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Phytochemical, Antinutrients, Food Toxicants Analysis and Comparative Efficacy of Methanol Extracts of Selected Vegetables of Adamawa State Nigeria on the Haematological Parameters of Alloxan Induced Diabetic Rats

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Abstract

Purpose: Phytochemicals present in vegetables has been associated with the health potentials of leafy vegetables. Diabetes has been implicated with increased oxidative stress leading to alteration of haematological parameters among other complications. This study investigated the phytochemicals, antinutrients and toxicants contents of methanol extracts of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora l*eaves and compared the effect of their leaves extract on some haematological parameters of alloxan – induced diabetic rats using standard methods of analysis.

Methodology: Fifty-five (55) male albino rats weighing 120 – 150g divided into 11 groups of five rats each were used. One diabetic untreated rat group, one diabetic group treated with standard drug ,8 diabetic groups treated with graded doses of the vegetable extracts and one normal group as control. All the groups received water and feed *ad libitum* together with their various treatments for 23 days. At the end of the experiment, blood was taken from the rats for determination of PCV, Hb, WBC, and RBC. ANOVA was used to separate the means of all the data collected with significance at $p< 0.05$ using Statistical Package for Scientist and Engineers (SPSE) version 9.1.

Results: Results showed reasonable levels of phytochemicals, antinutrient and toxicants levels within WHO safe limits. Results of animal studies showed that administration of 500mg of *Hibiscus cannabinus* leaf extract increased the PCV, HB, WBC and RBC of diabetic rats more than all the other vegetable extracts ($p<0.05$). It also improved the WBC and RBC better than standard drug. This shows that *Hibiscus cannabinus* leaf extract might improve some haematological parameters associated with diabetes mellitus, however its inhibitory effect on higher dose raised concern for further investigation.

Unique contribution to theory, practice and policy: *Hibiscus cannabinus* leaf extract could be used in the dietary management of haematological abnormalities associated with the pathophysiology of diabetes mellitus by health care systems.

Key Words: *Vegetables, phytochemicals, antinutrients, diabetes, haemotological parameters*

INTRODUCTION

Attention to vegetables as vital components of daily diets for the sick and the healthy has been on the increase. This is because of the increased awareness of the health protecting properties of the non-nutrient bioactive compounds (phytochemicals) found in vegetables. In sub–Saharan African population, this attention on vegetables as important components of African diets reinforces the significant roles that leafy vegetables play as vital dietary components (Ene-Obong, 2001; Smith and Patil, 2010). Vegetables include those leafy outgrowths of plants or parts of plants that are used in making soup or eaten with the principal part of the meal (Onimawo & Egbekun, 1998; Nwankwo *et al.,* 2015). They are important protective foods and highly beneficial for the maintenance of good health and prevention of diseases (Kubmarawa, Andenyang & Magomya, 2009). *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves are common vegetables in Northern part of Nigeria and popularly consumed in Adamawa state of Nigeria (Kubmarawa, Magomya, Yebojolla & Adebayos, 2011).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Oguntona, 1986, Nnam, 2011). Studies have shown that phytochemicals found in large quantities in fruits and vegetables are responsible for this protective effect (Sundarrayanan, Kumia & Sekar, 2011). Intake of antioxidant phytochemicals helps to improve endogenous antioxidants mechanisms and therefore reduce the vascular complications in diabetes and other effects of hyperglycemia (Fardoun, 2007). Vegetables are included in meals mainly for their nutritional values. Some are however reserved for the sick and convalescence because of their medicinal properties (Iloveindia, 2004; Nnam *et. al.,* 2012). Grover *et al*. (2002) identified that herbs play important role in diabetic therapy, particularly in developing countries where most people have limited resources. World Health Organization (WHO) recommends the evaluation of traditional plant extracts, for the management of diabetes as such extracts have fewer side effects and possess better glycaemic control over the synthetic medicines (WHO, 2007). Some plant extracts may have effects on the heamotological parameters of the diabetics.

Diabetes is fast becoming the most popular non-communicable disease (NCD) globally (Ayinla *et al*.,2015). Lack of nutritional information and adequate development of nutritionally improved products from local raw materials have direct bearing on the dietary management of diabetics. Many leafy vegetables and their extracts have been found to be effective in the management of non-communicable diseases (NCDs) including diabetes (Nnam *et* al.,2012). Phytochemicals found in large quantities in vegetables have been implicated to be responsible for this effect.

Antinutrients and toxicants are invariably present in vegetables; however, some antinutrients have beneficial antioxidant properties (depending on the dosage) necessary in the management of diabetes (Willy, 2003). Diabetic conditions among other complications affects blood glucose, lipid profile and haematological parameters of the affected individuals. Some researchers have reported that some vegetable extracts improve blood glucose and lipid profile of diabetic rats (Nwankwo *et al*.,2017; Onyechi, *et al.,* 2018). Lipid profile are often given much attention probably because it has direct bearing to cardiovascular complications of diabetes. The effect on haematological parameters is often neglected or not given much attention. Our laboratory has

investigated and reported that *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves improved the blood glucose and lipid profile of diabetic rats (Nwankwo *et al*., 2017). The antinutrients and phytochemical contents as well as the effects of the vegetables on the haemotological parameters need to be investigated. Chronic diseases like diabetes mellitus affect the blood cells severely due to damages mediated by free radicals which result in disruption of many blood components (Ayinla *et al*.,2015). Blood is a vital fluid which contains the red blood cells (RBCs), white blood cells (WBCs) and platelets suspended in the serum in homeostatic concentrations. The circulatory blood volume makes up about 8% of the weight of an average man. In diabetics, reactive oxygen species have been implicated in the mechanism of damage of blood cells (Angelousi & Lager, 2015), therefore it is necessary to source cheaper sources of antioxidants as a means of ameliorating the destructive effects of the reactive oxygen species in the diabetics. Hematological parameters are often important in routine medical evaluations. Analysis of blood parameters is relevant to risk evaluation of alterations of the haematological system in humans (Angelousi & Lager, 2015). Patients with diabetes often show a significant derangement in various haematological parameters (Akah *et al.,* 2009). In fact, several haematological changes affecting the red blood cells (RBC), white blood cell (WBC), platelet and coagulation factors are shown to be directly associated with diabetes mellitus (Akah *et al.,* 2009). Green leafy vegetables such as *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora leaves* abundant in Adamawa state of Nigeria contain valuable antioxidants and protectants (Nwankwo *et al.,* 2015), These vegetables may be cheaper and safer means of managing haematological abnormalities associated with the pathophysiology of diabetes mellitus. It is also necessary to compare their level of efficacy. As a result of the inadequate information on the comparative efficacy of these green leafy vegetables which are abundant in the state in relation to their contribution to the haemotology of diabetic subjects, this study sought to investigate the effects of the vegetables on some haemotological parameters of diabetic rats. There have been some renewed interests in hematological parameters as predictors of micro vascular complications of diabetes. However, some local vegetables have not been reasonably studied and some vegetables may be more effective than others in the management of haemotological parameters of diabetics. There is still need to investigate the level of efficacy of some common indigenous vegetables of Adamawa state, Nigeria in managing hematological parameters of diabetics in other to incorporate them in the dietary management of diabetics in the state. The present study investigated the phytochemical and antinutrient compositions of some common indigenous leafy vegetable extracts (*Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora)* of Adamawa state, Nigeria and the effect of their leaves extracts on the hematological parameters of alloxan - induced diabetic rats.

Statement of the Problem

Diabetes mellitus is one of the chronic non communicable diseases that have assumed epidemic proportion in most part of the world. It fast becoming the most popular non-communicable disease

(NCD) globally (Ayinla *et al*.,2015). It is a major source of morbidity and mortality in both developing and developed world (Fardoun, 2007). Currently, there are over 150 million diabetic patients worldwide. The number is likely to increase to 300 million or more by the year 2025 (Rajikeran *et al*., 2011). The International Diabetes Federation (IDF) (2013) reported that diabetes is no longer a disease of the poor as four out of five people (80%) that have diabetes in the world live in low- and middle-income countries. A country-by-country summary table by IDF 2012 showed that 3,165.31 million Nigerians between the ages of 20 and 79 years have diabetes, while 2,532.25million Nigerians living with the conditions are unaware and undiagnosed. Nigeria lost 88.681million persons in 2012 due to diabetes related illnesses and has a 4.83% comparative prevalence according to World Health Organization (WHO) standard (IDF,2012). As the global burden of diabetes accelerates the call to address the world wide care of diabetics intensifies daily (Ayinla *et al*.,2015).

There are several drugs for the management of diabetes. However, the drugs have prominent side effects and most often out of reach for most diabetics. No modern medicine has reached the satisfactory level in the management of diabetes. The next option is dietary management using foods that are locally available with antidiabetic effect. There has been increasing demand for the use of natural plant products with antidiabetic activity (Fuentes, Sagua, Morale & Bongue, 2005). This is because of their wide biological activities, high safety margins and low costs (Fuentes *et al*., 2005). Use of plant products to treat diabetes mellitus is of growing interest as most plant foods contain many bioactive substances with therapeutic potentials. Many leafy vegetables and their extracts are effective in the treatment of many non-communicable diseases (NCDs) (Fuentes *et al*., 2005; Nnam *et* al., 2012).

Scientific investigations of the phytochemical composition and antinutrient contents of of some indigenous vegetables of Adamawa state Nigeria -*Hibiscus cannabinus* , *Adansonia digitata*, *Sesamum indicum* and *Cassia tora* leaves has not been adequately done; and the effect of their leaves extract on the haemotological parameters of diabetics not yet studied. The study would provide evidence-based information on the effect of the leaves extracts on the haematological parameters in diabetes management.

LITRATURE REVIEW/ EMPIRICAL REVIEW

Vegetables in the diet have many positive effects upon health because of their constituents. Some vegetables have medicinal prosperities and can be used for the sick and convalescences (Kubmarawa, 2009; Nnam *et al*.,2012). Vegetables are naturally low in fat and calories. None have cholesterol; many are good sources of fibre, minerals and vitamins (lloveindia, 2004). Vegetables equally contain carbohydrates and protein. Vegetables may be eaten raw, cooked, fresh, frozen, canned or dried/dehydrated. Vegetables are nutrient dense; this means that for the small number of calories they contain; the level of nutrients is high. Vegetables are usually high in fibre and researches have shown that eating foods more in fibre lower blood sugar levels. In general, high fibre foods take longer to digest and therefore produce a slower rise in blood glucose levels (Balch & Balch, 1998).

Phytochemicals are group of chemicals found only in plant-based foods in very small amount (Nnam, 2011). They perform numerous preventive and healing functions within the body

(Pamplona-roger, 2005). Grover *et al*. (2002) identified that herbs play important role in diabetic therapy, particularly in developing countries where most people have limited resources and do not have access to modern medical treatment. WHO has also authenticated the use of herbal remedies for treatment of diabetes (WHO, 1980). Eboh (2006) reported that some African indigenous vegetables showed antihyperglycemia activity as antidiabetic agents. Fardou (2007) reported that a well-balanced antioxidant defense system through the use of dietary phytochemicals in vegetables is vital for proper prevention against diabetic damages. Flavonoids, tannins and Terpenes found in vegetables have been found to protect erythrocytes from oxidative stress and damage (Ayinla *et al*.,2015). Salehzadeh *et al.* (2020) suggested that traditional methods of food preparation could reduce certain anti nutrients and increase the nutritive value of vegetables.

Research Gaps

Diabetic conditions among other complications affects blood glucose, lipid profile and haematological parameters of the affected individuals. Some researchers have reported that some vegetable extracts improve blood glucose and lipid profile of diabetic rats (Nwankwo *et al*.,2017; Onyechi, *et al.,* 2018). Lipid profile are often given much attention probably because it has direct bearing to cardiovascular complications of diabetes. The effect on haematological parameters are often neglected or not given much attention. Our laboratory has investigated and reported that *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves improved the blood glucose and lipid profile of diabetic rats (Nwankwo *et al*., 2017). The antinutrients and phytochemical contents as well as the effects of the vegetables on the haemotological parameters of diabetic rats need to be investigated.

MATERIALS AND METHODS

Research design

The research design was experimental divided into two phases. The first phase involved the preparation of the leaves extract and determination of the LD_{50} of the vegetable extracts. The safest dose was used in graded doses in the second phase to determination the effect of the vegetable extracts on the haematological parameters of alloxan induced diabetic rats.

Procurement of materials

Two kilogramme each of the vegetables (*Hibiscus cannabinus* (*rama*), *Adansonia digitata* (*baobab*), *Sesamum indicum* (*karkarshi)*, and *Cassia tora* (*tabsa*) was bought fresh from Mubi daily market, Adamawa State Nigeria. The vegetables were identified at the Department of Agricultural Technology Federal Polytechnic Mubi, Adamawa state. Rat chow was bought from rodent diet retailers at Mubi town Adamawa state, Nigeria.

Preparation of the vegetables

The vegetables (2kg) each were sorted by removing extraneous materials and washed with deionized water. The leaves were shade dried at room temperature for two weeks. The dried vegetables were pulverized using gallenkamp mixer Kenwood –MPR 201 and used for leaves extract production. A part of the extract was used for chemical analysis and the rest for feeding the rats.

Preparation of vegetable extracts.

Preparation of the vegetable extracts was done using the standard procedure as described by Harborne (1998). Five hundred grammes each of the pulverized vegetables was soaked in the extracting solvent (methanol) in the ratio of 200ml solvent to 10 g of vegetable and stirred (with the aid of a magnetic stirrer) for one hour, then stored in the dark for 48 hours after which the mixtures were first filtered with muslin cloth and again with cotton wool in a funnel. The filtrates were concentrated with the aid of rotary evaporator, dried under vacuum, cocked in a glass tube and kept as the crude methanol extract of each vegetable. The desired consistency for feeding of the rats was derived by adding water to the crude extract in a ratio of 1gm:10ml (weight/volume). This provided 100mg / ml of each extract vegetable. The daily ration of each rat was calculated as

$$\textbf{Weight of rat} \times \frac{\textbf{dose}}{\textbf{Concentration}} \times \textbf{1000} = \textbf{daily ration}.$$

Toxicity level of extracts

Methanol extracts of the leaves were studied for Acute oral toxicity as per revised Organization for Economic Cooperation and Development (OECD) guidelines No. 423 for most commonly consumed vegetables (OECD 2001; Lorke,1983). The vegetable extracts were devoid of any toxicity in rats when given in doses up to 2000mg/kg b.w. by oral route. Hence for this study 500 and 1000mg / kg b.w. doses of the extracts were used.

Chemical analysis.

Antinutrients determinations

Oxalates

Oxalates was determined by AOAC (2005). About four (4g) grams of the sample was extracted with 6N, HCL. The oxalates in this extract were precipitated with $CuCl_2$ and calcium salts. The precipitated oxalates were washed with 25% H_2SO_4 and dissolved in hot water before titrating against 0.05N $KMNO_4$ (1ml of 0.05N $KMNO_4$) 2.2mg.

Phytates

Phytates was determined by photometric method adapted from the method of Latta and Eskin (1980). Five grammes (5kg) of each sample were extracted with 2.46 HCL. One tenth sodium chloride (0.1m NaCl) was added to elude inorganic phosphorus and seven tenths mol of sodium chloride was added to elude phytate. One millimeter (1ml) of Wade reagent was added and read at 500nm in a spectrophotometer.

Tannins

Tannins were determined by the modified Vanillin -HCL method as described by AOAC (2010). About 0.59g of each sample was extracted with 10ml of deionized water. The filtrate was mixed with 50ml water. Colour was developed with 3ml of 0.1m ferric chloride in 0.1N hydrochloric acid, followed by 3ml of 0.008m potassium ferrocyanate. Absorbance was read at 520nm in spectrophotometer within ten minutes of preparation. Tannins content was read at 520nm in

spectrophotometer within ten minutes of preparation. Tannins content was extrapolated from previously prepared tannic acid standard curve.

Toxicants determinations Cadmium and lead

Lead and cadmium were determined by the method of determination of lead and cadmium in vegetables by stripping chronopotentiometry as described by Lococo (2004). 20g of the samples were digested with concentrated sulphuric acid and dry ashed at high temperature with sulphuric acid as ashing aid. Metal ions were concentrated as their amalgams on a glass by carbon working electrode previously coated with a thin mercury film and then stripped by a suitable oxidant. Potential and time dials were digitally derived. Method of standard addition coefficient was determined as n-4, 0.998 for cadmium and n-4, and 0.993 for lead. Accuracy was matched with a reference sample.

Phytochemicals determination

Saponins

Saponins were determined by the method of Obadoni and Ochuko (2001). The samples were ground and 20g of each were put into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath at about 90^{0}C. The concentrate was transferred into a 250ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n – butanol was added. The combined n – butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavoniod determination

The flavoniod contents were determined by the method described by Bohn and Kocipai- Abyazan (1994). Ten grammes (10g) of each sample were extracted repeatedly with 100ml of 80% aqueous methanol at room temperature; the whole solution was filtered through Whatman filter paper N0 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a bath and weighed to a constant weight.

Calculation

Weight of empty beaker = WI

Weight of empty beaker + sample after drying = W2

Weight of empty residue = W_2-W_1

$$\text{Flavoniod} = \frac{W_2-W_1}{W_1} \times 100$$

Alkaloids

This was determined using Harborne (1998) method. About 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand

for 4 h. This was filtered and the extract was concentrated on a water bath to one –quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole situation was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Terpenoids

100mg (wi) of each extract was taken and soaked in 9ml of ethanol for 24 hours. The extract after filtration was extracted with 10ml of petroleum ether using separating funnel. The ether extract was separated in pre- weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula wi - wf / wi x 100).

Glycosides

Estimation of cyanogenic glycoside content of the samples was accomplished by determining the HCN (Hydrogen cyanide) released on hydrolysis. Extracts of each sample were obtained by homogenizing 30 g of sample in 250ml of 0.1m orthophosphoric acid for 5 minutes and clear supernant was taken. An aliquot of the supernant was used for estimation of hydrogen cyanide using an auto analyzer Technicon AA11, according to the method of Rao and Sung (1995).

Animal studies

Sourcing of animals and housing

Fifty-five male adult albino rats (150 - 200g) two months old were purchased from the Department of Biochemistry Adamawa state University Mubi. The rats were randomly allotted to eleven groups (one untreated diabetic group, one diabetic group treated with standard antidiabetic drug; eight diabetic groups treated with graded doses of vegetable extracts, and one normal group as control) of five rats each. The average body weight of each group did not differ by more than 5 grammes (5gm). The rats were housed in individual stainless - steel metabolic cages equipped to separate faeces and urine of the animals during the 23 - day study period in the Department of Nutrition and Dietetics, Federal Polytechnic Mubi, metabolic house**.** The study was carried out for 23 days consisting of 7 days acclimatization, 2 days of inducement and establishment of diabetes and 14 days on experimental diet.

Induction of Diabetes

Induction of Diabetes in the rats was done by intra - peritonealy injecting 5% freshly prepared aqueous solution of alloxan monohydrate to the rats at a single dose of 150mg / kg body weight of animal. The induced rats were allowed free access to feed and water as well as 5% glucose solution to avoid possible effect of hypoglycemia for 48hrs. After 48hrs of induction of diabetes (9TH day), blood was taken from each rat to confirm diabetes using an Accu- Chek glucometer. Rats with fasting blood sugar level ≥ 200mg / dl were considered diabetic and included in the study (Eze *et al.,* 2011; Yakubu *et al*.,2010; Meral *et al*., 2004).

Composition of Experimental diets and grouping of animals

Table 1: Diet Composition and Experimental Design

Diet groups	Diet Compositions	Dose of extracts or Drug (mg)	Number of Days	Number of Rats
G1D1**	RC		14	5
G2D2**	RCSD	0.5 (drug)	14	5
G3D3**	RCHB	500	14	5
G4D4**	RCAD	500	14	5
G5D5**	RCSI	500	14	5
G6D6**	RCCT	500	14	5
G7D7**	RCHB	1000	14	5
G8D8**	RCAD	1000	14	5
G9D9**	RCSI	1000	14	5
G10D10**	RCCT	1000	14	5
G11D11*	RC	-	14	5

G11D11 Group1 Diet1 to Group11 Diet 11

RC _ Rat chow

RCSD - Rat chow and standard drug

RCHB - Rat chow and *Hibiscus cannabinus* leaf extract

RCAD - Rat chow and *Adansonia digitata* leaf extract

RCSI – Rat chow and *Sesamum indicum* leaf extract

RCCT - Rat chow and *Cassia tora* leaf extract

* - control group

** - Experimental group

G11D 11 – normal rat + rat chow only

G1D1 - diabetic + rat chow only

G2D2 - diabetic + rat chow and 0.5mg standard drug

G3D3 - diabetic +rat chow and 500mg *Hibiscus cannabinus* leaf extract

G4D4 - diabetic +rat chow and 500mg *Adansonia digitata* leaf extract

G5D 5 – diabetic +rat chow and 500mg *Sesemum indicum* leaf extract

G6D6 - diabetic +rat chow and 500mg *Cassia tora* leaf extract

G7D 7 – diabetic + rat chow and 1000mg *Hibiscus cannabinus* leaf extract

G8D 8 – diabetic +rat chow and 1000mg *Adansonia digitata* leaf extract

G9D 9 – diabetic +rat chow and 1000mg *Sesemum indicum* leaf extract

G10D 10 – diabetic + rat chow and 1000g *Cassia tora* leaf extract

Feeding of the rats

Rat chow and water was given to the rats *ad libitum* throughout the period of the experiment. The vegetable extracts were given orally with an intubation tube to the rats in group 3 -10 every morning for the fourteen days daily. Rats in Group 1 were diabetics and fed rat chow only (negative control). Rats in Group 2 were diabetics and fed rat chow and 0.5mg of standard drug glibenclamide (standard drug control group). Rats in Groups 3-6 were diabetics and fed rat chow and 500mg / kg b.w. of each of the vegetable extract respectively. Rats in Groups 7-10 were diabetics and fed rat chow and 1000mg / kg b.w. of each of the same vegetable extract respectively. Rats in Group 11 were normal rats and fed with rat chow only (normal control) (Table1).

Blood sample collection.

Blood samples were collected on days 7th and 9th from the tail – end of the rats for blood glucose determination after an overnight fast. The samples were collected on reagent pad of the strip and then inserted into the glucometer. Blood glucose was tested on day 7 to confirm that the rats were not diabetic and on day 9 which served as base line information that the rats were diabetic. Blood for haemotological estimations were collected into heparinized tubes containing EDTA on day 23 after sacrificing the rats by decapitation and were immediately used for determination of haemotological parameters.

Biochemical indices determination

Fasting blood sugar determination

The fasting blood sugar level was determined on day 7th, 9th by conducting a tail- tip cut of the rats, a drop of capillary blood was obtained and the blood was allowed to cover the reagent pad of the strip which was then inserted into the glucometer. Diabetes was confirmed by glucose oxidase method using the one touch ultra-glucometer. The blood glucose level was read in mg / dl on the glucometer. Rats with fasting blood sugar level ≥ 200mg / dl were considered diabetic and included in the study (Eze *et al.*, 2011; Yakubu *et al*.,2010; Meral *et al*., 2004).

Determination of Haemotological (FBC) parameters

Full blood count comprising total red blood cell count (RBC), white blood cell count (WBC), percentage packed cell volume (PCV) and blood haemoglobin (HB)concentration indices were determined from the whole blood that was placed in EDTA test tubes using ABX Micros 60 Haemotolgy Analyzer (Horiba –ABX Montpellier, France). Thin blood film was prepared and stained using Fleishman stain for morphologic assessment of the red blood cells . The stained films were examined under the light microscope using x 40 objectives to select a good area for examination and a drop of oil placed on the film and examined with the x 100 objective.

Statistical analysis

All data collected were entered into the computer and the analysis ran with Statistical Package for Scientists and Engineers (SPSE,2012), analytical software manual version 9.1, USA. Means and standard deviation of phytochemical and antinutrient contents and standard error of the means of haematological parameters were calculated for the samples. One-way analysis of variance and All-pairwise comparism test was used to compare differences between means. The differences in means were considered significant at $p < 0.05$.

DATA ANALYSIS AND PRESENTATION

Antinutrient content of the vegetables

Table 2 presents the antinutrient and toxicant contents of methanol leaves extract of *Hibiscus cannabinus, Adonsonia, digitata, Sesamun indicum and Cassia tora* leaves. *Hibiscus cannabinus* leaf extracts contained oxalates 0.01mg, 5.10mg tannins, 0.66mg phytate, 0.34mg hydrocyanides, 0.02mg *cadimium* and 0.02mg lead. *Adansonia digitata* had 0.02mg oxalates, 4.57mg tannins, 1.75mg phytate, 0.22mg hydrocyanides, 0.03mg cadmium and 0.21mg lead. *Sesamum indicum*

leaves extracts had 0.01mg oxalates,6.96mg tannins, 1.21mg phytate, 0.45mg hydrocyanides, 0.01mg cadmium and 0.14m lead. *Cassia tora leaves extracts* had 0.01mg oxalates ,7.07mg tannins, 1.78mg phytate, 0.48mg hydrocyanides, 0.03 cadmium and 0.21mg lead.

Table 2 Anti nutrient and food toxicant contents of methanol extract of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves.

Antinutrient & Toxicant (mg/100g)	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamum indicum***	***Cassia tora***
Oxalate	0.01±0.00a	0.02±0.00a	0.01±0.00a	0.01 ± 0.00^{a}
Tannin	5.10 ± 0.02^{b}	4.57 ± 0.46^{bc}	6.96 ± 0.14^{ab}	7.07 ± 0.87^{a}
Phytate	0.66 ± 0.06^{c}	1.75 ± 0.05^{a}	1.21 ± 0.05^{b}	1.78 ± 0.10^{a}
Hydrocyanides	0.34 ± 0.03^{b}	0.22 ± 0.03^{bc}	0.45 ± 0.07^{a}	0.48 ± 0.03^{a}
Cadmium	0.02 ± 0.00^{ab}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.03 ± 0.01^{a}
Lead	0.02 ± 0.01^{b}	0.21 ± 0.01^{a}	0.14 ± 0.00^{ab}	0.21 ± 0.00^{a}

Mean ±SD, n=3, values on the same rows with different superscripts are significantly different ($p< 0.05$)

Phytochemical contents of the vegetables

Table 3 presents the phytochemical composition of methanol extract of *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves. *Hibiscus cannabinus* leaf extract contained 3.26mg saponins, 0.09mg flavonoid, 4.91mg alkaloids, 2.70mg glycosides, 1.09mg terpenes and 1.36mg phytosterols. *Adansonia digitata* had 2.05mg saponins, 0.14mg flavonoids, 5.22mg alkaloid, 2.40mg glycosides, 1.23mg terpenes and 2.39mg phytosterols. *Sesamum indicum* had 3.73mg saponins, 0.23mg flavonoids, 6.45mg alkaloids, 3.76mg glycosides, 1.43mg terpenes and 1.26mg phytosterols. *Cassia tora* contained 2.40mg saponins, 0.29mg flavonoids, 6.77mg alkaloids, 3.84mg glycosides, 2.30mg terpenes and 2.50mg phytosterols.

Table 3 Phytochemcial contents of methanol extracts of Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora leaves.

Phytochemicals (mg/100g)	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamum indicum***	***Cassia tora***
Saponins	3.26 ± 0.12^{b}	2.05 ± 0.17^{d}	3.73 ± 0.39^{a}	2.40 ± 0.08^{c}
Flavonoids	0.09 ± 0.01^{d}	0.14 ± 0.02^{c}	0.23 ± 0.03^{b}	0.29 ± 0.02^{a}
Alkaloids	4.91 ± 0.11^{cd}	5.22 ± 0.27^{c}	6.45 ± 0.05^{b}	6.77 ± 0.28^{a}
Glycosides	2.70 ± 0.20^{b}	2.40 ± 0.53^{c}	3.76 ± 0.09^{ab}	3.84 ± 0.16^{a}
Terpenes	1.09 ± 0.01^{d}	1.23 ± 0.02^{c}	1.43 ± 0.04^{b}	2.30 ± 0.06^{a}

Phytosterols	1.36 ± 0.04^{c}	2.39 ± 0.01^{b}	1	.26±0.04^{d}	2.50±0.46^{a}

Mean ±SD, n=3, values on the same rows with different superscripts are significantly different (p< 0.05).

Haematological parameters of the rats

Table 4 presents the haematological parameters of the aloxan induced diabetic treated rats and normal rats.

Groups	**PCV %**	**Hb g/dl**	**WBC x 10^3/µg**	**RBC x10^6 /µg**
G1D1	19.25^{e}	6.55^{f}	8600.00^{abc}	222.50^{ab}
G2D2	26.25^{b}	9.52b	8450.00^{abc}	193.75^{bc}
G3D3	24.50^{bc}	9.10^{bc}	9200.00^{ab}	202.50^{abc}
G4D4	22.50^{cde}	8.08^{cde}	7500.00^{bcd}	156.25^{cd}
G5D5	20.50^{de}	6.98^{ef}	8000.00^{abcd}	175.00^{bcd}
G6D6	23.50^{bcd}	7.10^{cde}	8000.00^{abcd}	181.25^{bc}
G7D7	20.75^{cde}	7.06^{ef}	6600.00^{d}	120.00^{d}
G8D8	24.00^{bcd}	8.59^{bcd}	7450.00^{bcd}	170.00^{bcd}
G9D9	22.00^{cde}	7.48^{def}	8000.00^{abcd}	190.00^{bc}
G10D10	21.25^{cde}	8.16b^{cde}	7300.00^{cd}	186.00^{bcd}
G11D11	39.00^{a}	13.26^{a}	9650.00^{a}	256.25^{a}
LOS	***	***	NS	*
±SEM	1.83	0.68	879.48	29.99

a,b,c,d,e,f Mean in the same column with different superscripts are significantly (p< 0.05) different. LOS- level of significance, ±SEM - standard error of the mean, *** = very highly significant, * = significant, NS = not significant

Hb - haemoglobin count
PCV - pack cell volume
WBC- white blood cell count
RBC- red blood cell count

G1D1 - diabetic + rat chow only
G2D2 - diabetic + rat chow and 0.5mg standard drug
G3D3 - diabetic +rat chow and 500mg *Hibiscus cannabinus* leaf extract
G4D4 - diabetic +rat chow and 500mg *Adansonia digitata* leaf extract
G5D 5 – diabetic +rat chow and 500mg *Sesemum indicum* leaf extract
G6D6 - diabetic +rat chow and 500mg *Cassia tora* leaf extract
G7D 7 – diabetic + rat chow and 1000mg *Hibiscus cannabinus* leaf extract
G8D 8 – diabetic +rat chow and 1000mg *Adansonia digitataleaf* extract
G9D 9 – diabetic +rat chow and 1000mg *Sesemum indicum* leaf extract
G10D 10 – diabetic + rat chow and 1000g *Cassia tora* leaf extract
G11D 11 – normal rat + rat chow only

Table 5 shows the Pearson correlations among haematological parameters of the rats. The PCV and Hb (γ = 0.91) were highly positively correlated (p<0.001). The PCV and WBC (γ = 0.48*)

and RBC ($\gamma = 0.47^{*}$) are slightly positively correlated ($p < 0.01$). The Hb and WBC ($\gamma = 0.44^{*}$) are slightly positively correlated ($p < 0.01$) but there is no correlation between the Hb and the RBC ($\gamma = 0.36^{NS}$) ($p > 0.05$).

Table 5: Shows the Pearson correlations among haematological parameters of the rats.

Parameters	PCV	Hb	WBC	RBC
PCV	0.00			
Hb	0.91***	0.00		
WBC	0.48*	0.44*	0.00	
RBC	0.47*	0.36 NS	0.58*	0.00

*** = highly significant $p < 001$
* = significant $p < 0.05$
NS = Not Significant

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

Discussions

Phytochemicals contents of the vegetable leaves extract

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Oguntona, 1986; Nnam, 2011). They are found generally in plants (Phytochemicals info). The results of this study showed that the phytochemicals (Table 4) were generally present in the vegetables in small quantities. However, in another study in our laboratory, the values of the phytochemicals present in the methanol extracts of *Hibiscus Cannabinus*, *Adansonia Digitata*, *Sesamum Indicum*, *Cassia Tora* Leaves (Saponins 2.05 - 3.73, flavonoids 0.09-0.29, alkaloids 4.91 - 6.77, glycosides 2.40-3.84, terpenes 1.09-2.30, phytosterols 1.26 - 2.50) per 100g were higher than the values in the fresh leaves (Saponins 0.06 - 0.12, flavonoids 0.01-0.04, alkaloids 0.03-0.21, glycosides 0.01-0.02, terpenes 0.09-0.21, phytosterols 0.09-0.16) per 100g by Nwankwo (2014). This might be due to the extraction process (Tijani,Aluyu and Balogun,2009). Saponins contents of *Adansonia digitata* (0.12mg/100g) and *Cassia tora* (0.12mg/100g) leaves in this study was comparable to saponin value of *Vernonia amagdalina* (0.13mg/100g) reported by Nnam *et al*. (2012). The methanol extracts of the leaves had higher levels of phytosterols (1.36mg, 2.39mg, 1.26mg, and 2.50mg / 100g) than the fresh leaves (0.09mg, 0.12mg, 0.08mg, and 0.16mg/100g) by Nwankwo (2014). The low levels of saponins and phytosterols in the fresh vegetables have the potential to lower cholesterol levels in humans due to their hypercholesteralmic effect (Nnam, 2011). Phytosterols are plant counterparts of cholesterol and thus inhibit its absorption. It lowers cholesterol level by blocking the uptake of cholesterol. The cholesterol is thus excreted from the body. This help to prevent heart diseases often associated with diabetes. Saponins form complexes with cholesterol to reduce plasma cholesterol levels. Whitney and Rolfes (2005) reported that tannins and saponins have the capability to lower serum cholesterol and also fight cancer when their concentrations are low in the body. The low levels of saponins in the vegetables are of interest and will boast the phytochemical properties of the

vegetables which will also impact on the haematological parameters. However, saponins are bitter and could reduce the palatability of food when present in large amounts. Simple food preparation methods could reduce the bitterness in the vegetables when consumed by simple method of preparation.

The flavonoid levels of the vegetable extracts (0.09mg, 0.14mg, 0.23mg, 0.29mg/100g) of *Hibiscus Cannabinus*, *Adansonia Digitata*, *Sesamum* Indicum, *Cassia* tora leaves were comparable to those of *Vernonia amagdalina* (0.04mg/100g), *0cimum gratissimum* (0.08mg/100g), *Gongronoma latifolium* (0.14mg/100g) by Nnam et al. (2012). The presence of flavonoids in the vegetables especially the levels in *Cassia tora* leaf extract (0.29mg/100g) are desirable. Flavonoids have antioxidant properties to protect the body against cardiovascular diseases and some forms of cancer (Nnam, 2011). They protect the cells against the breakdown of arachidonic acid, an unsaturated fatty acid that keeps cell membrane healthy and permeable. Flavonoids lower high blood pressures as well as cholesterol in animal studies and have strong anti-inflammatory properties (Nwankwo et al., 2017). They also inhibit low density lipoprotein (LDL) oxidation by free radicals (Verena, Mario and Karl, 2006). Based on these reports' consumption of the vegetables as constituents of human diet will be of health benefits. The alkaloid levels of the leaves extract of *Hibiscus Cannabinus*, *Adansonia Digitata*, *Sesamum indicum*, *Cassia tora* Leaves (4.91mg, 5.52mg, 6.45mg, and 6.77mg/100g) respectively were much higher than the values in the fresh leaves (0.12mg, 0.21mg, 0.03mg, and 0.15mg/100g) respectively reported by Nwankwo (2014). The high values may be due to the process and solvent used for the extraction. Flavonoids, tannins and Terpenes found in these vegetables have been found to protect erythrocytes from oxidative stress and damage (Ayinla *et al*.,2015). Antinutrient and toxicant contents of methanol extracts of Hibiscus cannabinus, Adonsonia digitata, Sesamun indicum and Cassia tora leaves.

The traces of oxalate observed in the vegetables could be that the plant constituents were not yet fully established (Bamishaiye *et al.*, 2011) at the time this study was carried out which is an advantage since oxalates if consumed in large amounts may be harmful to human health (Noonan & Savage, 2009). The phytates levels of the vegetables were below the safe limit (5.00mg/100g) (Aina et al., 2012). The low levels of phytate and traces of oxalates in the vegetable extracts are of interest because studies have shown that at lower doses these antinutrients show photochemical beneficial effects (Nnam *et al*.,2011). Phytates and oxalates at lower doses act as beneficial antioxidants. Phytic acid and oxalic acids in large amounts usually form insoluble salts with mineral elements such as zinc, calcium and iron to prevent their availability and utilization (Sariyan *et* al., 2010; Nnam and Onyeke, 2003). However, the low levels of phytate and oxalate present in these vegetable extracts would help to emeriolate the destructive effect of diabetes on the aloxan-induced diabetic rats as they would offer antioxidant photochemical effects. The *Cassia tora* leaf extract contained higher levels of most of the antinutrients and phytochemicals than Hibiscus *cannabinus leaf extract* studied but fail to impact positively higher on the haematological parameters of the diabetic rats. This might be because these phytochemicals and antinutrients are known to act much better in lower doses and so they synergistically act much better in the group that consumed *Hibiscus cannabinus* leaf extract that had lower levels of the phytochemicals and antinutrients (Table 2 and 3).

The tannin levels were higher than the safe level of tannins (0.15 - 0.20%) as recommended by Schiavono *et al*. (2007). The extraction process increased the tannin levels of the extracts in this study. However, Salehzadeh (2020) suggested that traditional methods of food preparation could reduce certain anti nutrients and increase the nutritive value of vegetables. In line with this, proper methods of food preparation could reduce the tannin levels and boost the phytochemical properties of the vegetables when consumed.Willy (2003) observed that tannins are antinutrients with antioxidant effects. They were traditionally considered antinutritional but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage. They act as cataion agents, preventing availability of certain nutrients and as well act as beneficial antioxidants. At lower levels they act as beneficial antioxidants while at higher levels they act as cataion agents, preventing availability of certain nutrients (Nnam *et al.*, 2012). Tannins form complexes with proteins, carbohydrates and certain metal ions (Nnam and Onyeke, 2003). The tannin - protein, tannic acid – starch and tannin metal complexes are resistant to enzyme hydrolysis, thus inhibiting the digestibility and absorption of nutrients (Nnam and Onyeka, 2003; Nnam *et al.*, 2012). The vegetables that had higher levels of tannins did not impact better on the haemotological parameters of the rats probably because of the tannin complexes that inhibited absorption of nutrients from the vegetables (Table 5) (Whitney and Rolfes, 2005).

The low levels of hydrocyanides in the vegetable's extracts (0,22mg – 0.48mg/100g) were below the safe levels (35.00mg/100g) by WHO, large quantities may prove toxic. Hydrocyanins produced from cyanogenic glycosides when consumed in large quantity over long periods may become toxic. Simple food preparation methods would remove the hydrocynins and leave the vegetables safe for human consumption. Cadmium and lead levels of the vegetable's extracts were within the safe levels (0.3 and 0.20/kg) allowed by WHO standard for food substances (SAFS). Cadmium and lead are inorganic metals that are naturally present in the environment. Excess of Cadmium and lead in the body course diseases including hearth diseases. The low values obtained in the study were similar to the report of Sobukola et al. (2010) that fruits and vegetables collected from production and market sites in Nigeria contained measured heavy metals contents within the safe limits prescribed by WHO/FAO. Bokenga (1994) reported that leafy vegetables when processed and cooked are often free of food toxicants. This suggests that the small quantity of the metals in the vegetables in the study would be removed during preparation of the vegetables for food.

Phytochemical contents of the vegetable leaves extract

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Oguntona, 1986; Nnam, 2011). They are found generally in plants (Phytochemicals info). The results of this study showed that the phytochemicals (Table 4) were generally present in the vegetables in small quantities. However, in another study in our laboratory, the values of the phytochemicals present in the methanol extracts of the leaves (Saponins 2.05 - 3.73, flavonoids 0.09-0.29, alkaloids 4.91 - 6.77, glycosides 2.40-3.84, terpenes 1.09-2.30, phytosterols 1.26 - 2.50) per 100g were higher than the values in the fresh leaves (Saponins 0.06-0.12, flavonoids 0.01-0.04, alkaloids 0.03-0.21, glycosides 0.01-0.02, terpenes 0.09-0.21, phytosterols 0.09-0.16) per 100g by Nwankwo *et al.* (2015). This might be due to the extraction process (Tijani,Aluyu and Balogun,2009). Saponins contents of *Adansonia digitata* (0.12mg/100g) and *Cassia tora*

(0.12mg/100g) leaves in this study was comparable to saponin value of *Vernonia amagdalina* (0.13mg/100g) reported by Nnam *et al*. (2012). The methanol extracts of the leaves had higher levels of phytosterols (1.36mg, 2.39mg, 1.26mg, and 2.50mg/100g) than the fresh leaves (0.09mg, 0.12mg, 0.08mg, and 0.16mg/100g) by Nwankwo et al. (2011). The low levels of saponins and phytosterols in the fresh vegetables have the potential to lower cholesterol levels in humans due to their hypercholesteralmic effect (Nnam, 2011). Phytosterols are plant counterparts of cholesterol and thus inhibit its absorption. It lowers cholesterol level by blocking the uptake of cholesterol. The cholesterol is thus excreted from the body. This help to prevent heart diseases often associated with diabetes. Saponins form complexes with cholesterol to reduce plasma cholesterol levels. Whitney and Rolfe (2005) reported that tannins and saponins have the capability to lower serum cholesterol and also fight cancer when their concentrations are low in the body. The low levels of saponins in the vegetables are of interest and will boast the phytochemical properties of the vegetables which will also impact on the haemological parameters. However, saponins are bitter and could reduce the palatability of food when present in large amounts. Simple food preparation methods could reduce the bitterness in the vegetables when consumed by simple method of preparation.

The flavonoid levels of the vegetable extracts (0.09mg, 0.14mg, 0.23mg, 0.29mg/100g extracts) are comparable to those of *Vernonia amagdalina* (0.04mg/100g), *0cimum gratissimum* (0.08mg/100g), *Gongronoma latifolium* (0.14mg/100g) by Nnam et *al*. (2012). The presence of flavonoids in the vegetables especially the levels in *Cassia tora* leaf extract (0.29mg/100g) are desirable. Flavonoids have antioxidant properties to protect the body against cardiovascular diseases and some forms of cancer (Nnam, 2011). They protect the cells against the breakdown of arachidonic acid, an unsaturated fatty acid that keeps cell membrane healthy and permeable. Flavonoids lower high blood pressures as well as cholesterol in animal studies and have strong anti-inflammatory properties (Nnam, 2011). They also inhibit low density lipoprotein (LDL) oxidation by free radicals (Verena, Mario and Karl, 2006). Based on these reports' consumption of the vegetables as constituents of human diet will be of health benefits. The alkaloid levels of the extracts (4.91mg, 5.52mg, 6.45mg, and 6.77mg/100g) were much higher than the values in the fresh leaves (0.12mg, 0.21mg, 0.03mg, and 0.15mg/100g) respectively reported by Nwankwo (2014). The high values may be due to the process and solvent used for the extraction. Flavonoids, tannins and Terpenes found in these vegetables have been found to protect erythrocytes from oxidative stress and damage (Ayinla *et al*.,2015).

Effect of the vegetable extracts on Haematological Parameters.

Diabetic rats not treated showed significant decrease ($p< 0.05$) in levels of PCV (19.25%) and Hb (6.55%) when compared with normal rats' group (PCV 39%; Hb 13.26g/dl). However, inclusion of vegetable extracts and standard drug increased the lowered PCV and Hb of the diabetic treated groups in the range of (20.50 – 26.25% and 6.98 - 9.52g/dl) respectively. Group 3 that consumed 500mg *Hibiscus cannabinus leaf* extract increased the PCV (24.50%) and Hb (9.10%) close to the increase showed by standard drug group (26.25 and 9.52%) respectively. The increase by Group 3 was significantly ($p<0.05$) higher than other vegetable treated groups though the increase was not dose dependent because the group that consumed 1000mg *Hibiscus cannabinus* leaf extract had significantly ($p < 0.05$) lower increase in PCV and Hb (20.75% and 7.10%) respectively than

the group that consumed 500mg *Hibiscus cannabinus leaves* extract (24.50 and 9.52%). Similar results were reported by Ayinla *et al. (*2015) that in alloxan - induced diabetic rats, PCV, RBC and Hb were decreased, but application of ethanolic leaf extract of *Senna fistula* increased the parameters ($p< 0.05$) in the treated rats. The Pearson correlations among haematological parameters of the rats in this study showed that the PCV and Hb ($\gamma =0.91$) were highly positively correlated ($p<0.001$). The increase shown by *Hibiscus cannabinus leaves* extract group might be related to its better antioxidant phytochemical content.

Packed cell volume (PCV) measures the percentage by volume of packed red blood cell in a whole blood sample after centrifugation. PCV can be used as a screening tool for anaemia and can also indicate the degree of fluid loss during dehydration. The significant decrease in the level of PCV in diabetic control rats (Table 4) may be as a result of cellular damage on the erythrocyte membrane or as a result of oxidative stress (Ayinla *et al.*,2015). The decrease may also be attributed to massive water loss from the diabetic rats in the form of urine. A drop in PCV indicates internal haemorrhage before any other symptoms become apparent (Mason, 2004).The vegetable extracts help to increase the PCV values in the treated diabetic rat groups. Haemoglobin test measures the amount of Hb in g/dl of whole blood and provides an estimation of oxygen carrying capacity of the red blood cells (Merlin *et al*.,2005). The decreased PCV and Hb concentration values of the diabetic rats in this study is in line with the work of Merlin *et al.* (2005) where anaemia in diabetic rats was documented.

The WBC count values of the untreated diabetic rat group, the standard drug treated rat group and all other vegetable leaf extracts treated rat groups were significantly ($p< 0.05$) lower than the normal rat control group. However, Group3 that consumed 500mg *Hibiscus cannabinus leaf* extract increased the lowered WBC (9200.00 x 10^3 /g) count to near the value of normal control rat group (9650.00 x 10^3/µg) and the increase was significantly ($p< 0.05$) higher than the WBC count value of the standard drug group. Also, there is no significant difference($p>0.05$) in the WBC count values of the untreated diabetic rat group and the standard drug treated rat group which shows that the *Hibiscus cannabinus leaf* extract at 500mg / kg b.w. might help to increase the WBC count values of diabetics better than the standard drug. Other researchers reported higher WBC count in diabetic control rat groups as in this study except group treated with 500mg/ kg bodyweight *Hibiscus cannabinus leaf* extract (Merlin *et al*.,2005; Ayinla *et al.*, 2015). It has been revealed that higher WBC count is one of the major components of inflammatory process that leads to atherosclerotic progress and cardiovascular disease (Owolabi *et al.*, 2007). The higher positive effect of the 500mg *Hibiscus cannabinus leaf* extract on the blood indices in this study might be attributed to its high phytochemical contents and low anti nutrient contents (Table 2 and 3). Phytochemicals help to improve endogenous antioxidants mechanisms and therefore reduce the vascular complications in diabetes and other effects of hyperglycemia (Fardoun, 2007; Nwankwo, *et al*.,2017). It is worthy of note that the group of rats fed with 1000mg of *Hibiscus cannabinus leaf* extract had significantly lower value of WBC count showing that the effect is not dose dependent which might be attributed to the fact that antioxidant and some antinutrients are more effective in small doses as shown in Table 4. Generally, there is no significant ($p>0.05$) difference between the WBC values of the normal rat group and all the treatment groups, suggesting that the leaves extract and the drug might not compromise the functional capacity of

the blood neither will they expose the rats to opportunist infection (Owolabi, 2013). The Pearson correlation of the haematological parameters of the rats showed a positive correlation between the WBC and the PCV, Hb, RBC of the rats (γ = 0.48,0.44, 0.58) respectively. Total white blood count determines the body's ability to fight infection which was high in the normal rats. Low WBC count in the diabetic groups may be caused by the problems with their production or with auto-immune disease due to diabetes. There is significantly ($p< 0.05$) lower level in the RBC value of the untreated diabetic rat group than the normal rat group. Administration of the standard drug and the vegetable extracts failed to improve the reduced RBC value of the treated diabetic rats instead they reduced the RBC more than the untreated group (Table 4) suggesting that the extracts have deleterious effect on the RBC. it has been reported that ingestion of medicinal plants or drugs can negatively alter some normal haemotological values of diabetics (Ajagbonna *et al.*, 1999; Ayinla *et al*.,2015). This decrease on RBC count seems to support this report. The groups that consumed lower doses(500mg) of all the extracts had lesser destruction in RBC values than those that consumed 1000mg of the vegetable extracts showing that higher levels of the extracts are not advisable as they may be deleterious on the RBC values. The group fed 500mg of *Hibiscus cannabinus leaf* extract had higher RBC value that is significantly ($p<.0.05$) higher than the standard drug group and other vegetable extract groups, however, it had lower RBC value than the untreated group showing that the drug and the higher quantity of vegetable extracts increased the destructive effect of diabetes on the RBC more than the group fed 500mg of *Hibiscus cannabinus leaf* extract. Red blood cell count can be a factor in erythropoietin process. Reactive oxygen species have been implicated in the mechanism of damage of red blood cells in diabetic patients (Ayinla *et al*., 2011), as a result haemotological complications develop as shown in this study. The effect on RBC count might be due to the binding of glucose to some of the red blood cells during diabetic condition and this was reversed during treatment with extracts and drug.

Conclusion

The *Hibiscus cannabinus and Ca*ssia *tora* leaves extracts in this study have better arrangement of phytochemical and antinutrient contents than *Adonsonia digitata and Sesamun indicum leaves* extract. All the vegetable extracts had toxicant levels within WHO safe limits. The increase in PCV, Hb, WBC and RBC of the diabetic rats following the consumption of the vegetable extracts especially 500mg of *Hibiscus cannabinus leaf* extract may suggest the positive effects of *Hibiscus cannabinus leaf* extract on haemotological systems of the experimental rats more than the other vegetables. *Hibiscus cannabinus leaf* extract at 500mg/kg b.w. might be capable of improving the haematological problems associated with diabetes in humans. Findings from this work suggests that Hibiscus *cannabinus and Ca*ssia *tora* leaves extracts have good phytochemical contents which can impart positively on the haemotological systems of diabetics and could be incorporated in the dietary management of diabetics by the health care systems.

Recommendations

More work should be done on the methanol leaf extract of the vegetables especially *Hibiscus cannabinus* to identify the component responsible for its haematoprotective property. Human studies are also needed to confirm the results obtained in this study.

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