



## **The Effects of the N-hexane Fractions of *Heinsia crinita* Leaf Extract on Electrolyte Balance and Some Haematological Parameters of Alloxan-induced Diabetic Albino Wistar Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author BIAM managed the project conception and design, coordination, interpretation of data and preparation of manuscript. Author EMA managed the experimentation, acquisition and analysis of data, statistical analysis. Author IAI managed the experimentation and acquisition of data. Author GOI performed the extraction and fractionation methodologies. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** Crude extracts of *Heinsia crinita* leaves have been found to have antidiabetic activity. The aim of this study was to evaluate the effects of the n-hexane fraction of *Heinsia crinita* leaf extract on electrolyte balance and some haematological indices of alloxan-induced diabetic albino Wistar rats.

**Methodology:** Thirty (30) albino Wistar rats of both sexes weighing 120-180g were shared into five (5) groups of six animals each. Group 1 and 2 served as the normal control (NC) and diabetic control (DC) respectively and received placebo. Groups 3, 4 and 5 were diabetic treated group, and received 500mg/kg bw Metformin, 400mg/kg bw of crude extract (HC-C) and n-hexane fraction (HC-H) of *Heinsia crinita* respectively.

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**Results:** The HC-H administered group, like the HC-C group, showed reversal of the observed increase in chloride ion (Cl<sup>-</sup>) and decrease in sodium ion (Na<sup>+</sup>) levels in DC group to close to those of the NC group and this effect compared well with the metformin-treated group. The HC-H group, unlike the HC-C group, did not show a reversal of the observed diabetic increase in potassium ion (K<sup>+</sup>). The WBC count was increased in DC compared to NC. On treatment with HC-C, HC-H and metformin, the WBC count decreased significantly (P<0.05) in all the treated groups compared to DC group. The RBC count was significantly decreased (P<0.05) in DC compared to NC. Similarly, there was a decrease in haemoglobin concentration in the DC group compared to the NC group. These decreases were significantly (p<0.05) reversed towards normal on treatment with HC-C and HC-H. The reversal was comparable to that of the standard drug metformin. Similarly the diabetes induced increase in platelet counts was significantly (P<0.05) reduced towards NC values on treatment with HC-H and HC-C.

**Conclusion:** The result show that the n-hexane fraction (HC-H), like the crude extract (HC-C), can protect against diabetes induced electrolyte imbalance and haematological disorders.

**Keywords:** Diabetes; electrolyte balance; haematological indices; n-hexane fraction; *Heinsia crinita*.

## ABBREVIATIONS

NC: Normal control; DC: Diabetic control; HC-C: *Heinsia crinita* crude extract; HC-H: *Heinsia crinita* n-hexane fraction; FBG: Fasting blood glucose; WBC: White blood cell; RBC: Red blood cell; bw: Body weight; K<sup>+</sup>: Potassium ion; Na<sup>+</sup>: Sodium ion; Cl<sup>-</sup>: Chlorine ion.

## 1. INTRODUCTION

Diabetes mellitus, a major global public health problem, is a metabolic disorder resulting from absolute or relative deficiencies in insulin secretion and/or insulin action [1,2]. A primary consequence of this is chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism [1]. Persistent hyperglycemia causes increased production of free radicals which may ultimately result in destruction of some vital body organs like the kidney, liver, pancreas and testis and in vascular complications [3-6]. Oxidative stress also results in the damage of the circulating red blood cells (erythrocytes) resulting in low haemoglobin (anaemia) [7,8]. Increased WBC and platelets count have been associated with diabetes [9-13] as is disturbance in electrolyte balance [14-20]. Current emphasis on the treatment of diabetes has shifted to medicinal plants as the available therapies with synthetic drugs is fraught with numerous side effects. Apart from its little or no side effects (at the therapeutic doses), medicinal plants are cheap and easily accessible to the rural poor. One of such plants *Heinsia crinita* has been shown in our laboratory to have hypoglycemic, hepatoprotective and nephroprotective effects in alloxan-induced Type 1 diabetes in albino Wistar rats [21]. Towards isolating the active diabetic components and standardizing the plants extract for treatment of diabetes, the crude leaf extract of the plant was

fractionated with n-hexane and its phytochemical composition evaluated using GC-MS [22]. The fraction contained phytochemicals that were known to have antihyperglycaemic, hypocholesterolemic, hypolipidemic and antioxidant activities suggestive that it may have antidiabetic activity as the crude extract. Further studies in our laboratory [23] has confirmed that the fraction indeed has antihyperglycaemic, and hypolipidemic properties. This study seeks to establish if the n-hexane fraction, like the crude leaf extract, can reverse the diabetic induced distortion of electrolyte balance and hematological indices.

*Heinsia crinita* also known as Bush apple belongs to the family Rubiaceae. It is a persistent scrambling shrub with very conspicuous leafy calyx-lobes, produces edible yellow or reddish fruits, sweet when ripe and pleasantly acidic [22-25]. The plant grows in the tropics and has over the years been used by traditional herbalists for the treatment of various ailments like sore throats, catarrh, hypertension etc. [21,26].

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Leave Extract

The plants material (leaves) were purchased from a local market in Akpabuyo Local Government Area of Cross River State, Nigeria. The leaves were authenticated in the

Department of Botany, University of Calabar, Nigeria as earlier published [21]. The collected leaf material were thoroughly washed with tap water, rinsed with distilled water, allowed to drain then air dried for 7 days after which the leaves were ground into powdered form with a manual blender. The powdered leaves were weighed using an electronic weighing balance and soaked in a 96% ethanol solution in the ratio of 1:4 for 48 hours with intermittent agitation. The mixture was doubly filtered using a cheese cloth and then Whatman filter paper. The filtrate was then concentrated using a rotary evaporator and residual solvent removed by placing in a water bath at a temperature of 40-45°C.

## 2.2 Fractionation of Plant Extract Using Column Chromatography

A standard column with a length of 60cm and diameter of 4.5cm was used for the chromatographic process. Silica gel of mesh 60-120 was suspended in distilled water to form a slurry and then loaded onto the column. The silica gel column was thereafter equilibrated with n-hexane. The crude extract (30g) was reconstituted in n-hexane, loaded onto the column and eluted using 500ml of n-hexane. The eluted fractions were pooled and concentrated at 40-45°C.

## 2.3 Experimental Animals and Treatment Protocol

Thirty (30) albino Wistar rats of both sexes weighing between 120-180g were used for this study. These animals were randomly divided into five (5) groups of six rats each and treated according to the schedule in Table 1. The doses used were based on the established LD<sub>50</sub> from preliminary studies. The extracts were reconstituted in normal saline (vehicle) and administered via gastric intubation, twice a day, at twelve hourly interval. The animals were maintained on pellets of normal animal feed obtained from Vital feed company from Jos, Plateau State, Nigeria and tap water. Both the

feed and water were provided *ad libitum* except where otherwise stated. Treatment lasted for 14 days.

## 2.4 Induction of Experimental Diabetes

Diabetes was induced in the experimental animals by intraperitoneal injection of 100mg/kg bw of alloxan monohydrate (Sigma–Aldrich, Inc, St. Louis, Mo, USA) in sterile saline after a 12hr fast. After five (5) days of alloxan injection, diabetes was confirmed in the rats if fasting blood glucose (FBG) as determined using a glucometer (Acon laboratories, Inc. San Diego U.S.A) on blood obtained from the tail vein of the rats, was above 180mg/dl.

## 2.5 Collection of Blood Samples for Analysis

At the end of 14 days, food was withdrawn from the rats and they were fasted overnight but had free access to water. The animals were then sacrificed and whole blood collected via cardiac puncture using sterile syringe and needle. The blood sample was divided into two fractions, one fraction was put into plain sample tubes while the other sample was put in ethylene diamine tetra acetate (EDTA) treated sample tubes. Sera were obtained from the clotted blood in the plain sample tubes by allowing it to stand for 2hours at room temperature to clot before centrifugation at 3,000rpm for 10minutes using a benchtop centrifuge to separate cell from serum. Sera obtained from the respective samples were carefully removed using Pasteur pipettes and put into respective dry labelled plastic specimen bottles. These were kept frozen in a refrigerator until when needed for various biochemical assays. The blood samples collected into the EDTA bottles were corked immediately and gently inverted severally to allow the blood mix with the anticoagulant and prevent clotting and cell haemolysis. The EDTA treated blood was used for haematological analysis and were carried out as soon as the blood was collected.

**Table 1. Experimental design and treatment schedule**

Group	Treatment	No of animals	Treatment dosage.
1	Normal control	6	Placebo(0.2%DMSO)
2	Diabetic control	6	Placebo(0.2%DMSO)
3	Metformin	6	Metformin(500mg/70kgb.w)
4	Crude extract	6	Crude extract (400mg/kg b.w)
5	n-hexane fraction	6	N-hexane fraction(400mg/kg b.w)

## 2.6 Electrolyte Determination

The concentration of electrolyte viz: sodium ( $\text{Na}^+$ ), chloride ( $\text{Cl}^-$ ), Potassium ( $\text{K}^+$ ) were assayed using standard commercial kits. Serum  $\text{Na}^+$  and  $\text{K}^+$  was determined using Teco kits, based on a colorimetric method, according to manufacturer's protocol.

Serum  $\text{Cl}^-$  concentration was estimated based on a modification of the colorimetric method of Skeggs and Hochstrasser [27] using Agape diagnostic kits.

## 2.7 Haematological Analysis

Haematological analysis was carried out using Syntex Analyzer KX-21N (Whole Blood mode).

## 2.8 Statistical Analysis

Data was presented as mean  $\pm$  standard error of mean. Data was computed and analysed using one way ANOVA and unpaired Student's t-test with the help of a statistical package, SPSS version 18.0 for Windows. Values were considered significant at  $P < 0.05$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Evaluation of Serum Electrolyte

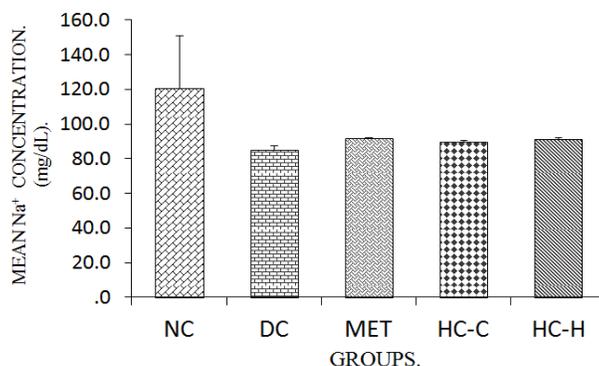
#### 3.1.1 Evaluation of serum sodium concentration

The serum sodium concentration of alloxan induced diabetic rats treated with crude extract and n-hexane fraction of *Heinsia crinita* is presented in Fig. 1. They was a significant

decrease in serum sodium concentration in diabetic control (DC) relative to normal control (NC) ( $P < 0.05$ ). Similar observations, in the diabetic state, have been reported in the literature [16-20]. Treatment with HC-H, like HC-C, showed an increase in sodium concentration when compared to DC ( $P < 0.05$ ). The extract treated groups also compared favourably with the group treated with standard drug metformin.

Alloxan is a cytotoxic analogue of glucose [Lenzen, 2008]. The deleterious action of alloxan is due to its selective destruction of pancreatic  $\beta$ -cells [28,29]. The result of this is the non-production of insulin leading to hyperglycaemia and lipid-peroxidation mediated damage, dysfunction and failure of various organs and tissues, including the kidney. Renal hypertrophy is also seen in the diabetic state as a result of accumulation of glycogen granules in the distal tubules of the kidney [30]. A consequence of kidney dysfunction is electrolyte imbalance [14-20].

Electrolytes play an important role in the maintenance of normal body fluid balance. Sodium [ $\text{Na}^+$ ], together with potassium [ $\text{K}^+$ ] and chloride ions [ $\text{Cl}^-$ ], are the major electrolytes located in all body fluids and they are responsible for the maintenance of acid/base balance, transmission of nerve impulses and regulation of fluid movement in and out of cells [31]. They are normally excreted by the kidneys, so disorders that decrease the functions of the kidneys can result in electrolyte imbalance. It has been shown that the administration of high doses of alloxan leads to kidney tubular cell necrotic toxicity [28, 32]. This may be as a result of the deleterious action of reactive species generated from the metabolism of alloxan on the



**Fig. 1. Sodium concentrations of the different experimental groups**  
 Values are expressed as mean  $\pm$ SEM. n=6

renal tissue. Another explanation for the diabetes induced lowering of serum sodium is that hyperglycaemia in the diabetic state results in an osmotic force that draw water to the extracellular space [16-20]. This dilutes extracellular sodium and leads to lower plasma sodium levels.

### 3.1.2 Evaluation of serum potassium concentration

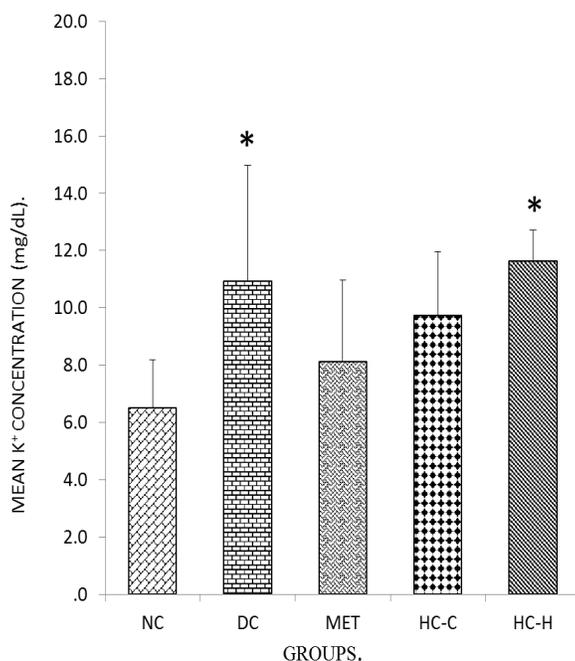
The serum potassium concentration of Alloxan induced diabetic rats treated with crude extract and n-hexane fraction of *Heinsia crinitia* is presented in Fig. 2. They was a significant ( $P < 0.05$ ) increase in the potassium concentration in the diabetic control relative to the normal control. This increase in potassium level in DC was significantly ( $P < 0.05$ ) reversed by treatment with HC-C and the standard drug Metformin. The n-hexane fraction, HC-H, unlike HC-C did not show any reversal. Similar observations, in the diabetic state, have been reported in the literature [16-20].

Unlike in the case of  $\text{Na}^+$  the movement of water out of cells, as a result of hyperglycaemia, leads to an increased intracellular potassium

concentration favouring a gradient for potassium to move out of the cells to the extracellular space causing hyperkalaemia [15,16,18-20]. Reduced glomerular filtration of  $\text{K}^+$  (due to acute kidney injury) may also be responsible for hyperkalemia.

### 3.1.3 Evaluation of serum chloride concentration

The serum chloride concentration of alloxan induced diabetic rats treated with crude leaf extract and n-hexane fraction of *Heinsia crinitia* and other experimental groups is presented in the Fig. 3. The DC group showed a slight increase in  $\text{Cl}^-$  concentration compared with the normal control. Similar observations, in the diabetic state, have been reported in the literature [16-20]. Treatment with the standard drug and plant extract and fraction resulted in decrease in  $\text{Cl}^-$  concentration towards normal values. The chlorine levels of crude extract group was comparable to that of the group treated with the standard drug. The decrease in  $\text{Cl}^-$  concentration in the HC-H group was more appreciable than the standard drug and HC-C and much lower than NC ( $P < 0.05$ ).



**Fig. 2. Potassium concentrations of the different experimental groups**  
 Values are expressed as mean  $\pm$ SEM. n=6

Kidney controls the levels of chlorine in the blood therefore any damage, disorder or failure of the kidney results in disturbance in blood chloride levels and will account for the high level of chlorine (hyperchloremia) in the diabetic state [14,18,20].

### 3.1.4 General discussions on the effect of administration of plant extract on diabetes induced electrolyte imbalance

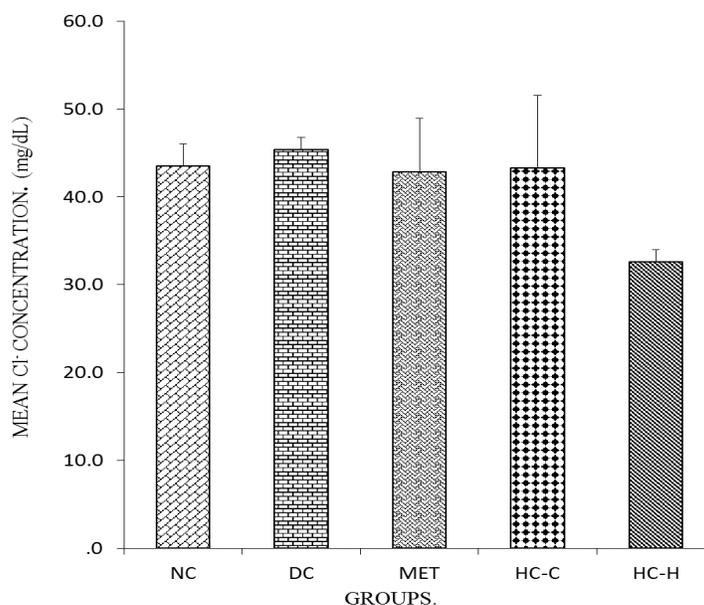
The HC-H group, like the HC-C administered group, showed restoration of these electrolyte values close to those of the normal control group (except for  $K^+$ ), and this effect compares well with the metformin-treated group. *Heinsia crinita* contains antioxidant principles [22,33]. These principles may also be responsible for mopping up cell damaging antioxidants thus restoring the integrity of the pancreatic B-cells with concomitant insulin production and restoration of the normal glycaemic state. Antioxidant phytochemicals present in the leaf crude extract and fractions may also have led to regeneration of the damaged kidney cells. This is not unprecedented. Histological studies in our laboratory has shown the regeneration of damaged tissues (kidney, pancreas, liver, testis) in diabetic animals on treatment with antidiabetic medicinal plant extracts and fractions [34-38].

## 3.2 Haematological Evaluation

### 3.2.1 Evaluation of red blood cells

The RBC count was significantly lowered in the DC compared to NC group (Table 2). Treatment with the *Heinsia crinita* n-hexane fraction, like its crude extract, reversed the levels towards normal though not as effective as the standard drug. Similarly, diabetes induction lowered the haemoglobin concentration compared to NC group. This was reversed by treatment with the plant crude extract and fraction.

Hyperglycemia in individuals with diabetes is also associated with changes in haematological parameters as well as micro and macrovascular complications. Haematological indices can be used to assess the extent of destructive effects on blood constituents of animals due to diabetes mellitus. Anaemia and increased erythrocyte fragility, are some of the conditions that have been associated with diabetes [7, 8, 39, 40]. In the current study, reduced RBC count, haemoglobin concentration and mean corpuscular haemoglobin level were observed in rats in the diabetic group compared to normal control (NC) group, indicating normocytic, hypochromic anaemia. This is consistent with earlier reports that observed significant decrease in this parameter in alloxan-induced diabetic rats [41-43].



**Fig. 3. Chloride concentrations of the different experimental groups**  
 Values are expressed as mean  $\pm$ SEM. n=6

Destruction of red blood cells leads to decrease in RBC count and low haemoglobin. Under normal conditions, the Kidney's peritubular interstitial cells release a hormone called erythropoietin (EPO) which signals the bone marrow to produce red blood cells. Kidney damage and failing kidneys occasioning diabetes (diabetic nephropathy) reduces the ability of the kidney to produce EPO resulting in reduced production of red blood cells [8,39]. Also in early diabetic nephropathy, damage to the peritubular fibroblasts can occur and lead to erythropoietin deficiency and anaemia prior to the loss of filtration [7]. The occurrence of anaemia in diabetes mellitus has also been suggested to be due to non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycaemia [44]. Oxidation of these glycosylated membrane proteins and diabetes mellitus induced hyperglycaemia causes an increase in the production of lipid peroxidases, resulting in haemolysis.

### 3.2.2 Evaluation of white blood cells

The haematological evaluation of alloxan induced diabetic rats treated with crude extract and n-hexane fraction of *Heinsia crinita* is presented in Table 2. The white blood cell count (WBC) of the DC was found to be significantly increased ( $P < 0.05$ ) compared with NC. Similar diabetes associated increase in WBC has been reported [11-13,45]. Treatment with the plant fraction HC-H, as with the plant crude extract HC-C, reversed the WBC levels towards the NC values.

An elevated WBC count is present in impaired glucose tolerance (IGT) [11] and WBC count is associated with macro- and microangiopathic complications in type 2 diabetes [12]. The increase in WBC count (leucocytosis) of DC group may be interpreted as increased cell-mediated immune response. There is increased

biosynthesis of cells involved in immunity when a living system is under biochemical insult [46].

### 3.2.3 Evaluation of platelet counts

Diabetes induction markedly increased the percentage platelet counts in the DC group compared to NC (Table 2). This increase was reversed by treatment with the plant extract and fraction.

The increased platelet count observed in diabetic rats study may be due to accumulation of products of advanced glycosylation, which caused reduction of membrane fluidity of platelets leading to platelet hyper function [47]. Also, it is known that insulin is a natural antagonist of platelet hyperactivity [10]. It sensitizes the platelet to  $PGI_2$  and enhances endothelial generation of  $PGI_2$  and NO. The defects in insulin action in diabetes creates loss of sensitivity to the normal restraints exercised by prostacyclin ( $PGI_2$ ) and nitric oxide (NO) generated by the vascular endothelium and presents as the major defect in platelet function [10]. Udvardy et al. [9] reported decreased platelet insulin receptor number and affinity in subjects with type 2 diabetes, which suggests that reduced insulin sensitivity may account for platelet hyperactivity in type 2 diabetes.

### 3.2.4 General discussions on the effect of administration of plant extract on diabetes induced haematological indices

Administration of the n-Hexane fraction of *Hensia crinita* (HC-H), as with the parent crude extract of *Hensia crinita* (HC-H), was effective in reversing the irregularities in the haematological parameters of diabetic rats (Table 2). The presence of bioactive antioxidant principles such as flavonoids, polyphenols and phytol in *Hensia crinita* n-Hexane fraction, as in the crude extract

**Table 2. Haematological parameters of diabetic animals treated with crude extract and n-hexane fraction of *Hensia crinita***

	WBC $\times 10^3/\mu\text{L}$	RBC $\times 10^6/\mu\text{L}$	HGB g/dL	MCH pg	PLT $\times 10^3/\mu\text{L}$	%N	%L
NC	14.0 $\pm$ 2.3	7.56 $\pm$ 0.99	14.2 $\pm$ 0.5	18.8 $\pm$ 0.70	934.5 $\pm$ 70.5	21.0 $\pm$ 3.8	76.85 $\pm$ 1.65
DC	16.26 $\pm$ 2.5	3.79 $\pm$ 0.38	11.97 $\pm$ 0.7	18.66 $\pm$ 0.3	1092.66 $\pm$ 79.69	17.23 $\pm$ 8.6	79.03 $\pm$ 9.81
Metformin	11.75 $\pm$ 0.85	7.69 $\pm$ 0.64	14.5 $\pm$ 1.4	18.85 $\pm$ 0.25	863.0 $\pm$ 100	13.2 $\pm$ 1.0	83.85 $\pm$ 1.35
HC Crude	11.3 $\pm$ 1.46	5.46 $\pm$ 0.61	15.46 $\pm$ 0.61	18.26 $\pm$ 0.55	905.0 $\pm$ 48.57	19.3 $\pm$ 3.57	77.56 $\pm$ 3.84
HC n-Hex	14.35 $\pm$ 0.65	5.0 $\pm$ 0.4	15.0 $\pm$ 0.4	17.05 $\pm$ 0.27	880.5 $\pm$ 1.5	13.95 $\pm$ 2.05	83.35 $\pm$ 2.75

Values are expressed as mean  $\pm$  SEM, n=6

[22], may have mopped up the cell damaging antioxidants leading to regeneration of the damaged pancreatic and kidney cells and subsequent restoration of insulin and erythropoietin production and attendant decreases in glucose levels and oxidative stress, increase in RBC, haemoglobin, and decrease in WBC and platelet count. Furthermore, the extract of *H. crinita* have been reported to be able to restore insulin sensitivity to cells [45,48]; this reduces the incidence of platelet hyperactivity. Also, the lowering of glucose concentration following treatments reduces the incidence of non-enzymatic glycosylation of RBC membrane proteins and thus a decreased susceptibility of RBCs to haemolysis.

#### 4. CONCLUSION

In conclusion, the results show that the n-hexane fraction (HC-H), as with the crude extract (HC-C), can reverse diabetes induced electrolyte imbalance and blood disorders. Taken together with our earlier study [23], the n-hexane fraction, like the leaf crude extract, has antidiabetic properties and represents a more refined preparation of the plant in the management of diabetes. It also represent a step towards isolating the active antidiabetic principle in the plant and standardization of the plant extract for management of diabetes.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

All authors hereby declare that the research has been determined exempt from review by the University animal research or ethics review committee and that the principles of laboratory animal care were followed.

#### COMPETING INTERESTS

The authors declare that no competing interests exist.

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