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DYSLIPIDEMIC AND HAEMATOLOGICAL EFFECTS OF CRUDE AND FRACTIONS OF *HENSIA CRINITA* IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

Iwara A. Iwara*, Godwin O. Igile, Godwin E. Okon and Patrick .E. Ebong

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P.M.B .1115, Calabar, Nigeria.

***Corresponding Author: Iwara A. Iwara**

Email: iwaraiwara83@gmail.com

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ABSTRACT

This study was carried out to evaluate the hypolipidaemic and haematological effects of extracts of *Hensia crinita* (H.C) in STZ-induced diabetic rats. Thirty Wistar rats weighing 120-150g rats were divided into six groups of 6 rats each. Diabetes was induced using 45mg/kg b.w of STZ in 0.1M sodium citrate buffer. Control groups were treated with 20% dimethylsulfoxide and test groups treated with glibenclamide (5mg/kg bw), 400mg/kg b.w crude H.C, 100% and 30% methanol fractions of H.C respectively. The results showed significant decrease ($p<0.05$) in concentrations of all lipid except for HDL-C concentration which showed no significant ($P>0.05$) change in diabetic rats treated with crude, 100% and 30% methanol fractions of H.C compared to the diabetic control group. Significant ($p<0.05$) changes were observed for haematological indices of all treated groups compared to diabetic and normal control groups except for RBC and Hb which showed no significant change ($P>0.05$) in all extract treated groups. The results thus suggest that administration of crude and extract fraction of H.C may contribute significantly to reduction of lipid parameters in diabetes and also could curtail haematological insults posed by diabetes and thus, useful in the management of diabetes.

Keywords: *Heinsia crinita*; Streptozotocin; Lipid profile; Haematological indices.

INTRODUCTION

Diabetes mellitus is a metabolic disorder with epidemiology attaining phenomenal frequencies. The most common forms of the disease are either due to a diminished production of insulin by the pancreas or insensitivity of the body's cellular receptors to insulin produced. It is a devastating disease that can lead to morbidity and mortality [1]. One major challenge in its management is maintaining blood glucose at near normal level with almost no episode of major fluctuation. A major metabolic derangement is that of lipid metabolism in both clinical and experimental diabetes [2]. The permanent cure for the disease has not yet been established due to its mechanistic complexity

and thus has so far been managed using various hypoglycaemic agents, most of which poses severe side effects such as exacerbating hyperinsulinaemia and causing weight gain in patients [3].

Heinsia Crinita is a plant belonging to the family Rubiaceae[4]. The common name is Bush apple [5]. *H. Crinita* is indigenous to West Africa especially Southern Nigeria but is now cultivated in Central Africa, South of Sahara desert and Francophone Africa [6]. The plant is classified as white and dark varieties with the two varieties different from each other only in terms of their taste [7]. Phytochemistry of the leaves of the plant reveals the presence of saponins, tannins, cardiac glycosides and alkaloids with the dark variety having a greater concentration of alkaloids and the white variety, a greater concentration of saponins [5].

Ingestion of a drug or medicinal plant can however alter the normal haematological values as such; assessment of haematological parameters could be useful in assessing the deleterious effects of drugs or medicinal plants [8]. Although phytochemistry of the plant has been established, evaluating the hypolipidemic effects of the crude and extract fractions of *H. Crinita* in STZ-induced diabetic rats, as well as assessing the haematological changes following administration of the plant extract is the concern of this study.

MATERIALS AND METHOD

Plant material

Mature leaves of *H. Crinita* were bought from Goldie market, Calabar South, Cross River State. The leaves were identified and authenticated by a botanist Dr Michael Eko of the Department of Botany, University of Calabar, Calabar, Nigeria. The specimen and their vouchers (EUDB S01/13) deposited in the department's herbarium.

Preparation of extract for animal administration

The leaves were washed and allowed to dry under shade for 7 days and were ground to powder form. One thousand grams of the powder sample were extracted with 3000ml of solvents mixture of methanol and dichloromethane in the ratio of 1:1 by boiling under reflux for 30 minutes and left overnight to cool. The extract was then double filtered first with a chess material followed by Whatman No.4 filter paper to obtain clear filtrate. The filtrate was concentrated *in vacuo* at low temperature (30-35^{o}C) to about one tenth the original volumes using a rotary evaporator. The concentrate was allowed to stand in a water bath (40^{o}C) for complete dryness.

Fractionation of plant extracts using column chromatography

The crude extract (250.grams) of H.C was chromatographically eluted with two different concentration of methanol solvent (30% and 100%) in a column packed with silica gel of mesh 60-120 (Oxford laboratory reagent, Mumbai-400 002, India, Batch No:1386). The fractions were collected and evaporated in rotary evaporator at 50^{o}C to 10% of its original volume, and were further evaporated to paste form in a water bath at 50^{o}C. The percentage yields for the fractions were: 9 grams (3.6%) 30% methanol and 12grams (4.8%) 100% fractions. The fractions and the remaining crude extract were stored in a freezer at -4^{o}C for further experiments.

Animal collection

Thirty healthy albino wistar rats weighing between 160g to 210g obtained from the healthy stock of the animal house, University of Calabar, were used for the study. The animals were acclimatized for two weeks prior to commencement of the experiment. The animals were housed in well ventilated wooden cages at standard conditions of temperature (25^{o}C).

Induction of diabetes

Diabetes was induced with 45mg/kg body weight of streptozotocin (Sigma Aldrich. Co.3050 Spruce Street, St Louis, MO 63103 USA.) in 0.1M sodium citrate buffer (pH 4.4). Animals with fasting blood glucose >7.8mmol/l or > 180mg/dl and above were enrolled for the study [9].

Experimental protocol

Animals were grouped as shown in the scheme below and also treated with extracts of H.C and metformin. The dosages of the plant extracts were as determined from preliminary work in our laboratory [10] whereas glibenclamide (5mg/kg b.w.) was administered. Treatment lasted for 21days and during the period rats were fed with pallets and water *ad libitum.* The protocol was in accordance with the guidelines of the National Institute of Health (NIH) publication (1985) for laboratory animal Care and Use and approved by the faculty of basic medical sciences Ethic Committee University of Calabar, Nigeria.

Experimental design

The experimental design consisted of six groups as shown below

Group	Number of animals	Treatments
NC	5	20%DMSO
DC	5	20%DMSO
DT	5	Glibenclamide
DT	5	400mg/kg b.w crude extract.
DT	5	400mg/kg b.w 30% Fraction.
DT	5	400mg/kg b.w 100% fraction.

NC= Normal control, DC= Diabetic control, DT=Diabetic treated, DMSO= Dimethylsulphuoxide.

Collection of samples for analysis

After 21 days experimental period, food was withdrawn from the animals with access to water only. The rats were then anaesthetized over chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles. Blood was divided into 2 portions: 1ml into EDTA bottle container for haematological analysis and the remainder emptied into another EDTA bottle and allowed for 2 hours and stored in a refrigerator at 4^{o}C. The refrigerated blood sample was then centrifuged at 3000rpm for 10 minutes. Plasma was separated using syringes and needle and stored frozen until used for biochemical analysis.

Biochemical analyses

Experimental assays were carried out for lipid profile parameters (Total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein and very low density lipoprotein). All analytical kits were purchased from Agappe diagnostics. (Konauerstrasse54.6330 cham, Switzerland Gmbh) while haematological parameters (red blood cell count, haemoglobin conc., platelet count, white blood cell count and lymphocyte count) was carried out using BC-2600 Automated Haematology Analyzer (Shenzhen mindray bio-medical electronics co., Ltd, made in china)

Statistical analysis

The results were analysed for statistical significance using ONE-WAY ANOVA with a Post.Hoc LSD's test. Values were expressed as mean ± SEM. $P<0.05$ were considered significant.

RESULTS

Effect of extracts on lipid parameters

Figure 1a-e below shows the result of 21 days administration of extract and fractions of *Hensia crinita* on the lipid profile of normal and diabetic rats (control and extract treated). The triacylglyceroland very low density lipoprotein cholesterol concentration in diabetic control group showed a significant ($p<0.05$) increase compared to normal control group. On treatment with extract, a significant reduction ($p<0.05$) was observed in triacylglycerol concentration was observed in all experimental groups compared to diabetic and normal control except for glibenclamide treated group that significant higher than the normal control. Total cholesterol level of diabetic control group increased significantly ($p<0.05$) when compared to normal control. Upon treatment with extracts of *Hensia crinita,* significant ($p<0.05$) decrease was observed in all experimental treated groups compared to the diabetic and normal control groups. However, high density lipoprotein cholesterol concentration showed no significant ($p>0.05$) change in experimental treated groups compared to diabetic and normal control groups. More so, LDL-C showed significant ($p<0.05$) increase in concentration in diabetic control groups compared to normal control which significantly decrease in all experimental groups compared to diabetic and normal control groups.

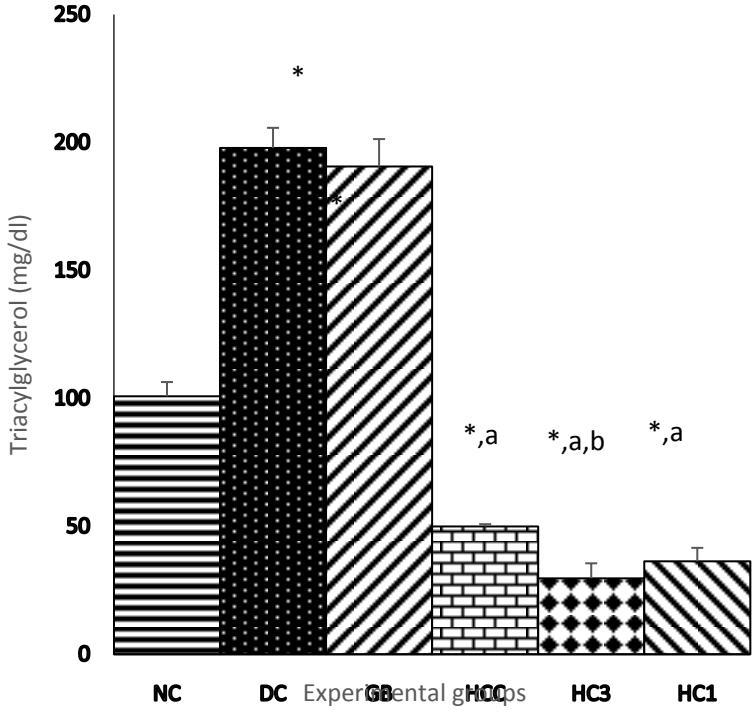

Figure 1a: Triacyglycerol concentration in extract and fraction treated experimental groups

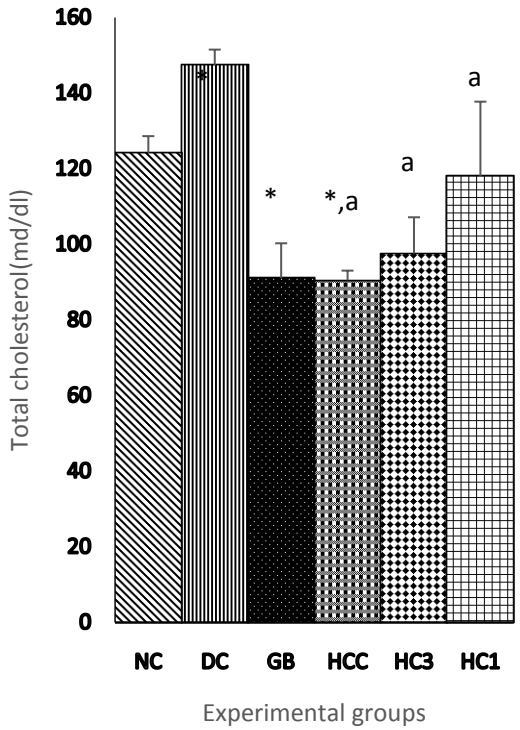

Figure 1b: Total cholesterol concentration in extract and fraction treated experimental groups

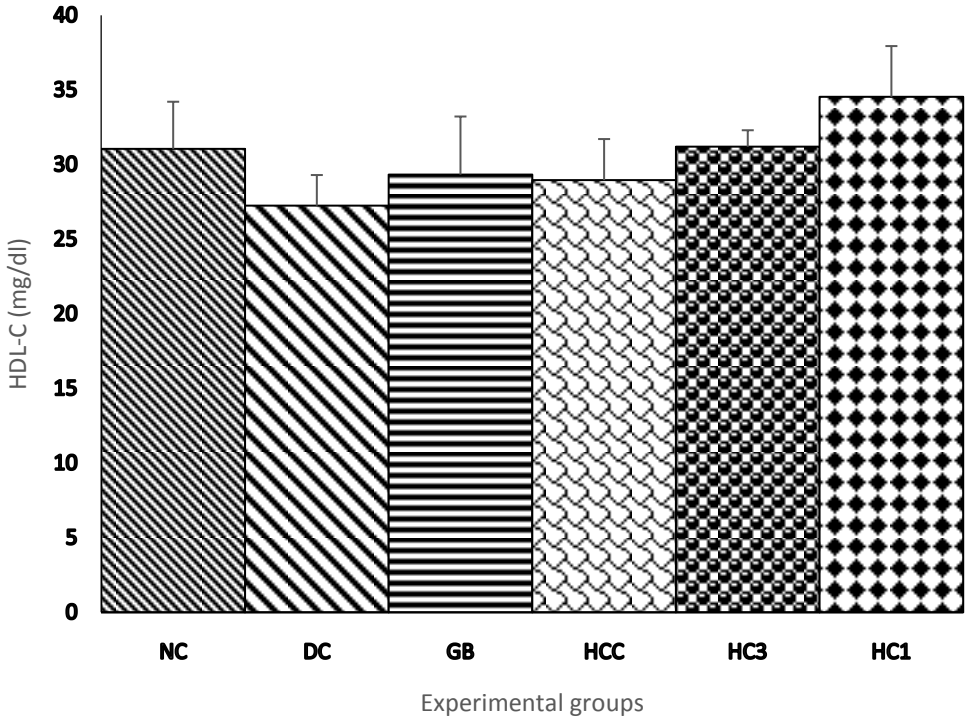

Figure 1c: High density lipoprotein concentration in extract and fraction treated experimental groups

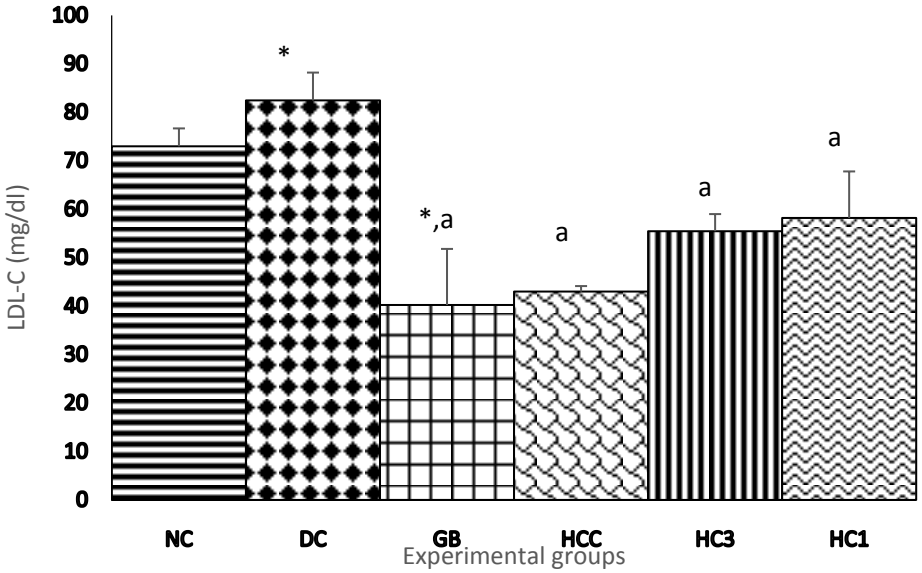

Figure 1d: low density lipoprotein concentration in extract and fraction treated experimental groups

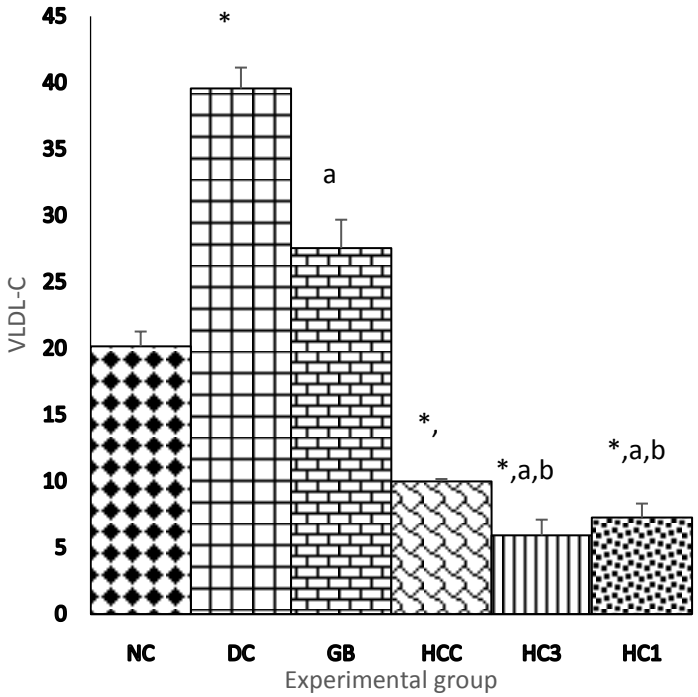

Figure 1e: low density lipoprotein concentration in extract and fraction treated experimental groups

Effect of extracts on Haematological parameters

Figure 2a-e below shows the effect of *Heinsia Crinita* extract on haematological parameters. From the result, no changes were observed in the red blood count and Haemoglobin levels of all experimental groups. A significant ($p<0.05$) reduction in white blood cell count was observed in diabetic control groups compared to normal control. On treatment with extract fractions and glibenclamide, a significant ($p<0.05$) increase in white blood cell and platelet levels compare to normal and diabetic control with H.C Crude extract observed to be the highest. Lymphocyte count was observed to be significantly ($p<0.05$) increase in diabetic control group compare to normal control which significantly ($p<0.05$) reduces in all experimental groups compared to diabetic and normal control groups.

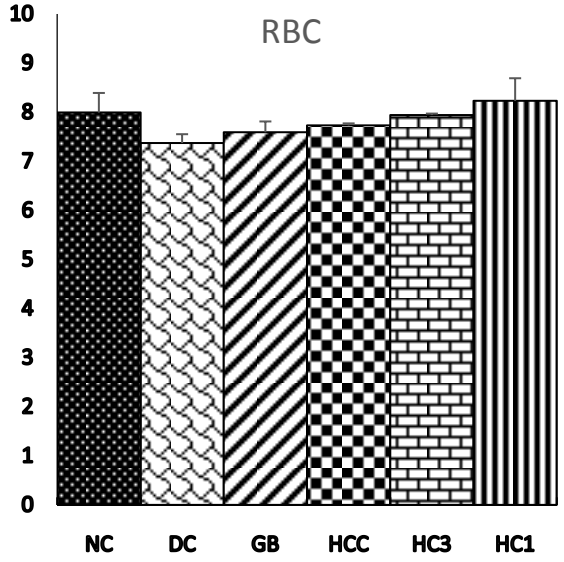

Figure 2a: Red blood cell (RBC) concentration in extract and fraction treated experimental groups

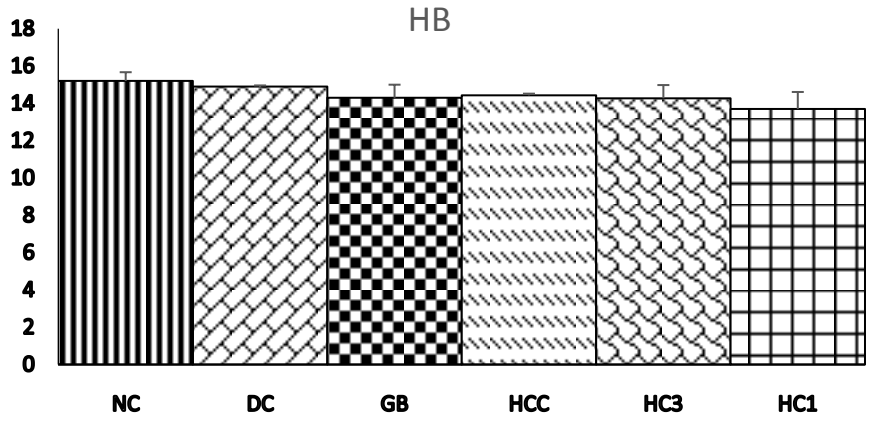

Figure 2b: Haemoglobin (HB) concentration in extract and fraction treated experimental groups

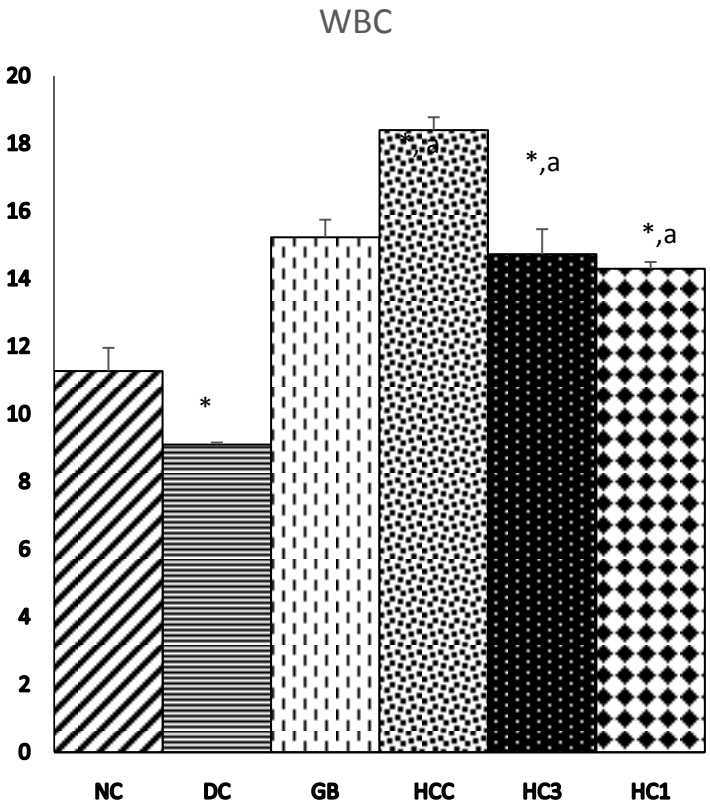

Figure 2c: White blood cell (WBC) concentration in extract and fraction treated experimental groups

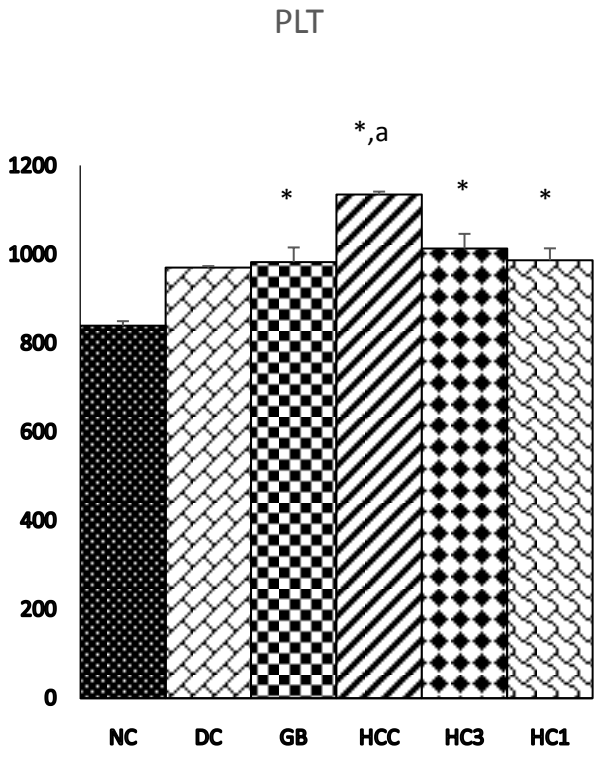

Figure 2d: Platelet concentration in extract and fraction treated experimental groups

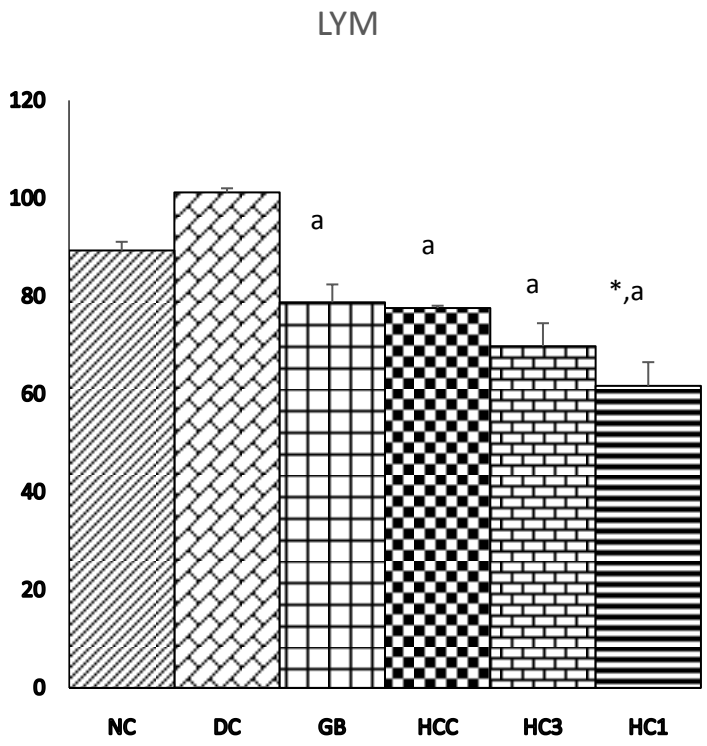

Figure 2e: lymphocyte concentration in extract and fraction treated experimental groups

DISCUSSION

Streptozotocin is a glucosamine-nitrosourea compound. It is toxic to cells by causing damage to the DNA. DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself [11]. Glucose transporter 2 (GLUT 2) is a type of transmembrane protein that facilitates glucose transport across cell membranes. It also carries glucosamine. Because streptozotocin is a glucosamine, its competes with glucose for GLUT 2 and this explains its relative toxicity to the beta cells since these cells have high concentrations of GLUT 2 [12]. Diabetes-induced hyperlipidemia is attributable to excess mobilization of fats from adipose tissue due to underutilization of glucose [13]. It has also been established by [14] that streptozotocin-induced diabetes raises lipid profile. From this study, it was observed that induction of diabetes significantly increase lipid profile which correlates with earlier report by [15]. Administration of *H. Crinita* crude and extract fractions was observed to significantly decrease TG, Total cholesterol, LDL-C, VLDL with no significant change in HDL-C against diabetic control. Phytochemical analysis by [15] reveals the presence of tannins, saponins, alkaloids and glycosides e.t.c with tannin present in significant concentration. Tannic acid, a major component of tannins, has been reported by [16] to have the capacity to decrease blood glucose level, by stimulating glucose transport, while inhibiting adipogenesis. Comparing the efficacy of these extracts with the patterns of glibenclamide, an anti-diabetic drug, it can be deduced that the extracts are potent in their hypolipidemic action. More so, reduction in lipid profile levels may be attributable to the presence this active phytochemicals in *H. Crinita.*

Notably in diabetic patientsis anaemia which is a common blood disorder that is seen to be in an increase [17]. It is a clinical condition characterized by reduction in hemoglobin concentration of

blood below the normal level (i.e., <13 g/dl for men and <12 g/dl for women) for the age, sex, physiological problem and altitude [18]. Also, incident of anaemia in diabetes has been reported to be as a result of elevated glycosylation of membrane proteins of red blood cells, which is connected with hyperglycemia [19].The primary reason for assessing RBC is to check anemia and evaluate normal erythropoiesis [20]. No significant change was observed ($p>0.05$) in DC rats against normal control for RBC. Groups treated with *H. Crinita* crude and 30% and 100% fractions showed no significant change ($p>0.05$) against normal control. This observation may imply that the extracts of *H. Crinita* may not alter the normal range of hematological parameters. Hemoglobin is protein essential to gas exchange. Any fluctuations in hemoglobin level in blood have significant influence on the metabolic performance and state of health of human or animals. It indicates the amount of intracellular iron. Hemoglobin levels were all within similar range as no significant change in NC, DC and extract treated group was observed. Platelets are fragment of cells that participates in blood clotting. They initiate repair of blood vessels walls and are also considered as an acute phase reactant to inflammation. This explains why PLT levels for DC rats significantly increased ($p<0.05$) against normal control as the body had to deal with inflammation caused by steptozotocin metabolism. Significant increases in glibenclamide, *H. Crinita* crude, 30% and 100% fractionate implies that they are potent as acute phase reactants to inflammation caused by steptozotocin metabolism in the diabetic rats. Diabetic control rats had significantly decreased values ($p<0.05$) for total WBC's when compared to normal control. Following treatment with *H. Crinita* crude, *H. Crinita* 30% and 100% fractionate, a significant increase against DC was observed. This indicates that this extracts may be anti-infective and has the abilities to boost the immune system as reported by [1]. Lymphocyte levels were observed to significantly increase ($p<0.05$) in the diabetic control group and significantly decrease in extract treated groups against diabetic control. This explains that these extracts have the abilities in curtailing hematological insults in the defense system of the diabetic rats.

CONCLUSION

It can be concluded that administration of crude and extract fractions of *Heinsia Crinita* may contribute significantly to reduction of lipid profile in diabetes and also could curtail hematological insults incited by diabetes and thus, useful in the management of diabetes.

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