Effects of *Achyranthes aspera, Bidens pilosa* and *Ajuga remota* Leaf Extracts on the Blood Glucose Levels and Hemoparameters in Male Goats Treated with Alloxan

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Summary

INTRODUCTION

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide thus the disease constitutes a major health concern. Presently, it is an incurable metabolic disorder which affects about 2.8% of the global population. Hyperglycemia brings about many complications in the body including production of changes in blood constituents. This study demonstrated the effect of hyperglycemia on different blood constituents with special emphasis on white blood cell and platelet count and hemoglobin and packed cell volume content as well as its influence on immunological changes on alloxan induced diabetes in the Small East African male goats. The study determined the phytochemicals present in ethanolic extracts of *Achyranthes aspera, Bidens pilosa* and *Ajuga remota* and their possible roles in reversing the hemoparameter changes in alloxan-induced diabetic Small East African goats.

MATERIALS AND METHODS

Eighteen mature 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 controls that received 4ml normal saline/day; group II diabetic controls that received 4ml normal saline/day; group III received conventional glibenclamide drug at 0.125mg/kg bw, group IV, V and VI received 250mg/kg bw/day of ethanolic leaf extracts of *A. aspera, B. pilosa* and *A. remota* respectively. Phytochemical constituents of the plant extracts were analyzed. Haematological parameters PCV, Hb, PLT and total WBC levels were determined. The data obtained were non parametric in nature and were transformed to normal distribution (parametric) using the log data in SPSS. Data was analyzed using inferential statistics and results expressed as Mean ± S.D. The probability values (p-values) were determined using t-test and ANOVA at 5% level of significance.

RESULTS

Results revealed the presence of the phytochemicals; phenols, flavonoids, alkaloids, glycosides, tannins, terpenes and saponins in the plant extracts. The alloxan treated goats became hyperglycemic (5.22±3.13) hence diabetic with significant increase in tWBCs

(13.80±3.63) and PLT (482.58±87.57) counts. There was significant decrease in PCV (37.47±3.53), and Hb (6.91±1.61) contents.

CONCLUSION

The present study showed that diabetes mellitus was associated with disruption of hemoparameters levels which may have been as a result of degenerative changes in the hepatic and renal systems. *B. pilosa* was able to restore almost all these aberrations to normal levels as observed in the healthy controls whereas *A. aspera* and *A. remota* moderately restored some parameters to normal levels. In summary, *B. pilosa* has strong antidiabetic activity and hence hemoparameter changes reversal in alloxan-induced diabetes in goats whereas *A. aspera* and *A. remota* ethanolic leaf extracts are moderate in the management of diabetes mellitus.

Keywords: *Goats***,** *A. aspera, B. pilosa, A. remota, Haematology, Antidiabetic Activity, Diabetes Mellitus*

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Introduction

Diabetes mellitus (DM) is a persistent metabolic disorder in carbohydrates, fats and proteins, and is attributed to complete or associated lack of insulin release with/without differing strength of insulin resistance [1]. It is a persistent endocrinological disorder that is indicated by an abnormally high blood glucose levels resulting from the pancreas secreting insufficient or improper utilization of insulin by the targeted cells that happen to resist insulin action in progression [2]. With insufficient insulin, the body tissues, specifically the adipose, muscle and hepatic tissues cease to take up and expend glucose from the circulatory system. This leads to increased blood glucose concentration, a state known as hyperglycaemia. If blood glucose concentration stay high for prolonged time this may lead to long-term destruction to organs like the heart, eyes, kidneys, blood vessels and nerves. Complexities in some of these organs could result in death [3]. It has currently turned out to be an epidemic with a global incidence of 5 percent in the whole population. Its global prevalence was predicted at 366 million adults i.e. 8.3% in 2030 [4] and is presently the fifth leading cause of death globally [5]. Epidemiologic patterns show that several factors play a role in distribution of diabetes mellitus; demographic, geographic, biologic, cultural, and other factors (the role of infections, early childhood diet, vitamin D exposure, environmental pollutants, increased height velocity, obesity, and insulin resistance [6].

Patients with diabetes mellitus show a significant derangement in various haematological parameters [7]. A high prevalence of anaemia was identified in type2 Diabetic patients where about 27% of diabetic patients were anaemic [8]. The mean values of total red blood cells (TRBC), haemoglobin (Hb), packed cell volume (PCV) and mean cell haemoglobin concentration (MCHC) for the diabetic patients were found to be lower than the values of control group [9]. Red blood cells (RBCs) are approximately 8µm in diameter and passes through 3µm capillaries without fragmenting and losing their haemoglobin. Research conducted by [9a], examined the effect of type 2 dibetes mellitus on red blood cells. They reported that there are changes in RBCs that include: anemia (or hypochromia) which is

observed as a paler than normal red blood cell colour attributed to a decrease in hemoglobin which lowers affinity for oxygen, anisocytosis or unequal RBCs sizes where some are elongated with some having increased diameter with many diabetic patients having between 10 and 15% rise in RBC diameter, raising viscosity. This occurs as a result of influx of glucose which flattens the biconcave disk and distends the cells [9a].

Packed cell volume (PVC) and haemoglobin are indices of red blood cells [10], conducted a research study on effects of diabetes on red blood cell parameters including PCV and hemoglobin (Hb). They reported mean values of total red blood cells (TRBC), haemoglobin (Hb), mean cell haemoglobin concentration (MCHC) and packed cell volume (PCV) for diabetic patients were found to be lower than the control group values demonstrating the presence of anaemia in the former group [10].

Studies have demonstrated that cellular innate immunity exhibit reduced functions (phagocytosis, chemotaxis, and killing) of diabetic monocytes/macrophages and polymorphonuclear (PMNs) cells as compared with cells of controls. For example in subjects suffering from type 2 diabetes mellitus (T2DM) or obesity, there were changes in reproduction of T cells and macrophages, and deterioration in the role of natural killer (NK) cells and B cells, which represented irregular adaptive and innate immunity [11]. A better control of the high glucose levels in DM patients results in enhancement of these cellular activities. In addition, some microbes become most infectious in a hyperglycaemic environment. Another process leading to the increase in prevalence of infections in patients that are diabetic is the heightened adherence of microbes to diabetic in comparison the nondiabetic cells [11]. A number of these infections are moreover, more probably to possess a complex route in diabetic compared with nondiabetic subjects. Diabetic ketoacidosis, for instance, is hastened or made complex by an illness in 75% of the incidences [12].

The challenge then arises as to the type of pathogenetic process that is concerned with this increased infection rate in DM patients.

Nevertheless, data concerning the bactericidal action of diabetic PMNs have produced conflicting findings [13]. In one research study an ineffective killing role of diabetic PMNs was discovered in all experiments by using Staphylococcus aureus microbe, though not within the experiments involving the killing of C. albicans [14], was utilized as the gauge. Therefore, according to these investgations we cannot deduce any conclusions concerning the nondiabetic serum effect on the killing of diabetic cells. No relationship was discovered with glucose level (Rajana, 2017), despite some experiments have indicated that bactericidal action was enhanced, in spite of failing to normalize following attainment of normoglycaemia [14].

People with diabetes, especially those with T2DM, show elevated platelet reactivity. Conflicting results have been reported for platelet count in diabetes [15]. Hyperglycaemia imparts higher platelet activity via direct effects and by enhancing glycation of platelet proteins [16]. Platelets play a significant function in the integrity of usual homeostasis and atherosclerosis process [16]. DM has been regarded as a ‘prothrombotic state’ having heightened platelet reactivity. [17], found the morpgological changes of platelet activity occurred in diabetic patients but found platelet count was not different between non-diabetic and diabetic population. However, they found

the mean platelet volume (MPV) being significantly higher in diabetic patients [17]. Among other reports on platelet indices, MPV findings were controversial [17].

Among the medicinal plants known to be used by some traditional communities in Kenya to manage diabetes mellitus are *Achyranthes aspera, Bidens pilosa* and *Ajuga remota*. [18, 19], examined the ethanolic and aqueous extracts of the grounded *A. aspera* whole plant that showed glucose lowering activity. Similarly, it has been reported that *B. pilosa* and *A. remota* can be used to manage diabetes mellitus in animals. However, the available information is not precise and conclusive hence it has been sparingly elucidated scientifically. The present study focused on *Achyranthes aspera, Bidens pilosa* and *Ajuga remota* which are locally common species. The main advantages of plant medicines are their potency, minimal cases of side effects, and affordable cost [20]. However, contradicting findings have been reported concerning antidiabetic effects produced by ethanolic leaf extracts of *Achyranthes aspera, Bidens pilosa* and *Ajuga remota*.

The present study investigated ethanolic leaf extracts of *A. aspera, B. pilosa*, and *A. remota* for presence of bioactive phytochemicals, blood glucose lowering potential and their ability to ameliorate the complications associated with diabetes mellitus with emphasis on disrupted levels of the blood haemoparameters WBCs and PLT counts and Hb and PCV contents.

Materials and Methods

Experimental animals' acquisition and maintenance

Eighteen reproductively mature Small East African (SEA) male goats from Kerio Valley in Rift Valley were recruited as model animals. The age of the animals lied between 10 and 13 months and weighing between 15.0--18.0 kg. The animals were bought and translocated to the University of Eldoret three weeks before start of research in order for the animals to acclimatize to the new environment. They were housed in a fly-proof pen at the Department of Animal Science premises. Each animal was given an antibiotic injection (procaine penicillin from Unisel Pharma (Kenya) Ltd), immediately on arrival as a prophylactic measure and multivitamin to boost protection against disease. They were also dewormed with valbazen (Ultravetis, East Africa limited), a broad spectrum antihelminthe at a rate of 4ml per animal 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 farm of Animal Science Department with salt lick and water provided *ad libitum.* Animal ethical standards in this experimental study were adhered to. The ethical approval for use of the goats in this study was obtained from the animal rights agency at Baraton University in Nandi County, Kenya.

Collection of the plants

The presently studied plants were picked from the confines of their natural habitats around Eldoret town in Uasin Gishu County on the basis of ethnobotanical information. Given that the leaves of Achyranthes aspera, Bidens pilosa and Ajuga remota are the predominantly used parts, leaves of these plants were particularly selected to be investigated herein. A taxonomist in the Department of Biological Sciences, University of Eldoret was consulted to authenticate the plant's botanical identities. Voucher specimen numbers were deposited at the University of Eldoret Herbarium as "MUH/Aas/35/97-

(Achyranthes aspera); MUH/Bp/06/96- (Bidens pilosa) and MUH/Ar/120/96-(Ajuga remota). Sufficient quantities (about fifteen kilograms) of fresh plant leaves were collected for each plant species.

Processing of the Plants For Extraction

The parts of the plants isolated in the present study were the shoot tips and/or leaves. The isolated plant materials (Aerial parts) were shade dried to retain maximum plant contents as much as possible since heat-labile compounds are preserved. It took four weeks for all the three different plant materials to be completely dry. This was ascertained by a constant weight after repeated weighing of the dry leaves. 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 grounded reducing sample particle sizes hence increasing surface contact between samples and the extraction solvent. The coarsely powdered plant materials were stored at room temperature in labeled air-tight and dry plastic bags and safeguarded from direct sunlight. This protocol is per [21].

Extraction

The efficacies of medicinal plants are highly dependent on the method of extraction or extract preparation. In the present study, and for maximum extraction of bioactive phytoconstituents, ethanolic extraction was carried out, the solvent being polar to extract most of the plant phytochemicals. The dry coarse powder approximately 7kg of each of *A. aspera, B. pilosa* and *A. remota* leaves were softened by soaking in analytical grade ethanol at (10:1 solvent to leaves powder v/v ratio) for duration of 72 hours in brown 4 litre bottles for each plant material with manual agitation two times a day. The maceration process was intended to weaken and break the plant's cell wall in order to free the soluble phytoconstituents. The brown glass bottles with the mixtures were wrapped in alluminium foils to minimize direct sunlight rays that may accelerate disintegration of the extracted chemical compounds. This protocol is per [22]. The extracts filtrate were then filtered by means of Whatman No. 1 (24cm) filter paper and filtrates concentrated *in vacuo* under reduced pressure to dryness by rotavac evaporator (Model Heidolp, Germany) at 66^{0}C, 100 r.p.m. to give a thick greenish paste extract of leaves and further concentrated by water bath at 40°C for about 2 hours.

The filtrates yielded 520.6g (7.4%), 487g (6.96%) and 432g (6.17%) of *A. aspera, B. pilosa* and *A. remota* leaves respectively where the disparities arose due to the varied quantities of the crude extracts of the plants after concentration. The resultant green gummy extract residues were stored in airtight tightly corked brown coloured glass bottles, labeled and stored in the fridge at 4°C until ready for use.

Experimental design and treatment protocol

The type of experimental design employed in this study was a laboratory based experiment. After four weeks acclimatization, the animals were randomly assigned different numbers tagged on their ears using Pat Pending Patent tagging machine (Copenhagen, Denmark). This was followed by random division of the animals into six groups of three (n=3) animals each and designated as group (I) - (VI).

Diabetic condition was then induced in the goats in groups II to VI. Goats in group I

served as healthy control. Induction of diabetes was done by a single intravenous (iv) administration of alloxan monohydrate (65 mg/kgbw) in 4 ml of normal/physiological saline. The healthy control animals were, however, injected with 4 ml of normal saline (vehicle). Diabetes induction was done 72 hours ahead of starting the experiment. Fasting blood glucose levels of each experimental goat was tested by drawing 4 mls of blood from the external jugular vein and goats with a fasting blood glucose (FBG) level more than 75 mg/dL or 4.2 mol/L (Gidado *et al*., 2005) were included in the study. After 86 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- Alloxan-induced diabetic control goats- Animals received only the vehicle, i.e. 4 ml/day of normal saline orally for 28 days

Group III- Alloxan-induced diabetic goats- After 4 days of alloxan induction, the animals were treated with 0.125mg/kg body weight/day of glibenclamide orally for 28 days.

Group IV- Alloxan-induced diabetic goats- After 4 days of alloxan induction, the animals were treated with *Achyranthes aspera L.* ethanolic leaf extract 250 mg/kg body weight/day orally for 28 days.

Group V- Alloxan-induced diabetic goats- After 4 days of alloxan induction, the animals were treated with *Bidens pilosa* ethanolic leaf extract 250 mg/kg body weight/day orally for 28 days.

Group VI- Alloxan-induced diabetic goats- After 4 days of alloxan induction, the animals were treated with *Ajuga remota* ethanolic leaf extract 250 mg/kg body weight/day, orally for 28 days.

Before administration of the different treatments, blood was obtained from each animal and the blood glucose levels determined. This was taken as the initial value at zero time. After the treatment, collection of blood was done sequentially after 1 hour, 2 hours and then 3 hours. During bleeding sessions, the animals continued to be fasted but were provided with water *ad libitum*. All treatments were done in the morning before the animals were released to the grazing field.

Qualitative phytochemical analyses of crude extracts

About 15ml of each of the stored three plant extracts were removed from the fridge for biochemical analysis. The extracts were investigated for the availability of some bioactive principles by employing the following standard procedures:

Test for flavonoids. For flavonoid identification in the collected plants, 1 ml of each plant extract was put in a test tube and added 1 millilitre of dilute sodium hydroxide, shaken gently, followed by addition of about 4 drops of dilute HCL.

Test for Phenols. To 1ml of each plant extract in a test tube was added 3 drops of ferric chloride solution.

Test for Alkaloids. To 1ml of each of plant extract in a test tube was added 1 ml of ammonia solution then 1ml of chloroform followed by 4 drops of Meryer's reagent.

Test for Terpenes. To 5 mls of each plant extract in a test tube was added 2ml of chloroform then 3 ml of concentrated sulfuric acid.

Test for Glycosides. To 2ml of each plant extract in a test tube was added 1 ml of acetic acid then a drop of 5% ferric chloride followered by 1 ml of conc.sulfuric acid.

Test for Saponins. To 2 ml of each of plant extract and 2 ml of distilled water in a test tube were vigorously shaken.

Test for Tannins. To 1ml of each of the plant extracts was added 4 drops of 2% ferric chloride solution.

Blood sampling and processing

Blood specimens were drawn by jugular venipuncture of the overnight (12 to 15h) fasted goats weekly for 28 days throughout the pre-treatment period. Drawing of the blood from the animals was done using 21 gauge needles attached to a test tube holder or hub in which is inserted a vacutainer tube to draw a predetermined volume of 4 ml of blood. Blood was drawn into anti-coagulant treated vacutainers for whole blood. The vacutainers were well labeled with the required information including experimental group of the animal, its number and date blood sample was drawn. Blood sample from each animal was drawn weekly for measuring fasting blood glucose levels and other biochemical variables for each group of animals. All measurements were taken in the morning (between 7:00 and 10:00 am) before the animals were released to the grazing field. 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 repeated on day 0, 7, 14, 21 and 28 during the treatment period. Beginning from the first day (4rd day of alloxan administration) referred here as day zero of extract treatment to diabetic goats, fasting blood glucose concentrations were determined on each seventh day using a glucose meter (On call-Plus Model, Angelhom, Sweden) [24]. The blood glucose level reading in millimoles per litre for each animal were taken and recorded. The remaining whole blood was immediately taken to the MTRH in Eldoret town for haematological parameters analysis. These included PVC, Hb, PLT and tWBCs. These parameters were assayed at MTRH in the Human physiology laboratory using an automatic analyzer (Reflectron Automated Analyzer, Beckman, U.S.A).

Data 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. The data obtained followed non-normal distribution or non-parametric in nature after failing to satisfy all the normality tests. Transformation of the data was carried out from non-normal distribution into normal distribution or parametric in nature using the log data in SPSS. Data was summarized and analyzed using descriptive and inferential statistics respectively. Results obtained were tabulated, coded and processed using SPSS software version 20.0 and on Excel platforms. The descriptive statistics utilized the use of tables, means ± standard deviations and bar charts 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 ($P<0.01$). The other groups were each compared with the Diabetic control group using ANOVA which also resulted in 1-tailed p-values with "bbb" as highly statistically significant ($P<0.01$).

Results

The results of the phytochemical analysis shows that *A. aspera, B. pilosa* and *A.remota* contain one or more of the tested metabolites namely: terpenes, alkaloids,

saponins, flavonoids, phenols, glycosides and tannins. The following were the plant extracts phytochemical analyses results: On addition of dilute sodium hydroxide to the extracts, the colour turned intense yellow and on addition of 4 drops of dilute hydrochloric acid turned colourless for *B. pilosa* extract. This is an indication of presence of flavonoids. There was no colour change in the other two extracts. On addition of 3 drops of ferric chloride solution to the extracts, there was a dark green colour with varying intensity observed in *B. pilosa* and *A. remota* extracts indicating presence of phenols. There was no colour change in *A. aspera* extract. Addition of 1 ml of ammonia solution and 1ml of chloroform and then addition of 4 drops of Meryer's reagent to the extracts resulted in cream coloured precipitates in *A.aspera* and *B. pilosa* extracts which is an indication of presence of alkaloid. There was no colour change in *A.remota* extract. When 2 ml of chloroform and then 3 ml of concentrated sulfuric acid were added to the extracts, formation of reddish brown ring at interface in *B. pilosa* and *A. remota* extracts was observed which confirmed presence of terpenes. Such colour change was not observed in *A. aspera*. Addition of 1 ml of acetic acid then a drop of 5% ferric chloride followed by 1 ml of concentrated sulfuric acid to the extracts resulted in a reddish brown ring at interface of *A. remota* extract only. This indicated presence of glycosides. There was no colour change in the other extracts. Addition of distilled water to the extracts and shaking vigorously resulted in formation of stable foam of varied intensity which lasted for a minute in *A. aspera* and *B. pilosa* extracts. This indicated presence of saponins. No foam was formed in *A. remota* extract. When 4 drops of 2% ferric chloride solution was added to each of the extracts, *B. pilosa* and *A. remota* extracts changed colour to blue with varied intensities indicating presence of tannins. There was no colour change in *A. aspera* extract. These results are summarized in table 1 below.

In table 1, it can be seen that *B. pilosa* was the richest in phytochemical constitution. Phenols, tannins, flavonoids, saponins, alkaloids and terpenes were all present in *Bidens pilosa* with at least moderate to above moderate concentrations.

Table 1 Phytochemical Constituents of A. aspera, B. pilosa and A. remota

Plants	***Phenols***	***Flavonoids***	***Alkaloids***	***Terpenes***	***Glycosides***	***Saponins***	***Tanins***
Achyranthes aspera	-	-	+	-	-	+	-
Bidens pilosa	++	+++	+	++	-	++	++
Ajuga remota	+	-	-	++	++	-	+

+ = means the phytochemical is present and
- = means the phytochemical is absent
+++ = means the phytochemical is present in high concentration.
++ = means the phytochemical is present in moderate concentration

Glycosides were only absent from its leaves. Only saponins and alkaloids were present in the leaves of *A.aspera*, whereas all the rest were absent. Flavonoids, alkaloids and saponins were absent in the leaves of *A. remota* whereas the rest studied biochemicals were present with the terpenes and glycosides occurring in moderate concentrations.

All the animals in groups II to VI became diabetic after intravenous injection of alloxan monohydrate. However, ninety-six hours (4 days) later after administration of alloxan monohydrate, all the animals had presented with glycaemic levels above 4.16 mM/L and were subjected to treatment. The diabetic goats exhibited highly significant (P < 0.01) increase (5.22±3.13) in serum fasting blood glucose levels.

Effect of A. aspera, B. pilosa and A.remota ethanolic leaf extracts and glibenclamide on the haematological parameters T/WBCs and PLT counts, and Hb and PCV content in alloxan induced diabetic goats.

Total WBCs and PLT count levels were observed to be significantly (P< 0.05) raised in normal saline treated diabetic control goats in comparison with the healthy control goats. In contrast, haemoglobin (Hb) and packed cell volume (PCV) contents were significantly (P<0.05) lowered as compared with the healthy control group. Administration of B. pilosa and glibenclamide effectively lowered the raised levels of total WBCs and PLT to regular levels and significantly increased the lowered levels of haemoglobin as did A. remota to normal levels. A. aspera significantly restored the raised concentrations of PLT in the normal saline treated diabetic control. Table 3 gives a summary of results from all the treatments and the healthy control group.

The *In vivo* effects of plant extracts on the haematological parameters T/WBCs and PLT counts, and Hb and PCV content at varying times is given in Figure 1.

Table 2: Effect of A. aspera, B. pilosa and A.remota ethanolic Leaf Extracts and glibenclamide on Fasting Blood Glucose Levels in Alloxan Induced Diabetic goats (mmol/L) 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 ± SEM. All blood glucose levels were recorded in mmol/L (n = 3)

Table 3: Effect of A. aspera, B. pilosa and A. remota ethanolic Leaf Extracts and Glibenclamide on the Haematological Parameters T/WBCs and PLT Counts, and Hb and PCV Content in Alloxan Induced Diabetic Goats for 28 days

Treatment	Total WBC's Count (x10^3)	PLT count (x10^3)	Hbcontent (mmol/L)	PCV content (mmol/L)
Healthy con + saline	11.16±0.55	361.08±64.50	8.99±0.627	50.80±5.115
Diabetic con+ saline	13.80±3.63 ^{aa}	482.58±87.57^{a}	6.91±1.61^{aa}	37.47±3.53^{a}
Diabetic + gilbenclamid	10.39±2.016 ^{b}	298.8333±61.48^{bb}	8.7±0.835^{b}	45.86±8.74
Diabetic + *A.aspera*	12.07±1.815	310.5±81.29^{b}	8.25±1.18	39.95±2.765
Diabetic + *B.pilosa*	11.29±1.83^{b}	348.83±66.95^{b}	9.39±0.64^{bb}	43.98±4.20
Diabetic + *A.remota*	11.54±2.39	359.167±88.136	9.837±0.685^{bb}	39.06±7.566

Significant codes: 'aaa' P<0.001 'aa' P<0.01 'a' P<0.05; Significant codes: 'bbb' P<0.001 'bb' P<0.01 'b' P<0.05. 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)

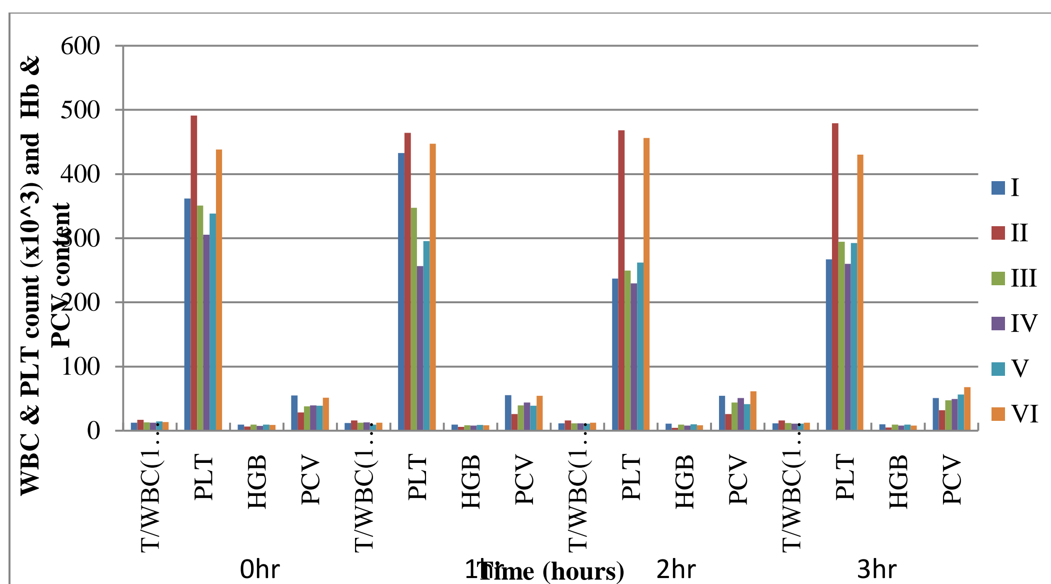

Figure 1: In vivo effects of A. aspera, B. pilosa and A.remota ethanolic leaf extracts and glibenclamide on the haematological parameters T/WBCs and PLT counts, and Hb and PCV content in alloxan induced diabetic goats at varying times. NB. Same axes were used for all the parameters despite the different units of measurement to display trends

Administration of the plant extracts reduced the WBCs in one phase where in the 1st hour the WBC levels were reduced to normal range and maintained to the 3rd hour, whereas PLTs were in two phases where in the 1st hour there were a gradual decline and a steep decline in the 2nd to 3rd hour reaching normal. All the plant extracts and glibenclamide elevated the reduced levels of Hb in the 1st hour to normal and maintained it through to 3rd hour.

Elevation of PCV levels by the extracts was gradual from 1st hour to the 3rd hour. However, it was only *B. pilosa* and *A. remota* that restored PCV levels to normal by the 3rd hour.

Discussion

Plant primary metabolites such as sugars, fats, ethanol, lactic acid and certain amino acids are microbial products produced continuously during the exponential phase of growth and are involved in primary metabolic processes such as respiration, development, reproduction and photosynthesis whereas secondary metabolites are not known to take part in these primary processes but are known to be toxins, antimicrobial, attractants/repellants, or as deterrents/defense against herbivores. This study showed that the diabetic animals treated with A. aspera, B. pilosa and A.remota ethanolic leaf extracts had their disrupted blood electrolyte levels restored to normal levels.

The analysis of the three plant extracts demonstrated the presence of the secondary metabolites; glycosides, phenols, flavonoids, saponins, tannins, terpenes, and alkaloids in varying proportions. These biomolecules brought about the reversal of the diabetic metabolic syndromes observed in the results of diabetic goats. The findings obtained in this experimental study hence proposes the phytochemicals identified are bioactive phytoconstituents and that these plants are demonstrating to be highly valuable store of bioactive principles of remarkable therapeutic significance. Treatment interventions and/or conventional management of diabetes have been impeded by inconviniences' such as unaffordability, inaccessibility, and possibility of likely toxic and adverse side effects [25] amongst others. The application of diabetic countering phytodrugs has increased, especially in the developing nations, possibly because of accessibility advantages and cost implications. In the present study, ethanolic leaf extracts of *A.aspera, B.pilosa and A. remota* were investigated for blood glucose lowering potential in alloxan induced diabetic goats.

In the present study, intravenous alloxan monohydrate administration to healthy goats remarkably induced diabetes as demonstrated by hyperglycaemia. As shown in table 1, the plasma glucose levels in alloxan induced diabetic goats were observed to be significantly increased in comparison with healthy control goats. The diabetic goats had approximately three to six times increases in blood glucose levels in relation to the healthy control goats. That the ethanolic extracts of *A.aspera, B. pilosa* and *A. remota* showed glucose lowering effect administered orally could be explained by the movement of their bioactive blood glucose lowering phytoconstituents across the gastro-intestinal mucosa. Oral administration of glibenclamide (0.125mg/kg bw/day) and *B.pilosa* at 250mg/kg bw/day for 28 days demonstrated significant ($P<0.05$) decrease in blood glucose concentrations. The capacity of *B. pilosa* extract to decrease the increased blood glucose levels to regular glycemic concentration is an important activator for the hepatic tissue to return to its regular homeostasis during induced diabetes.

In the present study, a broad array of laboratory haematological values changed considerably in the diabetic animals. The total WBC and PLT counts were significantly elevated amongst diabetics compared to normal control. A positive relationship was observed among raised total WBC and PLT on one hand and hyperglycaemia on the other in our study which could imply that a common process drives both the increased WBC and platelet counts in subjects having this disorder [26]. WBCs are constituted by polymorphonuclear cells (neutrophils, basophils and eosinophils) as well as lymphocytes and monocytes. The activity of

Mononuclear and polymorphonuclear leukocytes in diabetics may be elevated by reactive oxygen species (ROS) or advanced glycation end products (AGEs) and cytokines [27]. Activated total WBCs release cytokines and transcription factors which have an important inflammation role. The decrease in the tWBC counts in plant extract treated goats shows the anti-inflammatory property of the ethanolic plant leaf extracts through their active principles flavonoids and phenolic compounds which are both antihyperglycemic and antioxidants. This finding is consistent with that of Ayman M. Mahmoud (2013) who demonstrated that elevated WBCs counts in diabetic rats were restored by Citrus flavonoids.

Increased PLT count amongst diabetics, are attributed to decreased membrane variability, elevated intracellular calcium utility, reduced intracellular magnesium, reduced nitric oxide and prostacyclin production and reduced antioxidant concentrations [28]. Clinically high platelet counts are commonly found in diabetics with an extended period of the ailment. The findings of the present study is in harmony with [27] who demonstrated higher platelet counts which promote vascular events in human patients with accompanying insulin resistance. The vascular events include acceleration of atherosclerosis and could be linked to both macro- and micro vascular syndrome. It could as well be amplified myocardial infarction (MI), coronary artery sickness, cerebral ischemia and peripheral arterial malady [29]. Previous reports seem to propose the likelihood that increased platelet tally may be employed as a predictive pointer of upcoming diabetic complication [30].

In the current research study, it was demonstrated that the PCV and Hb were significantly lowered in diabetic goats in comparison with normal controls. Likewise, a preceding study found that the average values of total red blood cell (TRBC) count, Hb, and PCV content for diabetic animals are lesser than the values of normal control cluster [30]. PCV may be reduced in diabetic animals due to higher blood glucose concentration which may possibly lead to intracellular dehydration. In addition, the decrease in PCV value of diabetics may also be due to accumulation of proteins following diuresis and excretion of water excessively by the kidneys. Haemoglobin (Hb) concentration is sensitive to variation in hydration and renal, liver, and cardiac function. The decrease in Hb level may be due to the variation in plasma volume in diabetic animals that act in one direction on Hb and in opposite direction on blood volume because the Hb level depends on plasma quantity and erythrocyte mass [31]. The results of this study are in concurrence with further studies that demonstrated reduced hematological variables; PCV, Hb, and RBC in human patients with type 2 diabetes by [32]. However, inconsistent results were demonstrated by [33] who didn't find any significant effects on RBC, Hb, PCV, MCHC, MCH and MCV levels in alloxan induced diabetic dogs. In the present study, treatment with the ethanolic leaf extracts elevated the reduced Hb and PCV contents which propose that the extracts have anti-anaemic potency which could be owed to their antihyperglycemic property reversing osmotic diuresis hence intracellular hydration. The extracts could also be having the ability to stimulate secretion of erythropoietin which promotes bone marrow activities, a key location for hematopoiesis [34]. In a past study, it was demonstrated that the mechanism of action of flavonoids to maintain cellular integrity may be attributed to their ability to lower lipid peroxidation level [20].

Conclusion

In light of this information, it can be concluded that diabetes mellitus is associated with disruption of hemoparameter levels due to hyperglycemia induced osmotic imbalance and degenerative changes in the hepatic and renal systems. Ethanolic leaf extracts of *B. pilosa, A. remota* and *A. aspera* are an important source of the secondary metabolites; phenols, alkaloids, glycosides, flavonoids, terpenes, saponins and tannins in varying proportions and play important physiological therapeutic roles with respect to diabetes in the body including being antihyperglycemic, anti-inflammation, antioxidants, antithrombotic and antibacterial. *B. pilosa* has strong antidiabetic activity and ameliorates hemoparameter level disruptions in alloxan-induced diabetes in goats whereas *A. aspera* and *A. remota* ethanolic leaf extracts are moderate in restoration of the disrupted levels of hematological parameters in diabetic animals.

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