



# EFFECT OF *HIBISCUS SABDARIFFA L.*, *ZINGIBER OFFICINALE ROSCOE* AND *PIPER NIGRUM L.* ON THE HEMATOLOGICAL PARAMETERS OF ALLOXAN INDUCED DIABETIC WISTAR RATS.

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## ABSTRACT

**Background:** Diabetes is a disease condition of metabolism clinically expressed by chronic hyperglycemia and protein disorder; it has a link to several complications that cause morbidity and mortality and native plants with antidiabetic properties can be used to avoid further progression of the disease

**Objective:** To investigate the antidiabetic properties of combined extract of *hibiscus sabdariffa l.*, *zingiber officinale roscove* and *piper nigrum l.* as well as its effect on selected hematological parameters in alloxan induced diabetic wistar rats

**Methods:** Thirty (30) male wistar rats aged between 8-10 weeks, weighing 130g to 138g were divided into five groups of six rats each and acclimatized for a week prior to the commencement of the treatments. Of the 5 groups, 4 were induced with 170mg/kg body weight of alloxan. Diabetic rats were treated with 250mg/kg and 500mg/kg of the combined extract as well as 500mg/kg of metformin. The last diabetic group was left untreated and served as the negative control. All treatments were administered for 21 days with blood glucose level and body weight of the experimental animals regularly measured during the course of the treatment. At the end of the treatments, experimental rats were sacrificed and the blood collected was used to carry out hematological analysis including White Blood Cell count(WBC), Red Blood Cell count (RBC), Hemoglobin concentration (Hb), Hematocrit (Hct) or Packed Cell Volume (PCV) and Platelet (Plt). The results were subjected to analysis of variance (ANOVA) at  $P < 0.05$ .

**Results:** The combined plant extract was observed to cause a decrease in the blood sugar level and also helped to maintain the body weight of animals. Similarly, the level of the PCV and the HB significantly increased ( $p < 0.05$ ) after administering the combined extract at the two selected doses. Also the WBC count was significantly ( $p < 0.05$ ) lowered following the administration of the combined extract.

**Conclusion:** The combined extracts of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* have potential in the management of hyperglycemia as well as in the improvement of hematological parameters in alloxan induced diabetic rats.

**KEYWORDS:** *Hibiscus sabdariffa l.*, *zingiber officinale roscove*, *piper nigrum l.*, hematology, diabetes

## INTRODUCTION

Diabetes is known as a syndrome of altered metabolism due to genetics and environmental factors and it is said to be a chronic metabolic disorder known by increased blood glucose and glycosylated hemoglobin (Huan *et al.*, 2018, Abdulahad and Ismail 2016, Asmma *et al.*, 2018, Leonardo *et al.*, 2017, Zeinab *et al.*, 2015).

And it is linked to several complications that affect parts of the body such as eyes, kidney, heart, blood vessels, cause morbidity and mortality which is a common health problem for the aging population (Levent *et al.*, 2015, Jinling *et al.*, 2014). According to WHO by 2020, 300 million people will suffer from diabetes. (Ahmed, 2015), *Hibiscus sabdariffa l.* is the scientific name for zobo leaves which are used to make the most common

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Nigerian drink called zobo, and also as tea, water beverage and social drink, it is known to be a safe herbal medicine for several ailments such as diabetes mellitus, hypertension hyperlipidemia (Showande *et al.*, 2017 Nnanna *et al.*, 2018).

Ginger is botanically known as *Zingiber officinale* Roscoe, it is under the *Zingiberaceae* plant family, it is said to be originated in India, it is also cultivated in different countries such as Fiji, Australia, Taiwan, Nigeria etc and it has hypoglycemic effect that cannot be affected by heat making it an effective medical treatment in the management of diabetes. (Manhendra *et al.*, 2018, Mohamad *et al.*, 2019).

Black pepper is scientifically known as *Piper nigrum* L, it is under the *Piperaceae* family, it is mostly taken in few parts of West Africa such as Nigeria and Ghana. (Wodu, 2018). It is known to have function which is beneficial for the non-communicable metabolic condition called diabetes mellitus (Chetna *et al.*, 2016).

Alloxan is known as a diabetic inducing drug used to induce Type 1 diabetes in experimental animal models, it selectively destroys the beta-cells found in the pancreas where insulin is produced and it reduces body weight (Ankur and Shahjad, 2012, Ewenighi *et al.*, 2015).

Metformin is known as a medicine used to treat type 2 diabetes, its main function is to lower the blood glucose level by improving the way insulin function in the body. It is recommended when diet and exercise are not enough to regulate blood glucose level to reducing the amount of glucose the liver releases into the blood, it can also be called Bolamyn, Diagment, Glucophage and Glucient, the liquid metformin is called Riomet (Papanas and Maltezos, 2009).

Herbal plants like, *Hibiscus Sabdariffa* L, *Zingiber officinale* Roscoe and *Piper Nigrum* L have shown these hypoglycemic abilities with maximum effect and they are known to be locally available and assessable (Ojulari, 2014).

## MATERIALS AND METHODS

### Preparation of extracts

Healthy and organically grown *Hibiscus Sabdariffa* L, *Piper Nigrum* L and *Zingiber officinale* Roscoe were purchased from Ilishan market, Ogun State, South West of Nigeria. Extraction was carried out following standard guidelines reported in literature. The plant samples were washed, first and dried under low heat (40 degree centigrade). The dried samples 1000g were then grounded to powder and dissolved in 1000mL of 100% methanol (CH<sub>3</sub>OH) overnight. The extract was filtered and the filtrate was transferred to clean vessels and dried in the oven at 60<sup>o</sup>c for 6 hours (Mardiah *et al.*, 2018), the extracts of 32mg and 80mg were dissolved in 147ml of distilled water.

### Experimental animals

The study was done at the Babcock University Experimental Animal Facility (BUEAF), Ilishan-Remo, and Ogun State, Nigeria. Thirty male Wister rats aged 8-10 weeks with an average body weight (130g to 138g) were obtained from the rodent house of the BUEAF. The animals were kept at ambient room temperature and diurnal lighting conditions and were fed standard rat

pellet and water *ad-libitum*. Ethical protocols for the use of animals in research were strictly adhered to.

### Experimental design

Experimental animals were made diabetic by inducing them with alloxan; this was done by fasting the rats for a period of 12 hours after which they were intraperitoneally injected with freshly prepared alloxan solution at a dose of 65mg/kg body weight in 0.1M cold citrate buffer, PH 4.5. Animals were considered diabetic when blood glucose level values were above 200 mg/dl (Mardiah, 2014). The rats were treated with the extract by oral gastric intubation once every 24 hours as follows. Doses of 200mg/kg and 500mg/kg were selected for treatment (Otunola and Afolayan, 2015). The experimental rats were divided into five groups of 6 animals each and assigned different treatments that lasted for 21 days. Group 1 animals (Normal control) were orally administered with normal saline; Group II animals (negative control) were induced with alloxan and received water only. In Group III (T<sub>1</sub>) animals were induced with diabetes and treated with zobo and spice (ginger and black pepper) mixture extract at 200mg/kg body weight/day. Group IV animals (T<sub>2</sub> 500) were also induced and received the combined plant extracts of zobo and spice (ginger and black pepper) at 500mg/kg body weight/day. Lastly group V (Standard control) received the standard treatment drug (metformin) 500mg/kg after being induced with alloxan. The rats were weighed at the beginning of the study, following the induction of diabetes using alloxan, and at regular intervals during the treatment respectively. The blood glucose levels of the experimental rats were measured using a glucometer kit at the beginning of the study, when the rats were induced with alloxan, and at the end of the treatment (extract and metformin).

### Procedure for Hematological Parameters

After two weeks of treatment with extract and metformin, the rats were sacrificed by spinal dislocation, blood samples were collected using ocular puncture and put into bottles containing 10% of ethylene diamine tetracetic acid (EDTA) and the automatic blood analyzer was used to determine the following hematological values which includes: White Blood Cell count (WBC), Red Blood Cell count (RBC), Hemoglobin concentration (Hb), Hematocrit (Hct) or Packed Cell Volume (PCV), Platelet (Plt) (Ampa, 2017).

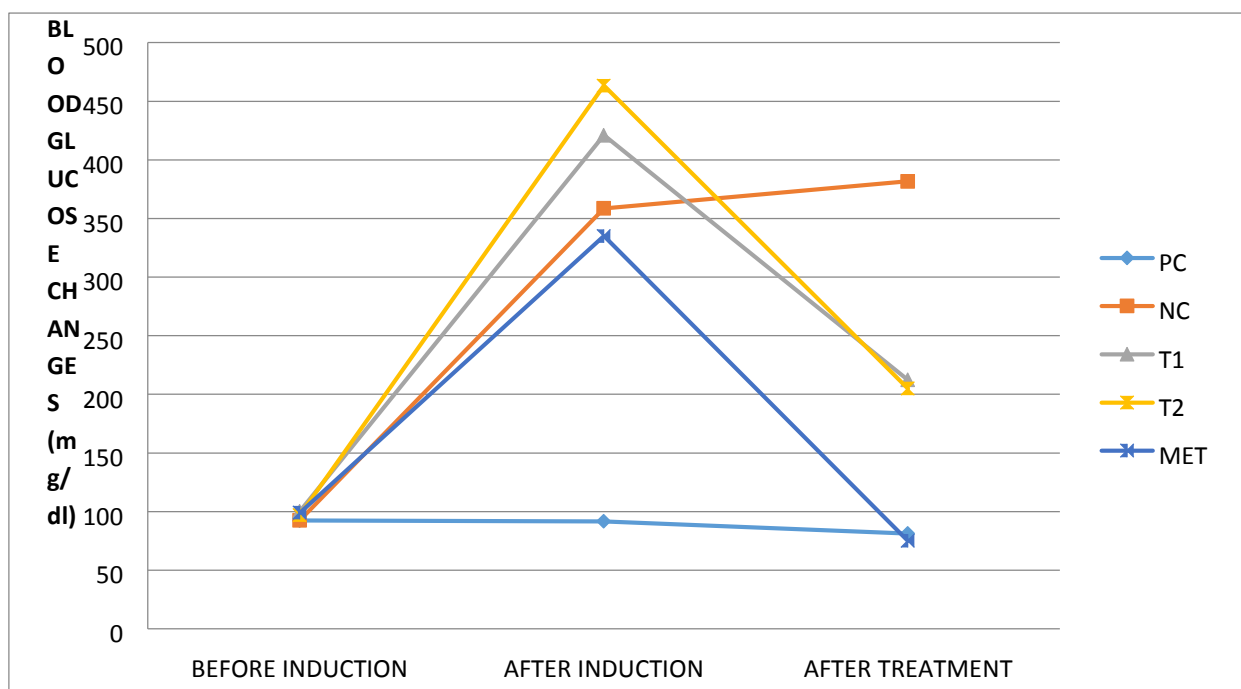
### Statistical analysis

All data collected were analyzed using the Statistical Package for Social Science (SPSS) in which the analysis of variance (ANOVA) was measured and levels of significance considered at p<0.05.

## RESULTS

### Effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the Blood glucose level

Result in figure 3.1 shows the effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the Blood glucose concentration of diabetic rats. Rats all the treatments reduced the blood glucose of diabetic rats. However, the blood glucose of diabetic untreated rats continued to increase up to the time of euthanization



**Figure 1:** Blood glucose level of the normal control group, negative control group and treatment groups during the oral glucose tolerance test. Results are presented as Mean  $\pm$  SEM.

PC: Positive (non-diabetic) control group

NC: Negative (diabetic) control group

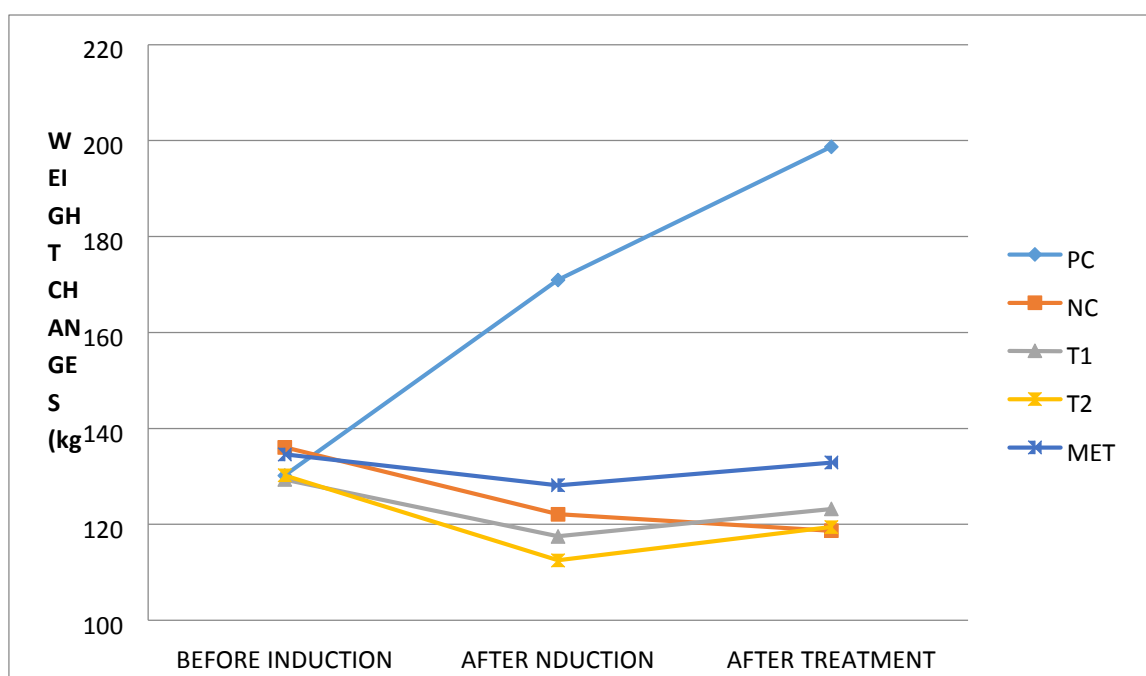
T1: diabetic group treated with 250mg/kg body weight of extract

T2: diabetic group treated with 500mg/kg body weight of extract

MET: diabetic group treated with metformin

#### Effect of combined leaf extract of *H. Sabdariffa*, *P. Nigrum L.* and *Z. officinale* on body weight changes of alloxan induced diabetic rats

Result in figure 3.2 shows the effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the body weight changes of diabetic rats. The results shows a decrease in the body weight of diabetic rats after induction, however, the treatments administered helped to minimize the loss in weight.

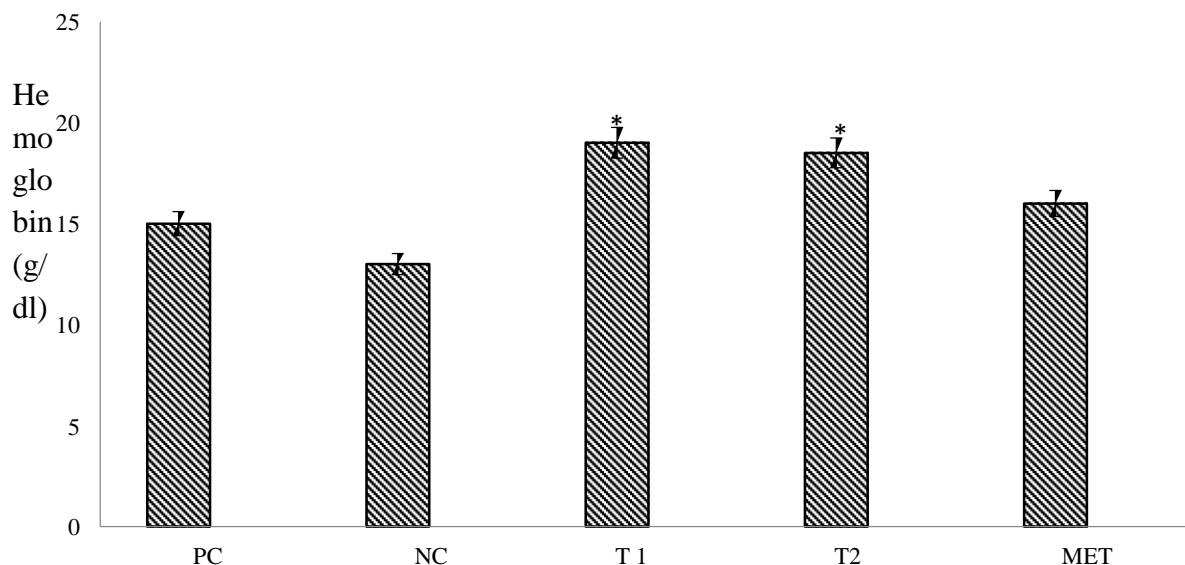


**Figure 2:** Blood glucose level of the normal control group, negative control group and treatment groups during the determination of change in weight. Results are presented as Mean  $\pm$  SEM.

PC: Positive (non-diabetic) control group  
 NC: Negative (diabetic) control group  
 T1: diabetic group treated with 250mg/kg body weight of extract  
 T2: diabetic group treated with 500mg/kg body weight of extract  
 MET: diabetic group treated with metformin

**Effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the PCV level of alloxan induced diabetic rats.**

Result in figure 3.3 shows the effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the PCV levels of diabetic rats. The results show a significant increase ( $p < 0.05$ ) in the PCV of diabetic rats treated with extracts at 250mg/kg and 500mg/kg when compared with the other experimental animals.

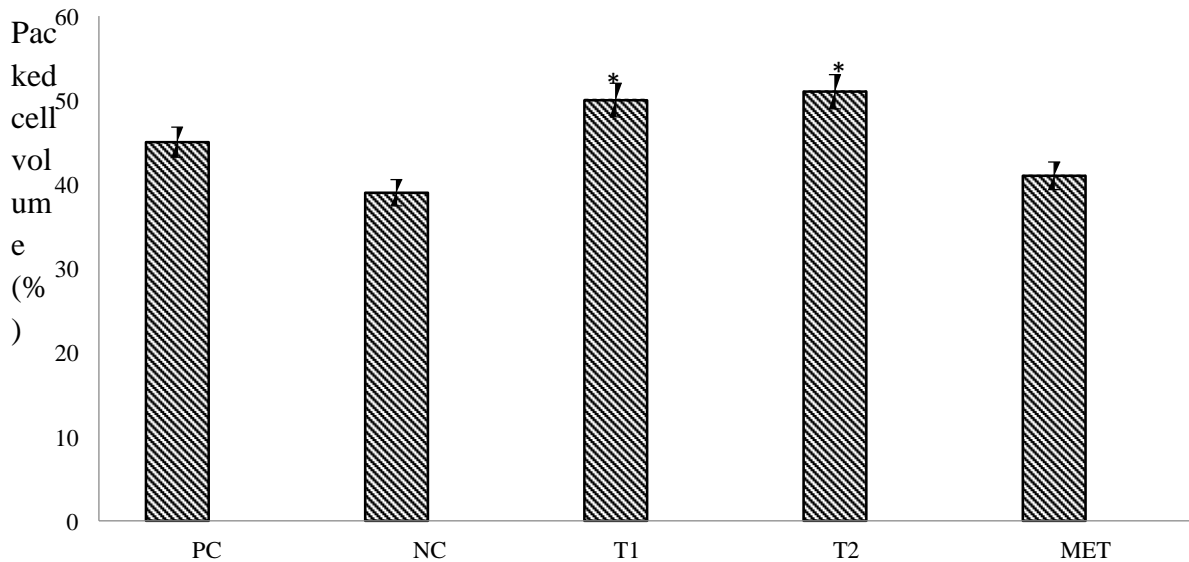


**Figure 3:** Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on packed cell volume (PCV) in alloxan-induced diabetic rats. Results are presented as Mean  $\pm$  SEM, \* $p < 0.05$  statistical significance.

PC: Positive (non-diabetic) control group  
 NC: Negative (diabetic) control group  
 T1: diabetic group treated with 250mg/kg body weight of extract  
 T2: diabetic group treated with 500mg/kg body weight of extract  
 MET: diabetic group treated with metformin

**Effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the Hemoglobin level of alloxan induced diabetic rats.**

Result in figure 3.2 shows the effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the Hb levels of diabetic rats. The results shows a significant increase ( $p < 0.05$ ) in the Hb level of diabetic rats treated with extracts at 250mg/kg and 500mg/kg when compared with the other experimental animals. The result also shows that the negative control recorded the lowest values for hemoglobin level.



**Figure 4:** Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on hemoglobin (Hb) concentration in alloxan-induced diabetic rats. Results are presented as Mean  $\pm$  SEM, \* $p < 0.05$  statistical significance.

PC: Positive (non-diabetic) control group

NC: Negative (diabetic) control group

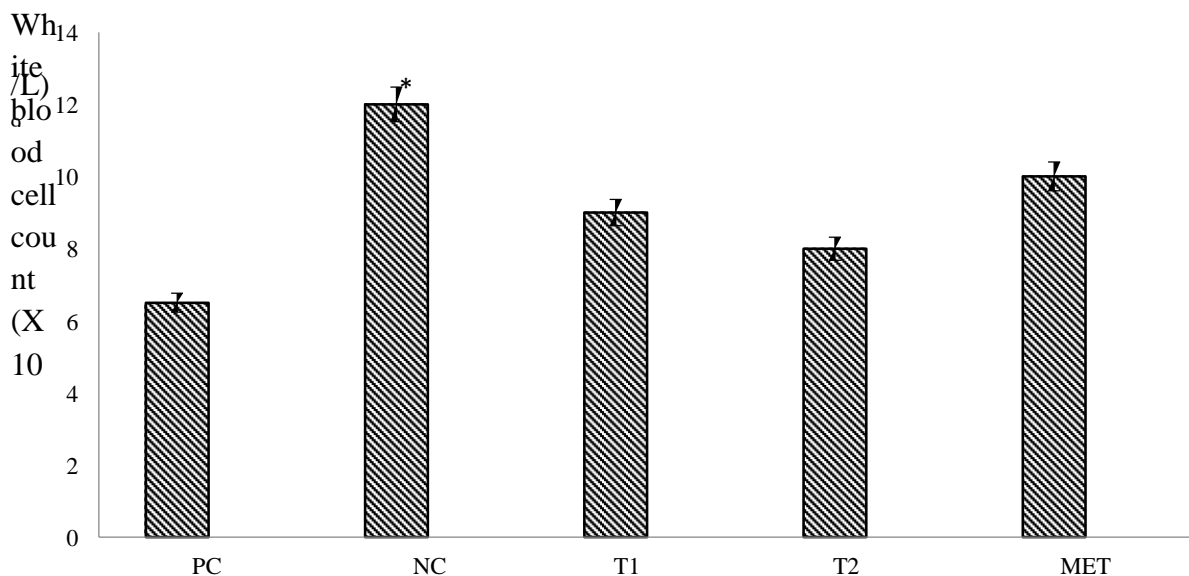
T1: diabetic group treated with 250mg/kg body weight of extract

T2: diabetic group treated with 500mg/kg body weight of extract

MET: diabetic group treated with metformin

**Effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the White Blood Cell (WBC) count of alloxan induced diabetic rats.**

Result in figure 3.5 shows the effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the WBC count of diabetic rats. The result shows an elevation in WBC levels in the Negative control group, however there is a significant decrease ( $p < 0.05$ ) in the WBC level of diabetic rats treated with extracts at 250mg/kg and 500mg/kg



**Figure 5:** Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on white blood cell counts (WBC) in alloxan-induced diabetic rats. Results are presented as Mean  $\pm$  SEM, \* $p < 0.05$  statistical significance

PC: Positive (non-diabetic) control group

NC: Negative (diabetic) control group

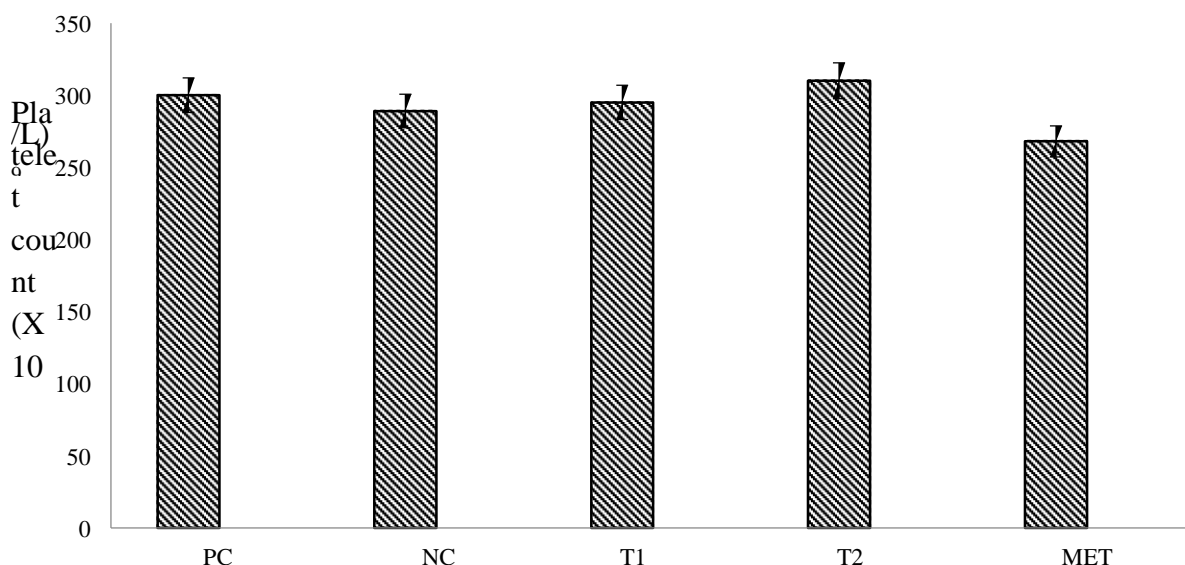
T1: diabetic group treated with 250mg/kg body weight of extract

T2: diabetic group treated with 500mg/kg body weight of extract

MET: diabetic group treated with metformin

**Effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the platelet count of alloxan induced diabetic rats.**

Result in figure 3.6 shows the effect of combined methanol extract of *Hibiscus Sabdariffa*, *Zingiber Officinale* and *Piper Nigrum* extract on the platelet count of diabetic rats. The result shows that the platelet count of the treatment groups were not significantly different ( $p > 0.05$ ) when compared to the diabetic untreated group (NC)



**Figure 6: Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on platelet count in alloxan-induced diabetic rats.** Results are presented as Mean  $\pm$  SEM, \* $p < 0.05$  statistical significance

- PC: Positive (non-diabetic) control group
- NC: Negative (diabetic) control group
- T1: diabetic group treated with 250mg/kg body weight of extract
- T2: diabetic group treated with 500mg/kg body weight of extract
- MET: diabetic group treated with metformin

**DISCUSSION**

Diabetes is a disease that can be distinguished by chronic hyperglycemia, whereby the body is unable to produce insulin or the body is unable to produce enough insulin to control the amount of glucose that enters the blood (Huan *et al.*, 2018). This study sought to evaluate if the combined extract of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* have the potential to counter hyperglycemia experienced in diabetes. Findings from this study showed that blood glucose level of all the groups induced with alloxan became elevated following induction of diabetes with only the non-induced control group maintaining a normal blood glucose level. However, while the glucose level for the negative control group continued rising until they were sacrificed, the blood glucose level of the T1, T2 and metformin control group decreased following administration of treatments, thereby showcasing the potential of the extracts as well as metformin in controlling hyperglycemia in diabetes. The hypoglycemic potential shown by the extract under study corroborates the findings of Jafri *et al.*, (2011). Though the difference between the T1 and T2 group was not significant, it was however observed that T2 ( $212.33 \pm 53.72$ ) group performed slightly better than the T1 ( $205.0 \pm 37.12$ ), this could be a pointer to the fact that a higher dose of the combined extract would even stabilize the blood glucose further.

Alloxan induced diabetic rats are known for weight loss and other diabetic symptoms such as hyperglycaemia (Bandigari *et al.*, 2018, Ewenighi *et al.*, 2015, Ojewumi and Kadiri 2013). Therefore, following induction, all induced animals recorded a gradual loss in body weight even though they were fed with the same amount of rat feed. Interestingly, their weight gradually increased following treatments with the metformin group showing the biggest recovery in weight amongst the treatment group. Conversely, the negative control group continued recording a loss in weight till the end of the experiment.

Studies have shown that changes in hematological parameters are a common complication of DM and this could create additional health burden in patients (Nwagu *et al.*, 2015). One of the most common hematological imbalances experienced in diabetics is anemia (Kotharia and Bokariya 2012). It is for this reason we evaluated possible alterations in the hematological parameters of experimental animals in this study. The result obtained for the packed cell volume (PCV) and Hb count showed that PCV and Hb level in the negative control group were at the lowest amongst all groups under study but this value was significantly increased ( $p < 0.05$ ) in the groups administered extracts. The reduced PCV level in the negative control group could be attributed to diabetes as it has been reported that diabetics have low PCV than non-diabetics, this reduction could be attributed to the dehydration and accumulation of protein experienced in diabetic patients (Jaman *et al.*, 2018). This finding is also consistent with what was reported by Lawrence *et al.* (2015). Also due to nephropathy that manifests in diabetes, a reduction in some red blood cell indices could be as a result of reduced and abnormal erythropoiesis, (Mohammed *et al.*, 2009). Similar to the observation in this study where RBC indices like PCV

and Hb levels were increased following administration of our combined extracts, there are reports that suggest that herbal extracts helped in the stimulation of production of haematopoietic regulatory elements such as erythropoietin and colony-stimulating factors by the stromal cells and macrophages in the bone marrow (Osigwe *et al.*, 2017)

White blood cells (WBC) are vital components of the body immune system. They help the body fight off diseases by acting as a scavenger that removes foreign substances (Osigwe *et al.*, 2017) and they are mobilized in response to either foreign invaders or a toxic environment (Ngogang, 2005). Findings in the present study show that the negative (diabetic) control group had an increased level of WBC indicating that there is a tendency of that group having chronic inflammation, most likely caused by the induction with alloxan, the WBC count was however seen to be reduced following treatment with our extracts and this decrease was significant ( $p < 0.05$ ).

There are reports suggesting that platelet count in diabetes become elevated (Uko, 2013) from the findings obtained in this study. The PC group had a slightly reduced platelet count compared to the NC group, the T1 and T2 also had slightly reduced platelets though there was no significance difference ( $p > 0.05$ ) across all the groups so our combined extracts had little effect on platelet counts.

## CONCLUSION

The combined extracts of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* have potential in the management of hyperglycemia as well as in the improvement of hematological parameters in alloxan induced diabetic rats.

**CONFLICTS OF INTEREST:** The authors declared no conflict of interest exist

## AUTHOR'S CONTRIBUTIONS:

All authors participated in research design. Author IFA and OAO conducted the research work and wrote the manuscript while Authors IJA and EIA revised the manuscript and the research design. All authors read and approved the final manuscript.

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