium (ETH) of Addis Ababa University, and voucher number 001 was given and deposited at the same institute for further reference. The dried leaves were manually grinded and the coarse powder was kept in polyethylene bags at room temperature until used for extraction.

Experimental animals

Adult male Wistar albino rats of body weight 150 to 230 g (initial) with no prior drug treatment were used for the study. Rats were purchased from Ethiopian Public Health Institute (EPHI). All the rats were acclimatized to the laboratory condition for one week before commencing the experiments and fed with pellete and tap water *ad libitum*. The animals were housed in 12 h light and dark cycle at room temperature (20 to 25°C). The experiment was performed in the laboratory of Pharmacology Department, School of Medicine, All animals employed in this study were handled as per the guidelines set by the national academies press, Washington, D.C., USA.

Extraction

The coarse powder (600 g) of *A. remota* leaves was macerated in 70% ethanol (1:10 leaves powder to solvent ratio) for 72 h with mechanical shaking twice a day. This was repeated three times until the extract gave faint or no coloration. The extract was then filtered through Whatman filter paper No.1 and the filtrate was evaporated to dryness under reduced pressure by Rota vapor and farther concentrated by water bath at 40°C. Then, the gummy residue extract was packed in air tight brown glass bottles with proper label and kept in a refrigerator at 4°C until used for the experiment.

Experimental induction of diabetes mellitus in rats

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared solution of streptozotocin (60 mg/kg body weight) dissolved in 0.1 M citrate buffer, pH 4.5. The negative control rats were injected with the same volume of citrate buffer only. Streptozotocin injected rats were allowed to drink 5% glucose solution overnight to overcome initial drug induced hypoglycemic mortality.

Diabetes mellitus in streptozotocin injected rats was confirmed by measuring the fasting blood glucose concentration, 72 h after injection. The rats with fasting blood glucose level above 250 mg/dL were enrolled in the study. Treatment was started on the third day after streptozotocin injection and considered as zero day of treatment.

Experimental design and treatment protocol

Rats were divided as Group I: non-diabetic or normal control,



Figure 1. Picture of *A. remota* benth.

received appropriate volume of vehicle, that is, 1% tween 80, 10 ml/kg body weight; Group II: diabetic control, received the vehicle, that is, 1% tween 80, 10 ml/kg body weight; Group III: diabetic treatment, received 200 mg/kg body weight of ethanol extract of *A. remota* leaves; Group IV: diabetic treatment, received 400 mg/kg body weight ethanol extract of *A. remota* leaves; Group V: diabetic treatment, received 600 µg/kg body weight of glibenclamide. Ethanol extract of *A. remota* leaves and glibenclamide were administered every morning for 28 days by gastric intubation with an oral gavage.

Measuring fasting blood glucose

FBG was measured with SensoCard glucometer after the collection of blood sample from the tail vein of the overnight (12 to 15 h) fasted rats on days 0, 7, 14, 21 and 28.

Determination of the weight of rats

Body weight gain or lost in each experimental rat was measured and recorded on days 0, 7, 14, 21 and 28 with triple balance.

Estimation of serum biochemical parameters

At the end of the experimental period (on day 29th), all five groups of rats were sacrificed after overnight fast, by anesthetizing with diethyl ether, and then blood was collected by direct cardiac puncture. Serum was separated after coagulated at room temperature for 30 min and centrifuged at 3000 rpm for 10 min, which was stored at -20°C until biochemical parameters were determined. Total cholesterol, triacylglycerol, high density lipoprotein-cholesterol, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea and creatinine were determined with 902 automated chemistry analyzer. Low density lipoprotein-cholesterol level was calculated using Friedwald equation. Serum TP was determined with A 25 BioSystems chemistry analyzer.

Statistical analysis

All the values of body weight, fasting blood sugar and serum biochemical parameters were expressed as mean ± standard error of mean (SEM) and were performed using SPSS software package Version 20.0. The values were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test.

RESULTS

Extraction

In preparation of crude ethanol extract of *A. remota* from 600 g coarse powder leaves, 11.8% (70.80 g) yield of gummy residue was obtained.

Effect of *A. remota* leaves extract on fasting blood glucose level in diabetic rats

The effect of different doses of ethanol extract of *A. remota* on fasting blood glucose level in diabetic rats is

Table 1. Effect of *A. remota* leaves extract on fasting blood glucose level in STZ induced diabetic rats.

Groups	Fasting blood glucose level (mg/dL)				
	0 day	7th day	14th day	21st day	28th day
Normal control	97.2±5.2	99.2±3.3	94.50±3.8	98.5±3.9	97.0±5.0
Diabetic control	383.7±15.1[a]	414.7±13.2[a]	446.5±9.7[ac]	473.17±11.1[ac]	398.8±21.5 [a]
Diabetic + AREt (200 mg/kg)	386.5±16.0[a]	394.7±13.4[a]	383.3±14.9[a]	313.3±18.1[abc]	275.7±14.8[abc]
Diabetic + AREt (400 mg/kg)	381.2±24.1[a]	387.0±20.9[a]	359.2±14.2[ab]	303.5±20.1[abc]	230.2±23.1[abc]
Diabetic + Glibenclamide (600 µ/kg)	393.0±12.8[a]	336.3±21.6[ab]	328.7±15.7[ab]	266.0±14.2[abc]	207.3±29.7[abc]

The values indicate mean ±S.E.M (n=6).[a] p<0.05 compared with normal control values, [b] p<0.05 compared with diabetic control values and [c] p<0.05 compared with the initial level of fasting blood glucose level (0 day) of the rats in the respective group.

Table 2. Effect of *A. remota* leaves extract on body weight of STZ induced diabetic rats.

Groups	Body weight (g)				
	0 day	7th day	14th day	21st day	28th day
Normal control	161.1±5.4	174.1±4.7[c]	188.7±5.5[c]	202.5±5.0[c]	215.5±4.06[c]
Diabetic control	142.6±3.4	132.6±2.6[a]	122.2±2.8[ac]	118.6±4.2[ac]	114.0±3.2[ac]
Diabetic + AREt (200 mg/kg)	147.0±5.3	135.8±4.0[a]	138.9±2.5[ab]	139.2±2.6[ab]	144.6±3.2[ab]
Diabetic + AREt (400 mg/kg)	150.2±6.9	137.8±5.8[a]	144.8±3.9[ab]	147.1±4.4[ab]	149.1±4.6[ab]
Diabetic + Glibenclamide (600 µg/kg)	145.8±2.9	137.1±2.7[a]	141.4±2.7[ab]	147.4±2.6[ab]	150.1±3.8[ab]

The values indicate mean ±S.E.M (n=6).[a] p<0.05 compared with normal control values, [b] p<0.05 compared with the diabetic control group and [c] p<0.05 compared with the initial weight (0 day) of the rats in the respective group.

Table 3. Effect of *A. remota* leaves extract on lipid profile of STZ induced diabetic rats after 28 days treatment.

Groups	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	86.0±1.3	79.5±1.0	28.3±0.7	41.77±1.7
Diabetic control	191.2±6.3[a]	163.50±3.3[a]	18.8±0.7 [a]	139.63±6.2[a]
Diabetic + AREt (200 mg/kg)	129.5±2.1[ab]	95.67±3.7[ab]	25.2±0.4[ab]	85.20±2.3[ab]
Diabetic + AREt (400 mg/kg)	98.2±1.5[b]	80.00±2.1[b]	29.77±0.5[b]	52.50±1.6 [b]
Diabetes + Glibenclamide (600 µg/kg)	88.7±2.1[b]	69.67±2.9 [b]	35.5±0.9[ab]	39.23±2.5[b]

The values indicate mean ±S.E.M (n=6). [a] p<0.05 compared with normal control values and [b] p<0.05 compared with diabetic control values.

given in Table 1. Fasting blood glucose level of diabetic control rats were significantly (p<0.05) higher than those of normal control rats on days 0, 7, 14, 21 and 28. Fasting blood glucose level significantly decreased in diabetic rats treated with 200 mg/kg and 400 mg/kg ethanol extract of *A. remota* and 600 µg/kg glibenclamide on 21st, 14th and 7th day of treatment, respectively as compared with diabetic control rats.

Effect of *A. remota* leaves extract on body weight in diabetic rats

As shown in Table 2, the body weights of untreated diabetic control rats were significantly reduced (p< 0.05) as compared to the normal control rats. Ethanol extract of *A. remota* treated diabetic rats for 28 days significantly (p< 0.05) improved the body weight gain at 200 and 400 mg/kg body weight as compared to the diabetic control group, comparable to that of the standard at 600 µg/kg. The body weight of normal control rats significantly (p< 0.05) increased on days 7, 14, 21 and 28 as compared to the 0th day of treatment, while the diabetic control rats significantly decreased on days 14, 21 and 28 as compare to 0th day. However, the body weight of diabetic rats treated with any of the doses of ethanol extract of *A. remota* did not significantly changed from the 0th day of treatment.

Effect of *A. remota* leaves extract on serum lipid profile in diabetic rats

The level of serum lipid profile of experimental rats is shown in Table 3. Serum total cholesterol, triacylglycerol and low density lipoprotein-cholesterol increased

Table 4. Effect of *A. remota* leaves extract on serum ALT, AST and ALP of STZ induced diabetic rats after 28 days treatment.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	30.0±1.1	55.8±1.35	51.833±0.8
Diabetic control	72.0±1.3[a]	94.8±3.5[a]	87.33±0.84[a]
Diabetic + AREt (200 mg/kg)	54.2±1.0[ab]	78.0±4.4[ab]	80.00±2.5[ab]
Diabetic + AREt (400 mg/kg)	47.7±2.4[ab]	75.0±3.1[ab]	72.83±1.0[ab]
Diabetic + glibenclamide (600 µg/kg)	42.8±1.6[ab]	71.5±5.5 [b]	65.83±1.2[ab]

The values indicate mean ±S.E.M (n=6). [a] p<0.05 compared with normal control values and [b] p<0.05 compared with diabetic control values.

Table 5. Effect of *A. remota* leaves extract on serum urea, creatinine and total protein of STZ induced diabetic rats after 28 days treatment.

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Total protein (mg/dL)
Normal control	38.2±0.6	0.8±0.03	5.8±0.1
Diabetic control	80.0±2.5[a]	1.7±0.2[a]	4.7±0.2[a]
Diabetic + AREt (200 mg/kg)	59.3±1.7[ab]	1.3±0.1[ab]	5.7±0.1[b]
Diabetic + AREt (400 mg/kg	52.5±1.3[ab]	1.1±0.1[b]	6.0±0.1[b]
Diabetic + glibenclamide (600 µg/kg)	49.2±1.8[ab]	1.0±0.0[b]	5.9±0.1[b]

The values indicate mean ±S.E.M (n=6). [a] p<0.05 compared with normal control values and [b] p<0.05 compared with diabetic control values.

significantly (p< 0.05) in diabetic control rats as compared to the normal control rats; while, the level of high density lipoprotein-cholesterol decreased significantly (p<0.05) in diabetic control rats as compared to the normal control rats. Serum total cholesterol, triacylglycerol and low density lipoprotein-cholesterol levels significantly (p<0.05) reduced whereas that of high density lipoprotein-cholesterol significantly (p<0.05) increased with 200 mg/kg body weight and 400 mg/kg body weight ethanol extract of *A. remota* leaves treatment as compared to the diabetic rats. Treatment with 400 mg/kg body weight ethanol extract of *A. remota* leaves for 28 days reversed the aforementioned values near normal. The effect was comparable with 600 µg/kg body weight glibenclamide with the same period of treatment. A dose dependent reduction in the levels of total cholesterol, triacylglycerol, low density lipoprotein-cholesterol and increase of high density lipoprotein-cholesterol was observed with both doses of ethanol extract of *A. remota* treatment.

Effect of *A. remota* leaves extract on serum alanine aminotransferase, aspartate amino transferase and alkaline phosphatase in diabetic rats

The serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline aminotransferase significantly (p<0.05) increased in diabetic control rats as compared to normal controls. Administration of 200 mg/kg body weight and 400 mg/kg body weight ethanol extract of *A. remota* for 28 days significantly (p< 0.05) reduced the activity of alanine aminotransferase, aspartate amino transferase and alkaline phosphatase in diabetic rats as compared to diabetic control group. Similar effect was also observed with 600 µg/kg glibenclamide treatment (Table 4).

Effect of *A. remota* leaves extract on serum urea, creatinine and total protein in diabetic rats

Table 5 illustrates that serum urea and creatinine levels were significantly (p<0.05) elevated in diabetic control rats as compared to the normal control rats. Whereas, a decrease in TP levels was found in diabetic control rats when compared with normal control rats. Administration of 200 and 400 mg/kg ethanol extract of *A. remota* leaves and 600 µg/kg glibenclamide significantly (p<0.05) reduced the serum urea and creatinine level as compared to the diabetic control rats. On the other hand, the level of serum total protein significantly (p<0.05) increased as compared to diabetic control rats after 28 days of treatment.

DISCUSSION

The present study was designed to investigate the

antihyperglycemic and antihyperlipidemic activities of ethanol extract of *A. remota* leaves in streptozotocin induced diabetic rats. Streptozotocin has been used to induce diabetes mellitus in experimental rats (Latha and Daisy 2011). A single intraperitoneal (IP) administration of 60 mg/kg streptozotocin effectively induced diabetes mellitus in rats, which was confirmed by elevated level of fasting blood glucose obtained from the tail of the rats after 72 h of injection.

The mechanism by which streptozotocin brings about diabetic mellitus includes selective destruction of insulin secreting pancreatic β-cells and minimize glucose uptake by peripheral tissues (Szkudelski, 2001). Ethanol extract of *A. remota* leaves reduced high fasting blood glucose level in streptozotocin induced diabetic rats. The mechanism antihyperglycemic of actions of ethanol extract of *A. remota* leaves might be due to an insulin mimetic effect on muscle and adipose tissues by either stimulating glucose uptake and metabolism (Daisy et al., 2010), by inhibiting hepatic gluconeogenesis (Cetto and Vázquez, 2010) and glycogenolysis (Rawi et al., 2011), by stimulation of regeneration process or increase pancreatic secretion of insulin from existing β-cells (Sharma et al., 2008) and/ or inhibition activity against α-glucosidase enzymes in small intestine which convert disaccharides into monosaccharaides for sake of absorption (Shinde et al., 2008).

The difference in the magnitude of the effect on fasting blood glucose between 400 and 200 mg/kg ethanol extract of *A. remota* leaves doses might be attributed to the higher concentration of the active component(s) responsible for more fall of fasting blood glucose in the former than the later.

Fasting blood glucose lowering effect of ethanol extract of *A. remota* leaves is similar to *Ajuga iva*, which belongs in the same family (Hilaly and Lyoussi, 2002). Phytochemical investigations of *A. remota* have reported the presence of bioactive compounds such as iridoid and flavonol glycosides and phytoecdysteroids (Kubo et al., 1983 and Manguro et al., 2006). It has been suggested that antihyperglycemic effect of ethanol extract of *A. remota* leaves extract probably attributed to these constituents through improvement of insulin level (Sundaram et al., 2012).

The loss of body weight in untreated diabetic rats is due to increased muscle wasting and catabolism of others tissue proteins (Flatt et al., 1990). However, ethanol extract of *A. remota* leaves treatment improved the body weight in diabetic rats perhaps, due to the antihyperglycemic effect which was an indication of proper glucose utilization (Pari and Satheesh, 2004), its protective effect in muscle wasting and controlling protein turn over and/or improvement in diabetes mellitus associated disorders (Oyedemi et al., 2012).

Abnormalities in lipid profile are common complications in diabetes mellitus (Gibbons, 1986). Such abnormality represents the risk factors for coronary heart diseases. Activation of hormone sensitive lipase during insulin deficiency causes an increase in free fatty acid mobilization from adipose tissue and result in synthesis of triacylglycerol in liver (Mooradian, 2009). In addition, hyperglycemia is accompanied by a rise in total cholesterol, triacylglycerol, low density lipoprotein-cholestreol and a fall in high density lipoprotein-cholesterol (Gao et al., 2009). In the present study, total cholesterol, triacylglycerol and low density lipoprotein-cholesterol levels decreased and high density lipoprotein-cholesterol increased in ethanol extract of *Ajuga remota* leaves treated diabetic rats.

The remarkable control of high serum triacylglycerol in ethanol extract of *A. remota* leaves treated diabetic rats could be due to inhibition of endogenous triacylglycerol synthesis in liver (Xie et al., 2007) or improvement in insulin level or the presence of active component(s) in ethanol extract of *A. remota* leaves that suppressed the activity of hormone sensitive lipase in adipose tissue or increased activity of hepatic lipase or lipoprotein lipase accountable for the hydrolysis of excess lipoprotein bound triacylglycerol into fatty acids (Pritchard et al., 1986).

Increased level of HDL-C in ethanol extract of *A. remota* leaves treated groups could be due to the enhancement of lecithin: cholesterol acyltransferase (LCAT) which plays a key role in incorporating the free cholesterol into high density lipoprotein which take back to the liver (Senoucia et al., 2012). LDL-C reducing effect of ethanol extract of *A.* remota leaves presumably attributed to increased expression of low density lipoprotein receptor (LDLR), which enhance low density lipoprotein particles uptake in liver from the circulation, through the depletion of intracellular cholesterol (Bursill et al., 2007).

Serum TC lowering property of ethanol extract of *A. remota* leaves could be attributed to the availability of hypocholesterolemic compounds in ethanol extract of *A. remota* leaves that may act as inhibitor for hepatic hydroxyl methyl glutaryl CoA (HMG CoA) reductase in liver, which take part in cholesterol synthesis (Kumarappan et al., 2007) or increasing the fecal content by inhibiting the absorption of cholesterol from intestine (Raederstorff et al., 2003). Isolated phytoecdysteroids and iridoid glycosides, phytochemical constituent of ethanol extract of *A. remota* leaves, from other *Ajuga* species have shown antioxidant activity (Fan et al., 2011). Thus, reduction of total cholesterol in ethanol extract of *A. remota* leaves ethanol extract of *A. remota* leaves extract treated diabetic rats might also attributed to the aforementioned phytochemical constituents by reducing lipid peroxidation by scavenging free radicals (Sharma et al., 2008).

The decrease in total cholesterol, triacylglycerol and low density lipoprotein-cholesterol and an increase in high density lipoprotein-cholesterol after 28 days treatment showed a dose dependent trend, indicating that

efficacy was proportional to the dose of ethanol extract of *A. remota* leaves. Similar hypocholestrolemia and hypotriacyleglycerol effects were observed by *A. iva* whole plant extract in streptozotocin induced diabetic rats (Hilaly et al., 2007).

The activity of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in serum are generally indicators of liver function. In diabetic rats, the level of these enzymes are elevated due to necrosis of liver cells by the injection of STZ (Hwang et al., 1996). However, ethanol extract of *A. remota* leaves treated diabetic rats showed decreased in the activity of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase enzymes that might support its hepatoprotective effect and normalization capability of impaired liver metabolism in diabetic rats.

There is increased protein catabolism with the flow of amino acids into the liver, which feeds gluconeogenesis as a result of insulin deficiency during uncontrolled diabetes mellitus. This might account for the decrease in serum total protein content in STZ induced diabetic control rats (Narendhirakannan et al., 2006). The results of the present study demonstrated that treatment of diabetic rats with ethanol extract of *A. remota* leaves caused a significant increase in serum total protein which might be attributed to an improvement in glycemic control and insulin secretion that increase protein synthesis or decrease protein degradation (Gao et al., 2009).

Negative nitrogen balance is manifested in diabetic rats associated with enhanced proteolysis in muscle and other tissues. Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentrations of urea and creatinine in serum (Basha and Subramanian, 2011) indicating progressive renal damage in diabetic rats (Anjaneyulu and Chopra, 2004). Treatment with ethanol extract of *A. remota* leaves resulted in a considerable reduction to near normal in serum urea and creatinine level indicating the renoprotective role of ethanol extract of *A. remota* leaves or delay diabetic nephropathy development.

Hepatoprotective and renoprotective effect of ethanol extract of *A. remota* leaves might be due to phyloecdysteroids, possibly through their antioxidant activity (Krishnan et al., 2007). In conclusion, these findings demonstrated that ethanol extract of *A. remota* leaves possesses antihyperglycemic and antihyperlipidemic properties which might support the traditional claim of its use in diabetes.

Conflict of Interests

The authors have not declared any conflict of interests.

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