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# *ARISTOLONCHIA REPENS* STEM BARK AMELIORATES LIVER AND KIDNEY FUNCTION IN HIGH-FAT DIET AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

**Omar, S. A., Soji-Omoniwa, O. and Osifade, J. T**

Phytomedicine, Toxicology and Reproductive Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Nigeria

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*Corresponding email: olaoye.sa@unilorin.edu.ng

## ABSTRACT

*Aristolonchia repens,* is among such herb utilized locally for managing diabetes and related problems. The study evaluated the ameliorative effects of aqueous extract of *Aristolonchia repens* stem bark on liver and kidney function in high-fat diet and streptozotocin-induced diabetic rats. Type 2 diabetes was induced in rats with high-fat diet and low dose of streptozotocin (35 mg/kg body weight). Non-diabetic male Wistar rats were treated with distilled water (Naïve control) while the diabetic male Wistar rats were treated with water, Metformin (100 mg/kg body weight), 50, 100 and 200 $mgkg^{-1}$ bodyweight of aqueous extract of *Aristolonchia repens* stem bark for 28 days. The rats were sacrificed and sera were collected and analyzed. High-fat diet and streptozotocin significantly altered ($P<0.05$) the alanine aminotransferase, aspartate aminotransferase activities, concentration of total bilirubin, total protein, albumin, globulin, creatinine, urea, potassium, sodium, bicaronate and chloride when compared with Naïve and positive control. Wistar rats treated with 50, 100 and 200 $mgkg^{-1}$ body weight of aqueous extract of *Aristolonchia repens* significantly did not altered ($p>0.05$) the alanine aminotransferase and aspartate aminotransferase activities, when compared with Naive and Metformin group. Similarly, the concentration of total bilirubin, total protein, albumin, globulin, creatinine, urea, potassium, sodium, bicaronate and chloride was not significantly altered ($p>0.05$) when compared with the Naïve and Metformin group. This study suggests that *Aristolonchia* repens stem ameliorates liver and kidney function in high-fat diet and streptozotocin-induced diabetic rats and hence may be safe for use in the management of diabetes.

**Keywords:** *Aristolochia repens*, Diabetes Mellitus, Streptozotocin, High-fat diet.

## INTRODUCTION

Diabetes mellitus (DM) is a prevalent metabolic disorder marked by hyperglycemia (elevated levels of blood sugar), polyphagia (excessive eating), and polydipsia (excess thirst) (Blair, 2016; Alam *et al.,* 2021). The hyperglycemia, which is the hallmark symptom of DM, could be a result of either dysfunction of pancreatic beta cells leading to deficiency in insulin production or desensitization of insulin receptors on target organs (insulin resistance) (Camarillo, 2023). If left uncontrolled, diabetes can result in damage to the kidneys, nerves, and eyes. Other complications that might occur as a result include cardiovascular diseases such as heart attacks and strokes, hypertension, and imbalanced osmolality of blood (Ignatavicius and Workman, 2016). Based on the underlying cause or mechanism, there are two major types of diabetes mellitus: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) (World Health Organization, 2019). T1DM is characterized by immune-mediated destruction of pancreatic beta cells resulting in an absolute insulin deficiency while T2DM (more common) is characterized by different levels of beta cell dysfunction and insulin resistance (Alam *et al.,* 2021). Since 1980, there has been a substantial escalation in the

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prevalence of diabetes. The World Health Organization's data indicates a substantial surge in the number of diabetic adults from 108 million diabetic adults in 1980 to 422 million in 2014 with the global prevalence increasing from 4.7% to 8.5% (Mekala and Bertoni, 2020). According to the International Diabetes Federation's statistics, as of 2021, an estimated 537 million adults were diabetic, reflecting a prevalence of 10.5%. Projections indicate a further escalation to 783 million by 2045, corresponding to a prevalence of 12.2% (Sun *et al.,* 2022). Several medications are often recommended for the management of diabetes mellitus, T2DM especially, such as metformin (most commonly used), acarbose (an α-glucosidase inhibitor), sulfonylureas, rosiglitazone, and GLP-1 agonists (e.g exenatide and liraglutide) (Inzucchi *et al.*, 2012; Nanjan *et al.,* 2018). However, the use of the medications has some side effects like hypoglycemia, abdominal pain, diarrhea, and lactic acidosis (Filippatos *et al.*, 2014; Kalra, 2014). Moreover, a majority of them are not easily accessible due to cost considerations. Consequently, several ongoing research endeavors are investigating the antidiabetic activity of phytoconstituents derived from medicinal plants. This avenue is particularly promising, as these plant sources present a more accessible alternative, coupled with the advantage of reduced costs and diminished adverse side effects (Ansari *et al.,* 2022).

*Aristolochia repens* is a perennial vine of the family Aristolochiaceae, and it is commonly known as "Dutchman's Pipe" (Onuminya *et al.*, 2022). The plant is native to southeastern United States of America, and it survives in a variety of habitats. It is now being grown in West Africa (Bankole *et al.,* 2021), known by its local name, "Akogun," and its dried leaves and roots are used in Northern Nigeria as an anthelminthic (Afoloyan and Sowemimo, 2022). Studies have established the use of *A. repens* in treating piles, stomach aches, diarrhea, hypertension, and diabetes (Obode *et al.,* 2020; Bankole *et al.*, 2021). Furthermore, its stem and seed have been used in combination with other medicinal plants like *Aristolochia albida* in the treatment of intestinal worms. Traditionally, *Aristolochia spp* has been used to treat gastrointestinal disorders, sexually transmitted diseases, and cardiovascular and respiratory conditions (Afolayan and Sowemimo, 2022). This evidence suggests its traditional use in treating various diseases; however, there is a noticeable gap in knowledge regarding its hypoglycemic activity. Therefore, the objective of this study was to evaluate the ameliorative effects of aqueous extract of *Aristolonchia repens* stem bark on liver and kidney function in high-fat diet and streptozotocin-induced diabetic rats.

## MATERIALS AND METHODS

### Plant Materials

Fresh stem of *Aristolochia repens* obtained at Oja oba in Ilorin Nigeria was identified and authenticated at the Herbarium section (Voucher No: UILH/001/1254), Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

### Experimental Animals

Thirty (30) Male Wistar rats (150.50 - 155.35 g) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were kept in well ventilated cages and allowed to acclimatize to animal house condition of temperature 28 – 31 ℃, 12 hours of natural light and 12 hours darkness and humidity (50 – 55%) for fourteen (14) days. The rats were fed with rat pellets (Vita Feed, Grand Cereals, Vom, Jos, Nigeria) and allowed free access to water.

### Assay kits and Reagents

Streptozotocin was obtained from Sigma-Aldrich, St Louis, Mo, USA. AccuChek Glucometer strips was obtained from Nature's Choice™ Wholefood specialist, Meyerton, South Africa. Metformin was a product of Austell laboratories Pvt. Ltd., Johannesburg, South Africa. Assay kit for bilirubin and albumin were obtained from Agape diagnostics, Switzerland. Aspartate aminotransferase and Alanine aminotransferase assay kits were obtained from Randox Laboratories Ltd., Antrim, UK. Urea, creatinine and albumin assay kits were products of Fortress diagnostic. All other chemicals were of analytical standard.

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**Plant Preparation**

Fresh stem barks of *A. repens* was collected, washed to remove all contaminants, air dried to a constant weight and then grinded into fine powder using a mechanical grinding machine. The powdered form of the stem was pretreated and air dried, then it was stored in a cool dry container prior to analysis.

**Aqueous Extract Preparation of *Aristolochia repens* Stem Bark**

A known weight of five hundred grams (500 g) of the *A. repens* stems were percolated in 2000 ml of distilled water for 48 hours with continuous stirring at intervals. It was filtered using whatman (no. 1) filter paper. The resulting filtrate was lyophilised with a Zirbus Lyophiliser, (Model VaCo 5-11, Zirbus Technology, Stephensonstraat, Germany) to obtain 12.20 g corresponding to a yield of 2.44%.

**Diet Constituents**

Corn, soybeans and maize husk were purchased from Olufadi Market, Ilorin, Nigeria. Lard was sourced from a pig abattoir at Obbo Road Ilorin, Kwara State. Sucrose was a product of Sigma-Aldrich Inc., St. Louis, MO, USA. Soybean oil was a product of Sunola Foods Limited, Isolo, Lagos. The vitamin and mineral mix were product of Wealth Agro Allied, Ibadan, Oyo State, Nigeria.

**Diet Formulation**

The maize seeds were rinsed and dried for 48 hours rinsing and drenching and then milled. The milled product was then placed in cheese cloth, drained overnight, and air-dried. Similarly, the soybean seeds were soaked for 6 hours to facilitate the removal of the seed coat. After dehulling, the soybeans were air-dried and milled. The lard was melted to eliminate impurities and left to cool at 27°C. The percentage nutrient composition adhered to the diet formulation this was as outlined by Brotolin *et al*. (2018), with slight adjustments (Brotolin *et al*. (2018) included salt and cholesterol in the composition of diet which wasn't included in this study) as detailed in Table 1.

**Table 1: Diet constituents of the control and high-fat diets**

| Ingredients | Control diet (Low fat diet) (g) | High-fat diet (g) |
|---|---|---|
| Soybean | 200 | 200 |
| Lard | 0 | 300 |
| Soybean oil | 40 | 40 |
| Corn starch | 552 | 252 |
| Sucrose | 100 | 100 |
| Corn hulk | 50 | 50 |
| Vitamins | 10 | 10 |
| Mineral mix | 40 | 40 |
| L-Lysine | 4 | 4 |
| D-Methionine | 4 | 4 |
| Total | 1000 | 1000 |

**Induction of Type II diabetes**

Twenty-five Wistar rats were subjected to an eight-week regimen of a high-fat diet, while an additional five rats (serving as the positive control group) were maintained on a low-fat diet. Following the high-fat diet period, the animals underwent an overnight fast, after which each rat from the high-fat diet group received an intraperitoneal injection of a low dose of streptozotocin (STZ) at 35 mg/kg body weight. Precisely three days of post-STZ injection, fasting blood glucose levels were assessed using an AccuChek active glucometer and compatibility strips, with blood obtained from the caudal vein of the rat's tail. Rats exhibiting glucose concentrations exceeding 200 mg/dl were classified as diabetic.

**Experimental Design**

Thirty (30) Wistar rats were randomly selected into six (6) groups of five (5) animals each. The grouping is as follows:

Group 1 (Naïve control) – Low-fat diet + Distilled water

Group 2 (Negative control) – High-fat diet + Diabetic induced rat

Group 3 (Positive Control) – High-fat diet + STZ + Metformin –100 mg/kg body weight

Group 4 (AEARSB 50) – High-fat diet + STZ + 50 mg/kg body weight of the aqueous extract of *A. repens* stem bark

Group 5 (AEARSB 100) – High-fat diet + STZ + 100 mg/kg body weight of aqueous extract of *A. repens* stem bark

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Group 6 (AEARSB 200) – High-fat diet + STZ + 200 mg/kg body weight of aqueous extract of *A. repens* stem bark

**Preparation of Serum**

The methodology outlined by Yakubu and Omar (2019) was employed. Animals were sacrificed 24 hours post the final day of treatment, following an overnight fasting period. They were anaesthetized with diethyl ether and sacrificed by simply incising the jugular vein. Blood samples were collected in plain sample bottles for subsequent biochemical serum analysis, followed by centrifugation for 10 minutes to obtain the serum. The serum were transferred into plain bottles and stored in the freezer until further analysis was required.

**Determination of Biochemical Parameters**

The determination alanine aminotransferase (ALT) activity was carried using the method described by Wright *et al*. (1972). The method described by Reitman and Frankel (1957) was used to determine the aspartate aminotransferase (AST) activity. The method described by Jendrassik and Grof (1938) was adopted for determination of bilirubin concentration. Albumin concentration was determined using Doumas *et al*. (1997). Creatinine concentration was determined using Bartels and Bohmer (1972) method. Urea concentration was determined using the method described by Veniamin and Vakirtzi (1970). Potassium concentration was determined using the method described by Terri and Sesin (1958). Sodium concentration was determined using the method described by Maruna (1958). The chloride concentration was determined using the procedure described by Skeggs and Hochestrasser (1964).

**Statistical Analysis**

All data are expressed as the mean of five replicates ± standard error of mean (SEM). The results obtained were analyzed with IBM SPSS Statistics 20 using one-way ANOVA (Analysis of Variance) by Duncan's post HOC test for multiple comparisons. Values were considered statistically significant at $p < 0.05$.

**RESULTS**

**Fasting Blood Glucose Level**

The fasting blood glucose (FBG) concentration of STZ and high-fat diet-induced diabetic rats with *A. repens* extract is shown in Figure 1. The FBG concentration increased significantly upon administration of STZ and high-fat diet. However, a significant reduction ($p < 0.05$) was observed with treatment with aqueous extract of *A. repens* stem bark when compared with the negative control. The treatment with 200 mg/kg body weight showed the most profound reduced fasting blood glucose concentration comparing favourably with the control.

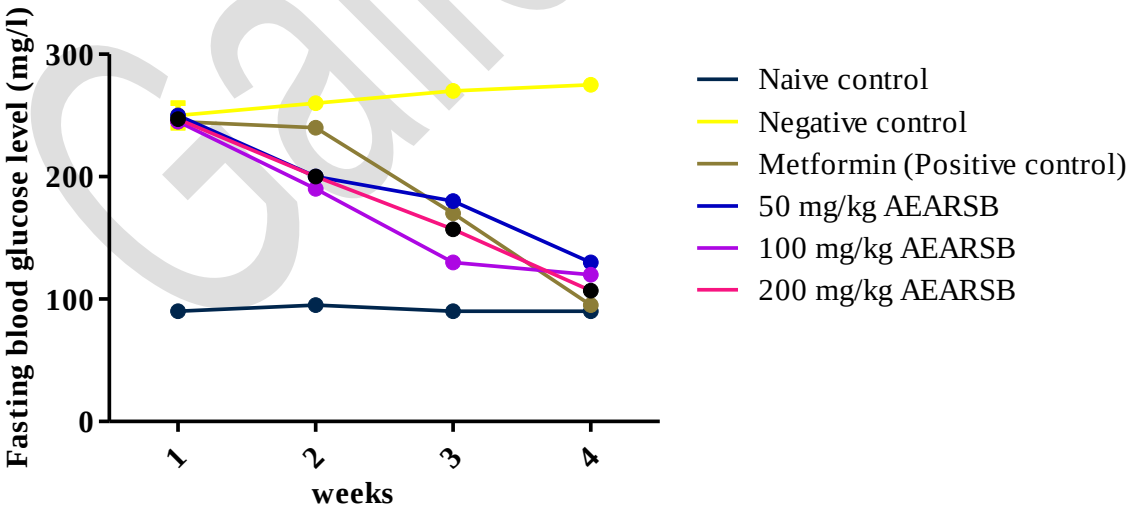



**Figure 1: Fasting blood glucose level of Streptozotocin and high-fat diet induced diabetic rats treated with aqueous extract of *A. repens* stem bark**

AEARSB: aqueous extract of *A. repens* stem bark

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Table 2 depicts the significant increase ($p < 0.05$) of alanine and aspartate aminotransferase activity when compared with positive control. Treatment with aqueous extract of *A. repens* at 50, 100 and 200 mg/kg showed no significant difference ($p > 0.05$) when compared with Naïve and positive control. The bilirubin concentration of negative control was significantly increase when compared with the Naïve and positive control. The treatment at 50, 100 and 200 mg/kg did not significantly ($p >0.05$) altered the bilirubin concentration when compared with the Naïve and positive control. The serum concentration of protein (6.64 ± 0.78 mg/dl), albumin (10.95 ± 0.70 mg/dl) and globulin (4.58 ± 0.28 mg/dl) of negative control was significantly reduced ($p < 0.05$) when compared with the positive control (total protein was 23.13 ± 1.01 mg/dl, albumin was 11.95 ± 0.65 mg/dl and globulin was 11.09 ± 0.76 mg/dl). While the serum concentration of protein, albumin and globulin of animals treated with 50, 100 and 200 mg/kg body weight of aqueous extract of *A. repens* stem bark was not significantly altered ($p >0.05$) when compared with Naïve and positive control (Table 2).

Urea (11.68 ± 0.61 mg/dl) and creatinine (3.92 ± 0.21) concentration of negative control was significant increased ($p<0.05$) when compared with the positive control (Urea was 8.28 ± 0.77 mg/dl and creatinine was 0.84 ± 0.14 mg/dl). Treatment with 50, 100 and 200 mg/kg body weight of aqueous *A. repens* stem bark extracts significantly reduced ($p<0.05$) the urea and creatinine concentration when compared with the negative control but compared favourably with positive control (Table 3). The level of serum bicarbonate ions of negative control was significantly reduced when compared with the Naïve and positive control. Treatment with aqueous extracts of *A. repens* stem bark at 50, 100 and 200 mg/kg body weight significantly decreased ($p<0.05$) the sodium when compared with negative control but compared favourably with the positive control. The potassium and chloride concentration of STZ and high-fat diet induced diabetic rats was significantly increased when compared with the positive control. Treatment with aqueous extracts of *A. repens* stem bark at 50, 100 and 200 mg/kg body weight significantly reduced ($p < 0.05$) the potassium and chloride concentration when compared with negative control but compared favourably with the positive control (Table 3).

## DISCUSSION

The 28 days treatment with aqueous extracts of *A. repens* at 50, 100 and 200 mg/kg body weight reduced the blood glucose concentration in STZ induced diabetic rats. Therefore, the reduction in the blood glucose levels of STZ and high-fat diet induced diabetic animals treated with the aqueous *A. repens* suggests anti hyperglycemic effect of the extract. This is an indication of hypoglycemia activity of the extract.

The serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are involved in the reductive transfer of amino acids, specifically alanine and aspartate, respectively. This process occurs by transferring these amino acids to alpha-ketoglutarate, forming pyruvate or oxaloacetate, respectively. When hepatocytes are damaged, they release their contents, including ALT and AST, into the extracellular space. Therefore, elevated levels of these enzymes are indicative of liver damage, as they are responsible for the metabolism (transamination) of alanine (Desai *et al*., 2012). The observed reduction in the activity of ALT and AST in the serum as well as corresponding decrease in the activity of the enzyme in the serum of STZ and high-fat diet diabetic rats treated with aqueous extracts of *A. repens* stem bark may imply that the extract have the ability to reverse the enzyme activity. The non-significant difference in the activities of the enzymes in STZ and high-fat diet diabetic rats with the control further suggest an indication that the integrity of the organs is not compromised and also the extract might not pose any toxicity effects on the organs.

Bilirubin is the product of hemoglobin catabolism within the reticuloendothelial system. Heme breakdown determines the formation of unconjugated bilirubin, which is then transported to the liver (Hansen *et al*., 2020). In the liver, UDP-glucuronyltransferase conjugates the water-insoluble unconjugated bilirubin to glucuronic acid, and conjugated bilirubin is then excreted into the bile (Zhou *et al*., 2010). Increased bilirubin causes jaundice and can indicate metabolism problems in the liver, for example reduced hepatocyte uptake, impaired bilirubin conjugation, or reduced bilirubin secretion

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(Nwikwe and Balogun, 2021). Therefore, serum bilirubin concentration is a real liver function biomarker, which measures the ability of liver to clear bilirubin from the blood as it circulates through the liver. In contrast, serum transaminase levels indicate the rate of enzyme release from injured cells and the Total bilirubin rates of enzyme degradation (mainly by the mononuclear phagocyte system) (Yang *et al*., 2014). The observed reduction in the bilirubin after 28-days oral treatment with aqueous extracts of *A.repens* stem bark of STZ and high-fat diet shows no derangement in the liver. It also denotes that the ability of the liver to clear circulating blood bilirubin is unaltered. Total protein is composed of albumin, bilirubin and globulin and reflects the balance of protein biosynthesis and catabolism. Albumin and globulin are useful indicators of synthetic function of the liver (Yakubu *et al.*, 2003). Inflammatory responses are mostly triggered by a decrease in plasma albumin levels (Ruot *et al.*, 2000). The absence of any significant effect comparing with the controls on the total protein, albumin and globulin concentrations suggests that aqueous extracts of *A.repens* stem bark had no toxic effect on the synthetic function of the liver in the diabetic rats.

Serum levels of urea and creatinine can be used as prognostic markers and predictors of renal damage in diabetic patients (Pathan *et al.*, 2020). Serum creatinine is a more sensitive index of kidney function compared to serum urea level. This is because creatinine fulfills most of the requirements for a perfect filtration marker (Pathan *et al.*, 2020). Urea is a nitrogen-containing substance normally cleared from the blood by the kidney into the urine. Diseases that compromise the function of the kidney e.g. diabetes, often leads to increased serum levels of urea (Gounden *et al.*, 2021). Therefore, treatment with aqueous extracts of *A.repens* stem bark to STZ and high-fat diet induced diabetic rats resulted into reduction of creatinine and urea concentrations, this implies that the extract was able to reduce the urea and creatinine concentration. Thus, suggesting that treatment with aqueous extract of *A. repens stem* bark might help restore optimal kidney function in diabetic rats.

Serum electrolytes including sodium, potassium, chloride and bicarbonate ions are often used to assess acid-base imbalance and monitor the effect of treatment on a known imbalance that is affecting bodily organ function (Gowda *et al*., 2010). In this study, the non-significant difference in the serum sodium, potassium, chloride and bicarbonate of the treated animals with 50, 100 and 200 mg/kg body weight of aqueous extract of *A. repens* stem compared well with the control may suggest normal renal function.

In conclusion, this study suggests that aqueous extract of *Aristolonchia repens* stem bark exhibits ameliorative effects on liver and kidney function in a diabetic rat model induced by high-fat diet and streptozotocin. These findings suggest the extract's potential as a therapeutic agent for diabetes management. However, further investigation is necessary to elucidate the underlying mechanisms and ensure safety in humans.

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**Table 2: Selected liver function indices of streptozotocin and high-fat diet induced diabetic rats treated with aqueous extract of *A. repens* stem bark**

| Parameters | ALT (U/L/mg protein) | AST (U/L/mg protein) | Total bilirubin (mg/dl) | Total protein (mg/dl) | Albumin (mg/dl) | Globulin (mg/dl) |
|---|---|---|---|---|---|---|
| **Naïve control** | $20.72 \pm 3.68^{a}$ | $17.59 \pm 1.10^{a}$ | $7.45 \pm 1.17^{a}$ | $22.05 \pm 1.57^{a}$ | $12.56 \pm 0.42^{a}$ | $10.39 \pm 0.82^{a}$ |
| **Negative control** | $55.25 \pm 3.97^{b}$ | $50.92 \pm 0.64^{b}$ | $15.27 \pm 1.80^{b}$ | $6.64 \pm 0.78^{b}$ | $10.95 \pm 0.70^{b}$ | $4.58 \pm 0.28^{b}$ |
| **Positive control** | $22.41 \pm 2.20^{a}$ | $15.08 \pm 1.49^{a}$ | $7.92 \pm 1.75^{a}$ | $23.13 \pm 1.01^{a}$ | $11.95 \pm 0.65^{a}$ | $11.09 \pm 0.76^{a}$ |
| **50 mg/kg body weight of AEARSB** | $30.54 \pm 3.81^{c}$ | $16.26 \pm 1.38^{a}$ | $7.61 \pm 1.31^{a}$ | $25.64 \pm 1.62^{a}$ | $12.45 \pm 1.08^{a}$ | $12.19 \pm 1.03^{a}$ |
| **100 mg/kg body weight of AEARSB** | $29.54 \pm 3.81^{a}$ | $17.50 \pm 2.28^{a}$ | $6.69 \pm 0.78^{a}$ | $24.13 \pm 0.70^{a}$ | $12.62 \pm 0.40^{a}$ | $11.51 \pm 0.92^{a}$ |
| **200 mg/kg body weight of aqueous extract of AEARSB** | $23.62 \pm 2.73^{a}$ | $17.80 \pm 1.13^{a}$ | $7.11 \pm 0.57^{a}$ | $23.46 \pm 0.65^{a}$ | $12.87 \pm 0.64^{a}$ | $10.97 \pm 0.75^{a}$ |

Values are mean ± SEM of five determinations and are considered statistically significant at $p < 0.05$. Values with different alphabetical superscripts across the groups for each parameters are significantly different ($p<0.05$).
AEARSB: aqueous extract of *A. repens* stem bark

**Table 3: Selected kidney function indices of streptozotocin and high-fat diet induced diabetic rats treated with aqueous extract of *A. repens* stem bark**

| Parameters | Urea (mg/dl) | Creatinine (mg/dl) | Sodium (mmol/l) | Potassium (mmol/l) | Chloride (mmol/l) | Bicarbonate (mmol/l) |
|---|---|---|---|---|---|---|
| **Naive control** | $8.28 \pm 0.77^{a}$ | $0.84 \pm 0.14^{a}$ | $28.15 \pm 2.53^{a}$ | $14.63 \pm 0.28^{a}$ | $8.97 \pm 2.54^{a}$ | $28.30 \pm 0.43^{a}$ |
| **Negative control** | $11.68 \pm 0.61^{b}$ | $3.92 \pm 0.21^{b}$ | $19.71 \pm 2.88^{b}$ | $23.96 \pm 0.92^{b}$ | $14.52 \pm 1.14^{b}$ | $11.74 \pm 0.63^{b}$ |
| **Positive control** | $8.21 \pm 0.48^{a}$ | $0.71 \pm 0.24^{a}$ | $25.64 \pm 2.12^{a}$ | $13.70 \pm 0.16^{c}$ | $10.08 \pm 0.13^{c}$ | $22.81 \pm 1.05^{c}$ |
| **50 mg/kg body weight of AEARSB** | $8.84 \pm 0.40^{a}$ | $0.64 \pm 0.12^{c}$ | $20.75 \pm 3.50^{c}$ | $18.42 \pm 2.30^{c}$ | $11.44 \pm 1.28^{c}$ | $18.54 \pm 1.16^{d}$ |
| **100 mg/kg body weight of AEARSB** | $8.98 \pm 0.57^{a}$ | $0.77 \pm 0.18^{a}$ | $23.63 \pm 2.55^{a}$ | $15.75 \pm 1.19^{c}$ | $10.71 \pm 1.00^{c}$ | $20.70 \pm 0.92^{c}$ |
| **200 mg/kg body weight of AEARSB** | $9.03 \pm 0.75^{a}$ | $0.81 \pm 0.11^{a}$ | $25.22 \pm 2.00^{a}$ | $14.69 \pm 1.52^{a}$ | $10.34 \pm 0.12^{c}$ | $21.70 \pm 0.78^{c}$ |

Values are mean ± SEM of five determinations and are considered statistically significant at $p < 0.05$. Values with different alphabetical superscripts across the groups for each parameters are significantly different ($p<0.05$).
AEARSB: aqueous extract of *A. repens* stem bark

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