Effects of *Achyranthes Aspera, Bidens Pilosa* and *Ajuga Remota* Leaf Extracts on Serum Glucose and Electrolyte Levels in Alloxan Treated Male Goats

Lagat J.K. [1*], Ng'wena A.G.M.[2] and Mwaniki D.M. [2]

1. *Department of Biological Sciences, School of Science, University of Eldoret, Eldoret, Kenya*
2. *Department of Medical Physiology, School of Medicine, Maseno University, Maseno, Kenya*

***Corresponding author:** Prof. Ng'wena Gideon Magak, Department of Medical Physiology, School of Medicine, Maseno University, Maseno, Kenya. Email: gngwena@hotmail.com

Summary

BACKGROUND

Diabetes mellitus (DM) is a metabolic disorder that causes a major health concern and whose prevalence has continuously increased globally over the past few decades. It has been considered as an incurable non-communicable metabolic disorder of multiple etiologies affecting about 2.8% of the population worldwide. Derangement of water and electrolyte balances was found to occur in subjects with diabetes mellitus, resulting from insulin deficiency, hyperglycemia, and hyperketonemia. Electrolytes imbalance contributes to complications observed in diabetes and pose a significant risk of contracting many diseases. In the present study, we compared the anti-diabetic activities of ethanolic leaf extracts of *A. aspera, B. pilosa and A. remota* and their influence on serum levels of glycemia, natremia, calcemia, kalemia, and chloremia in diabetic Small East African male goats.

MATERIALS AND METHODS

Eighteen young goats aged between 10 and 16 months were divided into six groups comprising of three animals each and given oral treatments as follows: Group I healthy control that received 4ml normal saline/day; group II diabetic control that received 4ml normal saline/day; group III received conventional glibenclamide drug at 0.125mg/kg bw/day; group IV, V and VI received 250mg/kg bw/day of leaf extracts of *A. aspera, B. pilosa* and *A. remota* respectively. Serum electrolytes Na^+, Cl^-, Ca^{2+} and K^+ levels were determined. Results obtained were tabulated, coded and processed using SPSS software.

RESULTS

Data was summarized and analyzed using descriptive and inferential statistics respectively. The probability values (p-value) were determined using t-test and ANOVA at 5% level ($P<0.05$) of significance. The results showed diabetic goats became hyperglycemic with significant increase in Na+ (139.89±16.25), K^+ (9.16±3.01), Cl^- (121.29±5.56) and a concomitant significant decrease in Ca^{2+} (1.024±0.62). *B. pilosa* was able to restore almost all these aberrations to normal levels whereas *A. aspera* and *A. remota* moderately restored some parameters to normal levels.

CONCLUSION

The results demonstrated antidiabetic activity of *B. pilosa* and moderately by *A. aspera* and *A. remota* crude leaf extracts in the management of diabetes mellitus in alloxan-induced diabetic goats and hence restoration of electrolytes balance.

Keywords: *Electrolytes, Hyperglycemia, Na^+, K^+, Cl^- Ca^{2+}, Diabetes Mellitus*

***[Afr. J.** Health Sci. 2021 34(4):537-549]*

Introduction

Diabetes mellitus (DM) is a metabolic disorder that causes a major health concern and whose prevalence has continuously increased worldwide over the past few decades. It has been considered as an incurable non-communicable metabolic disorder of multiple etiologies affecting about 2.8% of the global population [1]. Most cases of diabetes mellitus fall into one of two broad categories; "type 1 diabetes mellitus (T1DM)" and "type 2 diabetes mellitus (T2DM)". About 10% of patients with diabetes have type 1 and 90% have type 2 diabetes mellitus. T1DM is usually juvenile onset and results from the autoimmune destruction of the pancreatic β-cells. T2DM is usually a progressive disease that remains undiagnosed in a significant percentage of patients for several years. Traditionally, it is predominant among elderly people of over 40 years [2].

The prevalence of diabetes is increasing rapidly, and type 2 diabetes now accounts for 20 to 50% of cases diagnosed as new-onset diabetes in young people [1]. The number of persons affected by diabetes continues to rise globally. Estimates suggest that 438 million individuals will be affected in 2030 [3]. Essentially, emerging and developing countries will experience this large increase. Higher rate of mortality and morbidity are observed in low-income countries due to their weak health systems and limited infrastructures. More than 80% of the mortality due to diabetes is registered in these countries [3].

Diabetes syndrome is associated with multiple medical conditions such as hypertension, retinopathy, cardiomyopathy and neuropathy in various degrees [4]. It is known that diabetes is associated with serum electrolytes disturbances. These electrolyte disturbances are common in patients with diabetes mellitus and may be the result of an altered distribution of electrolytes related to hyperglycemia-induced osmotic fluid shifts brought about by osmotic diuresis [4]. Complications from end-organ injury and the therapies used in the management of diabetes may also contribute to electrolyte disturbances.

Electrolytes and Lipids have always played significant roles and changes in their concentrations gives good indications of disease progression in a number of non-communicable diseases. Hospitalized patients and also community subjects have displayed electrolyte disorders and are frequently affected [5]. The diabetic ketoacidosis and hyperosmolar hyperglycemic state, the two serious acute metabolic complications, are involved in the osmotic diuresis in diabetes. Although these two disorders often occur together, diabetic ketoacidosis is typically related to T1DM and hyperosmolar hyperglycemic state is typically associated with T2DM. Osmotic diuresis leads to loss of water, sodium, potassium, calcium and other electrolytes [6].

The electrolytes disturbances should be taken into account in the surveillance of DM. Currently in Kenya, the fasting blood glucose test is the main test done when considering diabetes management. The patient lipid profile is sometimes added to this test. The objective of this study was to establish and highlight the ways in which specific electrolytes may be influenced by dysregulation in glucose homeostasis and determine the ability of *A. aspera, B. pilosa* and *A.remota* in correcting these electrolyte aberrations.

Materials and Methods

Plant collection

The plants used in this study were collected from their natural habitats around Eldoret town in Uasin Gishu County on the basis of ethnobotanical information. A traditional medical practitioner provided information concerning the stage of growth when curative potency is maximal. This was given as the near flowering stage of the plants. Given that the shoot tips and/or leaves of *Achyranthes aspera, Bidens pilosa* and *Ajuga remota* are the part predominantly used by the local medicine practitioners, leaf extracts of these plants were specifically chosen to be studied herein. An acknowledged authority in taxonomy authenticated the botanical identity of the plants and voucher specimens were deposited at the University of Eldoret Herbarium. Fifteen kilograms of fresh plant leaves were collected for each plant. The voucher specimen numbers for the three collected and studied plants were recorded as *"MUH/Aas/35/97-*(*Achyranthes aspera); MUH/Bp/06/96- (Bidens pilosa) and MUH/Ar/120/96-(Ajuga remota).*

Processing and extraction

The collected plant materials (Aerial parts) were shade dried to retain as maximum plant contents as possible since heat-labile compounds are preserved. It took four weeks for all the three different plant materials to be completely dry. The rate at which the leaves dried up was based on the succulence of the plant materials where *Ajuga remota* took the longest time of 4 weeks.

The dried leaves were manually grinded and the powdered plant materials were kept at room temperature away from direct sunlight in closed labeled dry plastic bags. This protocol is per [7].

It has been reported that ethanol being polar, ethanolic extracts of most plants extracted higher concentrations/amount of phenolics compared to acetone, water, and methanol [8]. It is for this reason that in the present study, and for maximum extraction of bioactive phytoconstituents, ethanolic extraction was carried out. The dry coarse powder approximately 7kg of each of *A. aspera, B. pilosa* and *A. remota* leaves were macerated in ethanol (1:10 leaves powder to solvent ratio) for 72 hours in brown 2 two litre bottles for each plant with mechanical shaking twice a day. This protocol is per [9]. The extracts were then filtered through Whatman filter paper No.1 and solutions evaporated to dryness under reduced pressure by rotavac evaporator (Heidolp, Germany) at 66^0C, 100 r.p.m. to obtain a thick greenish mass of leaves extract and further concentrated in a water bath at 40^OC. The resultant green gummy extract residues were packed in air tight brown glass bottles, labeled and kept in the refrigerator at 4^oC until used for the experiment.

Preparation of plant extracts

A dose of 250mg/kgbw of the plant extracts were used. This dose was selected on the basis of previous reports on the same herbs by [10] A report showed that plant extracts of

A. aspera 250mg/kg bw and 500mg/kg bw did not show any difference in their efficacy, that is, not dose dependent [11]. A similar report was given for B. pilosa as being dose independent in its efficacy within a range of 150mg/kg bw to 300mg/kg bw [10]. The desired doses of the extract were administered according to the body weight of the goats in respective groups. The freeze-dried plant extracts were prepared for oral treatment to the goats as follows: The 250mg/kg body weight dose were prepared by dissolving 420 gm of the solvent free gummy residue of each of the three plants extracts in 420 ml of physiological saline in separate glass flasks to prepare stock solutions. A daily oral dosage of 10ml of each of the extract solutions of *A. aspera, B. pilosa* and *A. remota* were administered to each goat accordingly in the three groups of experimental animals respectively.

Preparation of Glibenclamide

Since the active principle in glibenclamide may not be evenly distributed in the 150mg tablet, a number of tablets were crashed using small pestle and mortar, mixed thoroughly and the powder weighed using an electronic analytical balance. Glibenclamide powder weighing 50mg were packed in aluminium foils to be reconstituted in 5ml of normal saline and administered to each goat in the appropriate group daily. Glibenclamide is sparingly soluble in water and therefore dissolving 50mg of the powder gives a suspension of the drug which was administered orally using 5 ml disposable syringes.

Experimental animals and ethical clearance

Eighteen reproductively mature small East African male goats from Kerio Valley in Rift Valley were used as model animals. The animals were aged between 10 months and 16 months and weighing between 15.0--20.0kg. The animals were purchased and transported to University of Eldoret three weeks before start of study in order for the animals to acclimatize to the new environment. They were housed in a pen at the Department of Animal Sciences premises. Each animal was given an injection of an antibiotic (procaine penicillin from Unisel Pharma (Kenya) Ltd), immediately on arrival as a prophylactic measure and multivitamin to ensure the study starts with healthy goats. They were also dewormed with valbazen (11.36% or 113.6mg/ml) (Ultravetis, East Africa limited), a broad spectrum antihelminthe at a recommended dosage rate of 20mg/kg (approximately 4mls per 20kg goat) and sprayed with an acaricide (Ectomin) (Ultravetis, East Africa limited) to control ectoparasites. This procedure was repeated monthly for similar reasons. The goats were grazed free range at the Animal Sciences farm with water and salt lick provided *ad libitum.*

Animal ethical standards in this experimental study were adhered to. Permission for handling and care of the goats was sought from animal rights agency at University of eastern Africa, Baraton no. REC: UEAB/11/8/2018.

Experimental design and treatment protocol

After four weeks acclimatization period, the animals were randomly identified by different numbers tagged on their ears using Pat Pending Patent tagging machine (Copenhagen, Denmark). This was followed by random division into six groups of three (n=3) animals each and designated as group (i) - (vi). Blood sample from each animal was also drawn weekly for determining fasting blood glucose levels and

serum electrolytes for each group of animals. The first day of blood withdrawal was taken as day 0. Assaying of the blood samples during this pre-treatment period was done for four weeks for the purpose of taking the baseline data. The same procedure of measurements was, therefore, repeated on day 7, 14, 21 and 28 during the pre-treatment period. Diabetic condition was then induced in the goats, except those in group (i) (which served as healthy control) by intravenous injection of alloxan monohydrate (65 mg/kgbw) in 4 mls of normal/physiological saline. Diabetes induction was done 96 hours before the start of the experiment. Fasting blood glucose level of each experimental animal was determined by taking the blood from the external jugular vein and animals with a fasting blood glucose level above 75 mg/dl [12] were recruited in the study. Animals described as fasted were deprived of food for at least 12 hours before the start of bioassay but had free access to water. After 96 hours (4 days), the six groups of animals were treated as follows:

Group I- Non-diabetic or healthy control- Animals received only the vehicle, i.e. 4 ml/day of normal saline orally for 28 days

Group II- Diabetic control- Animals received only the vehicle, i.e. 4 ml/day of normal saline orally for 28 days

Group III- Diabetic treatment- After 4 days of alloxan induction, the animals were treated with 0.125mg/kg bw/day of glibenclamide orally for 28 days.

Group IV- Diabetic treatment- After 4 days of alloxan induction, the animals were treated with *Achyranthes aspera L.* ethanolic leaf extract 250 mg/kg bw/day orally for 28 days.

Group V- Diabetic treatment- After 4 days of alloxan induction, the animals were treated with *Bidens pilosa* ethanolic leaf extract 250 mg/kg bw/day orally for 28 days.

Group VI- Diabetic treatment- After 4 days of alloxan induction, the animals were treated with *Ajuga remota* ethanolic leaf extract 250 mg/kg bw/day, orally for 28 days.

Collection of blood samples

Before administration of the different treatments, all the animals were bled by collecting blood samples via jugular venipuncture into plain vacutainer tubes (without the anticoagulant for chemistry tests) and EDTA treated vacutainers (for haematology tests). The venipuncture was performed by first identifying the jugular puncture site by making a smooth circular pass over the site with a 70% alcohol pad. Gauge 21 needles were attached to the hub by removing the plastic cap over the small end of the needle and inserting into the hub, twisting it tight. The plastic cap over the needle was then removed and gently inserted into the skin at the appropriate venipuncture site. Holding the hub securely, the first vacutainer plain tube was then inserted to draw the predetermined volume of blood until the blood stops flowing into it. After collecting blood into the second ETDA tube, the vacutainer tube was then removed by holding the hub securely and pulling the tube off the needle and inverting it gently 3-4 times. The gauze pad was then placed over the puncture site and the needle removed. The tubes were then well labeled to show the animal's identity, the group and date. This was the initial measurement at time zero. The animals were then given their respective treatments and again bled hourly until the third hour to determine the pharmacodynamics of the plant extracts. During this blood collection period, the animals continued to be fasted but were allowed free access to water. All treatments were done in the morning before the

animals were released to the grazing field. The parameters that were studied in this research were serum electrolytes Na^+, Cl^-, Ca^{2+} and K^+.

Measuring fasting blood glucose and separation of sera from hematocrit

Collection of blood samples from each animal was done once a week for four weeks before and after treatment periods. Fasting blood glucose (FBG) was measured using a glucometer (On Call Plus model) after the collection of blood samples from the jugular veins of the overnight (12 to 15h) fasted goats weekly for 28 days during the pre-treatment period and on day 0, and immediately after treatment on the 7^{th}, 14^{th}, 21^{st}, and 28^{th} days of the treatment period. Removal of the blood from the animals was done using plain (lacking anticoagulant) (red top) vacutainers to provide the sera and the anti-coagulant treated vacutainers (pink top) for whole blood.

The blood samples in the red plain tubes after collection were immediately put in the test tube stands and left undisturbed for about three hours to allow the sera and the blood cellular component to separate. This gave two layers: (from top to bottom) serum and cell layers. Carefully the supernatant (serum) was then aliquoted using sterile serological pipettes into cryovials and stored in a freezer at -20^0C. The cryovials were adequately labeled with the relevant information including experimental group of the animal, its number and date blood sample was drawn. The serum samples obtained were used to determine the electrolytes; Na^+, K^+, Ca^{2+} and Cl^- levels. These electrolytes were assayed at MTRH in Eldoret at the Human physiology laboratory using an automatic analyzer (Reflectron Automated Analyzer, Beckman, U.S.A).

Statistical analysis

Data collection method was quantitative and relied on random sampling where data was collected from groups assigned to different treatments with a view to testing the hypotheses. Results obtained were tabulated, coded and processed using SPSS software and on Excel platforms. Data was summarized and analyzed by using descriptive and inferential statistics respectively. The descriptive statistics used included means ± standard deviations, tables and bar plots to display trends. The probability values (p-value) were determined using t-test and ANOVA at 5% level ($P<0.05$) of significance. The Diabetic control group was compared with the Healthy control group using t-test which resulted in 1-tailed p-values with ‘‘aaa’’ as highly statistically significant. The other groups were compared with the Diabetic control group using ANOVA which also resulted in 1-tailed p-values with ‘‘bbb’’ as highly statistically significant.

Results

The single dose of alloxan monohydrate (65mg/kg bw) administration lead to significant increase in glucose concentration from (2.07±0.26 mmol/l) healthy control to (5.22±3.13 mmol/l) diabetic control. Administration of glibenclamide at a dose of 0.125mg/kg bw/day and *B. pilosa* at a dose of 250 mg/kg bw/day for 28 days ably corrected this anomaly significantly ($P<0.05$) to (4.00±1.49 mmol/l) and (4.02±2.13 mmol/l) respectively. The other two plants extracts *A. aspera* and *A.remota* were also able to lower the blood glucose concentrations to (4.81±2.68 mmol/l) and (5.00±3.18 mmol/l) respectively though statistically insignificant ($P> 0.05$). The findings of all the various treatments are displayed in table 1.

Table 1: Effect of A. aspera, B. pilosa and A. remota ethanolic Leaf Extracts and Glibenclamide on Fasting Blood Glucose Levels (mmol/L) in Alloxan Induced Diabetic Goats for 28 Days

Treatment group	Blood glucose levels (mmol/L)	
	Pre-treatment	Post treatment
Healthy control + saline	1.93±0.14	2.07±0.26
Diabetic control + saline	2.06±0.16	5.22±3.13[a]
Diabetic + Glibenclamide	2.00±0.22	4.00±1.49[ab]
Diabetic + *A.aspera*	2.18±0.31	4.81±2.68
Diabrtic + *B.pilosa*	1.92±0.17	4.02±2.13[ab]
Diabetic + *A.remota*	1.94±0.08	5.00±3.18

[a]P<0.05 with respect to normal control; [b]P< 0.05 with respect to diabetic control; The data was analyzed using one-way ANOVA. All results were expressed as mean ± SD. All blood glucose levels were recorded in mmol/L (n = 3)

Effects of A. aspera, B. pilosa and A. remota ethanolic leaf extracts on serum electrolyte levels (Mmol/L) in Alloxan induced diabetic goats

Hyperglycemia sets the internal environment for osmotic diuresis while causing a dilutional effect on electrolyte concentrations. The osmotic effect of glucose results in decreased circulating blood volume and fluid shift from the intracellular spaces causing cellular dehydration.

The results table showed that there was a significant difference between the blood electrolyte concentrations in the different treatment groups which indicated a major shift from the vehicle treated healthy control. Alloxan monohydrate (65mg/kg bw) administration resulted in significant elevation of serum Na^+, K^+ and Cl^- levels from (129.15±5.511), (5.14±0.44) and (109.69±3.43) mmol/l in healthy control to (139.89±16.25), (9.16±3.01) and (121.29±5.56) mmol/l in vehicle treated diabetic control respectively. In contrast, Ca^{2+} level of diabetic control was reduced significantly from (2.1± 1.28) to (1.024±0.62) mmol/l (P< 0.05) as shown in table 2.

Administration of *B. pilosa* at a dose of 250 mg/kg bw/day and the reference drug glibenclamide at a dose of 0.125mg/kgbw/day for 28 days was able to correct all these aberrations significantly (P< 0.05) except the glibenclamide which was unable to lower chloride ions concentration significantly.

A. aspera extract was able to lower K^+ significantly (P< 0.05) and Cl^- highly significantly (P<0.01). The *A. remota* extract was able to lower Cl- significantly (P<0.05) and it's correction of the other aberrations were statistically insignificant.

Table 2: Effect of A. Aspera, B. Pilosa and A. Remota Ethanolic Leaf Extracts on Serum Electrolyte Levels in Alloxan Induced Diabetic Goats (Mmol/L) for 28 Days

	Na^+	K^+	$Ca2^+$	Cl^-
Healthy control + saline	129.15±5.511	5.14±0.44	2.1±1.28	109.69±3.43
Diabetic + saline	139.89±16.25^{aa}	9.16±3.01^{aaa}	1.024±0.62^{a}	121.29±5.56^{aa}
Diabetic +gilbenclamide	130.37±6.3^{bb}	5.86±1.48^{b}	1.26±0.13^{b}	113.49±7.11
Diabetic + *A.aspera*	156.59±30.8	5.99±1.12^{b}	1.11±0.35	104.18±5.80^{bb}
Diabetic + *B.pilosa*	131.35±2.26^{bb}	5.82±1.12^{bb}	1.70±0.32^{b}	109.81±6.42^{b}
Diabetic + *A.remota*	134.30±4.89	6.95±1.44	1.48±0.38	108.78±5.21^{b}

Signif. codes: 0.001 'aaa' 0.01 'aa' 0.05 'a' 1; Signif. codes: 0.001 'bbb' 0.01 'bb' 0.05 'b' Statistical comparison: a: Group 1 and Group 2 b: Group 3,4,5,6 and Group 2 *p<0.05. Values are expressed as Mean± SD (n=3)

These results indicated that *B. pilosa* extract exhibited a more potent property in correcting the electrolytes disturbance as compared with *A. aspera* and *A.remota* leaf extracts. The results of all the treatments and placebo are presented in table 2.

In vivo effects of the plant extracts on serum electrolyte levels at varying times were given in Figure 1. In the 1st and 2nd hour, all the plant extracts maintained the elevated levels of $Na^{+.}$ However, there was a steep reduction of Na^+ concentration in the 3rd hour to normal level by the extracts except *A. aspera.* Glibenclamide was able to reduce Na^+ levels from the 2nd hour to 3rd hour to normal levels.

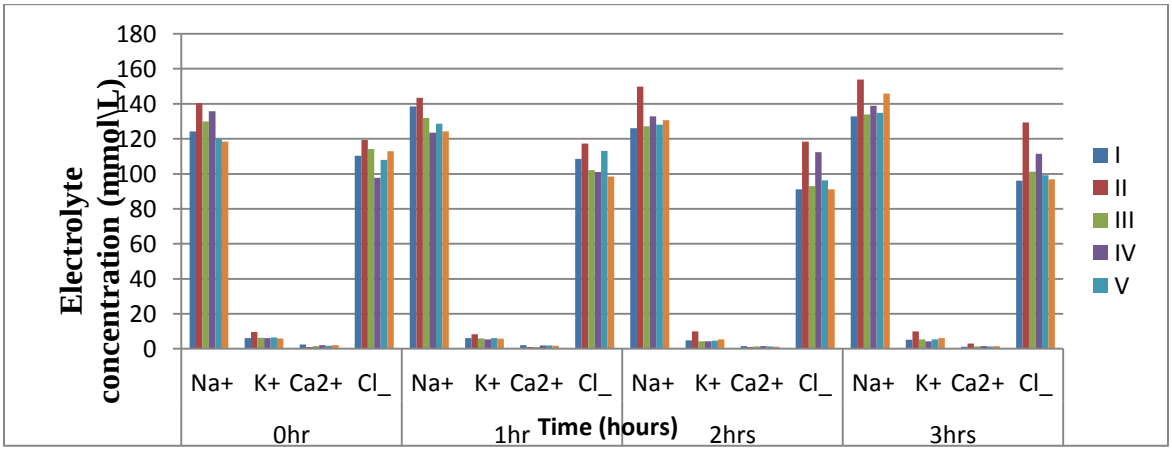

Figure 1: In vivo effects of A. aspera, B. pilosa and A. remota ethanolic leaf extracts on serum electrolyte levels in alloxan induced diabetic goats (mmol/L) at varying times

Elevation of the decreased levels of Ca^{2+} (1.024±0.62mmol/L) as demonstrated by diabetic control goats, from its observed normal level (2.1±1.28 mmol/L) occurred in two phases; there was gradual elevation in the 1st hour by all the extracts and glibenclamide and then a sharp rise in the 2nd and 3rd hour to normal levels by *B. pilosa, A. remota* and glibenclamide except *A.aspera*. Similarly, all the plant extracts reduced the elevated levels of Cl- (121.29±5.56 mmol/L) gradually in the 1st hour and then a steep reduction in the 2nd and 3rd hours to normal levels.

Discussion

The analysis of *Achyranthes aspera*, *Bidens pilosa* and *Ajuga remota* ethanolic leaf extracts in this study demonstrated the availability of glycosides, phenols, flavonoids, saponins, tannins, terpenes, and alkaloids though variable for the three plants. These biomolecules brought about the reversal of the diabetic metabolic syndromes observed in the diabetic goats results. The findings obtained in this experimental study hence assumed the phytochemicals identified are bioactive phytoconstituents and that these plants are suggested to be a highly valuable store of bioactive principles of remarkable therapeutic significance. Phenolic substances are among the leading and very extensive groups of plant compounds. They have biological characteristics like anti-inflammation, anti-atherosclerosis, anti-hyperglycaemia, anti-angiogenetic as well as possession of anti-oxidant properties [13]. Tannins stick to proline loaded protein and interrupt the manufacture of proteins and decrease serum lipid levels Flavonoids are phenolic compounds demonstrated to be anti-hyperglycemic and anti-oxidant compounds [14]. Saponins are reported to generate preventive property on cholesterol and inflammation binding activities whereas alkaloids possess analgesic, anti-hyperglycaemic and anti-bacterial properties [14]. Glycosides have been shown to be anti-hyperglycaemic, anti-oxidant and lowering of blood pressure [15]. Findings indicated that terpenes exhibit anti-inflammatory, anti-oxidant and retardation of cholesterol synthesis [15].

Electrolytes (meaning carrying a minute electrical charge-potential), are found inside the human body. The role played by the electrolytes in several body processes cannot be overemphasized. These processes include control of fluid levels, conduction by nerves impulses, acid-base balance (pH), contraction of muscles, and blood clotting process. Sodium, calcium and potassium are all significant for suitable electrolyte balance. Imbalance of electrolytes leads to kidney failure, fever, dehydration, and vomiting. This has been considered as among the factors contributing to complications seen in DM and other endocrine problems [16]. High glucose levels promote osmotic diuresis in the internal environment while at the same time causing electrolyte levels dilutional effect. Osmotic effect of the glucose causes a decrease in flowing blood volume and a shift of fluid from the intracellular compartment leading to cellular dehydration [16].

The electrolytes sodium, potassium, calcium, and chloride were selected because these are the most common macro electrolytes and correlated with Diabetes mellitus owing to the latter disrupting their levels. The serum electrolyte levels of Na+, K+, and Cl^- for diabetic subjects showed a highly significant ($P < 0.001$) increase. In contrast, serum Ca^{2+} levels exhibited a statistically significant ($P < 0.05$) decrease. These results were consistent with those reported by previous studies conducted by

[17] on diabetic human patients. Derangement of water and electrolyte balances occurs in subjects with diabetes mellitus, resulting from insulin deficiency, hyperglycemia, and hyperketonemia. Under physiological conditions, most of the Na^+ is reabsorbed in the proximal tubule of the kidney [17].

Hyperglycemia is restricted to the extracellular space so water moves from the intracellular to the extracellular compartment initially, diluting plasma sodium. During the accompanying osmotic diuresis, water is generally lost in excess of sodium until eventually the loss of water is in equilibrium between extracellular and intracellular compartments. As a result there is resultant reduced volume of blood with a proportionate elevated sodium concentration. Therefore, in diabetes mellitus, plasma sodium concentrations may be abnormally high (hypernatremia) as water loss exceeds sodium loss. Such a physiological state leads to thirst, brain dysfunction (due to rapid decrease in intracellular water content and brain volume caused by an osmotic shift of free water out of the cells) and in severe cases can lead to confusion, muscle twitching, seizures, coma and death (18). In this study, hyperglycemia-induced osmotic diuresis which increased water excretion is thought to be the primary mechanism underlying the increased serum concentrations of Na^+ observed in response to elevated glucose levels.

Diabetes associated elevated levels of potassium (hyperkalemia) could be as result of multiple causes such as reduced glomerular filtration or diminished kidney capacity to excrete potassium to urine, tissue destruction where dying cells release potassium into the blood circulation, redistribution of potassium from intracellular to extracellular compartment and alterations in the $Na^{+/}K^+$ ATPase that maintained the transmembrane gradients of sodium and potassium [19]. Implications of abnormally high potassium can lead to irregular heart beat which may cause heart attack. Consistent with our finding, several studies reported elevated values of serum potassium among human diabetic populations. [17] Demonstrated in India an elevated potassium mean value of 5.73 ± 0.07 in ketoacidosis diabetics. [15] Reported in a study conducted in Pakistan a higher level of potassium (7.41±1.8) in diabetic human patients with stable glycemic control [19]. The present results are however not consistent with that reported by some previous study conducted by [20], where there was significantly reduced levels of sodium and potassium in diabetic human subjects compared with normal ones.

Chloride is an important electrolyte responsible for maintaining the acid-base (pH) balance in the body, fluid regulation and transmission of nerve impulses. Its concentration is carefully regulated by the kidneys. An excess of chloride levels in the body can be dangerous as it is linked to higher than normal acid in the blood and may lead to kidney stones, kidney failure or even coma [21]. The basis for kidney stone formation is supersaturation of the urine with crystal-forming substances such as calcium; oxalate, uric acid and too much salt than the fluid in the urine can dilute [20]. The observed high levels of chloride (hyperchloremia) could be due to dehydration (as a result of diuresis) and acidosis (where the pH of the body was abnormally low as a result of elevated triglycerides) [21]. High levels of serum chloride could also be attributed to the observed increased levels of serum sodium since chloride is the major anion associated with sodium in the extracellular fluid (ECF) which

comprises the blood plasma (or serum) compartment and the interstitial fluid compartment [21, 22]. The elevated levels of chloride could also be a pointer to a chronic or an acute kidney disease.

Reduced calcium levels (hypocalcaemia) in blood serum of alloxan induced diabetic goats may suggest under-active parathyroid gland (hypoparathyroidism), renal disorder or hypoproteinemia [22]. Hypoproteinemia was an observation in this study and renal disorder could have occurred with persistent hyperglycemia. In the distal convoluted tubule, Ca^{2+} absorption is regulated independently of Na^{+}, where numerous factors, such as calcitonin, parathyroid hormone, and vitamin D, can have marked effects on Ca^{2+} reabsorption and secretion [22]. Hypocalcemia could also be due to renal failure in diabetic animals which is linked to hypomagnesemia [22]. The reduced levels of calcium (hypocalcemia) among the diabetic groups in our study are consistent with the results of [23] who reported 43% of diabetic patients suffering from hypocalcemia in Diwaniya-City in Iraq. In contrast to our finding, a study conducted in Nigeria showed no difference between the mean level of serum calcium in diabetic patients and control subjects [24]. However other authors reported a significant decrease in serum calcium level in diabetic subjects [25]. Based on the findings of this study, electrolyte disturbances should be taken into account in the surveillance of diabetes.

Conclusion

In summary, whereas *B. pilosa*, *A. remota* and *A. aspera* all exhibit antidiabetic properties, *B. pilosa* demonstrates a highly potent ability to ameliorate the hyperglycemic condition of diabetic subjects. *B. pilosa* leaf extract reverses the diabetic-associated electrolyte level disturbances to normal concentrations comparable to the conventional drug, glibenclamide, due to its ability to restore blood glucose level. However, the leaf extracts of *A. aspera* and *A. remota* only moderately restores these diabetes-associated metabolic syndromes. Therefore, *B. pilosa, l*eaf extract possess significant antidiabetic activity and moderately by *A. aspera* and *A.remota* crude leaf extracts in the management of diabetes mellitus. Changes in electrolyte levels found in diabetics may have a great potential as a diagnostic tool in clinical practice. Electrolyte imbalance also has a significant effect upon the risk of contracting many other diseases.

References

1. **Domingueti CP, Sant'Ana Dusse LM, das Graças Carvalho M, Pires de Sousa L, Gomes KB, *et al***. (2016) Diabetes mellitus: The linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *J Diabetes Complications* 30: 738-745.
2. **Zimmet P, Alberti K, Shaw J** (2001). Global and societal implications of the diabetes epidemic. *Nature* 414: 782-787.
3. **Shaw JE, Sicree RA, Zimmet PZ** (2010). Global estimates of the prevalence of diabetes mellitus for 2010 and 2030; Diabetes Atlas, Volume 87, issue 1,p4-14.
4. **Deshpande AD, Harris-Hayes M, Schootman M.** (2008). Epidemiology of diabetes and diabetes-related complications. *Phys Ther*; 88(11):1254-64.
5. **Palmer BF, Clegg DJ** (2015). Electrolyte and Acid-Base Disturbances in Patients with Diabetes Mellitus. *N Engl J Med.* ;373(6):548-59.

6. **Kitabchi AE, Umpierrez GE, Murphy MB, Kreisberg RA** (2006). Hyperglycemic crises in adult patients with diabetes: a consensus statement from the American Diabetes Association. *Diabetes Care;* 29(12):2739-48.
7. **Ngugi M Piero, Murugi N Joan, Kibiti M Cromwell, Ngeranwa J Joseph, Njue M Wilson, Maina Daniel, *et al*.**, (2011). Hypoglycemic Activity of Some Kenyan Plants Traditionally used to Manage Diabetes Mellitus in Eastern Province
8. **Koffi, E.; Sea, T.; Dodehe, Y.; Soro, S.** (2010). Effect of solvent type on extraction of polyphenols from twenty three ivorian plants. *J. Anim. Plant Sci*; 5, 550–558.
9. **Kondeti VK, Badri KR, Maddirala DR** (2010). Effect of Pterocarpus santalinus bark, on blood glucose,serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin- induced diabetic rats. *Food and Chemical Toxicology* 48 (5),1281-1287.
10. **Debella Asfaw, Aberra Geyid, Dawit Abebe, Zewdneh Makonnen** (2005). Screening of some medicinal plants of Ethiopia for their antibacterial properties and chemical profiles. *Journal of ethnopharmacology* 97 (3), 421-427.
11. **Vidhya R, Rajiv G, Jothi G, Radhika J and Brindha** P (2012). Evaluation of Achyranthes aspera on alloxan induced diabetic rats); *International journal of Pharmacy and Pharmaceutical Sciences*,vol4 suppl 5.
12. **Gidado A, Ameh DA, Atawodi SE** (2005). Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. *Afr J Biotechno* 4(1):91–93
13. **Han, X., Shen, T., Lou, H.** (2007). Dietry polyphenols and their biological significance. *Int. J. Mol. Sci*., : 950-988.
14. **Okwu, D.E.** (2004). Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. *J.Sustain. Agric. Environ.*, 6(1): 30-37
15. **Kappers IF, Aharoni A, van Herpen TW, Luckerhoff LL, Dicke M, et al**. (2005) Genetic engineering of terpenoid metabolism attracts bodyguards to Arabidopsis. *Science* 309: 2070-2072.
16. **Husain, F.; Arif Maan, M.; Sheikh, M.A.; Nawaz, H.; Jamil A** (2009). Trace elements status in type 2 diabetes. *Bangladesh J. Med.* Sci. 8, 52–56. [CrossRef]
17. **Holkar S, Vaishnav D, Hivre M.** (2014). Study of serum electrolytes levels in patients with diabetic ketoacidosis. *Int J Health Sci Res*; 4(9):154-7.
18. **Carl Nierenberg** (2019). Hypernatremia and Hyponatremia: Causes and Risks of Sodium Imbalance. http//www.livescience.com/65222-1.
19. **Shahid SM, Rafique R, Mahboob T**. (2005). Electrolytes and sodium transport mechanism in diabetes mellitus. *Pak J Pharm Sc*; 18(2):6-10
20. Eugenie A. A. Anago, Thierry C. M. Medehouenou, Casimir D. Akpovi, Henri Tchehouenou (2016). Electrolyte disturbances in diabetic patients in Cotonou, Benin; *Int J Res Med Sci. Dec;4(12):5430-5435*
21. **Leslie K. Williams, Chunmin Li, Stephen G. Withers, and Gary D. Brayer** (2012). *Journal of Medicinal Chemistry***;** *55* (22), 10177-1018
22. **Mader and Sylvia S.** (2004). Human Biology. *New York: McGraw- Hill* (3) 114-123.
23. **Al-Yassin AMH, Nimnima A, Challab ED.** (2009).Calcium and diabetes mellitus type two: a prospective study done on people with type 2 diabetes in Diwaniya

teaching hospital. *Kufa Med Journal*; 12(1):468- 75.

24. **Ugwuja E, Eze N. A** (2006). Comparative Study of Serum Electrolytes, Total Protein, Calcium and Phosphate Among Diabetic and HIV/AIDS Patients in Abakaliki, Southeastern, Nigeria. *Internet J Lab Med*; 2(1)
25. **Wang S, Hou X, Liu Y, Lu H, Wei L, Bao Y** (2013).. Serum electrolyte levels in relation to macrovascular complications in Chinese patients with diabetes mellitus. *Cardiovasc Diabetol*; 12:146.