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EFFECT OF ETHANOL EXTRACT OF THE FRUITING BODIES OF ORGANICALLY CULTIVATED *PLEUROTUSOSTREATUS* ON THE HAEMATOLOGICAL INDICES OF HIGH SUCROSE- HIGH FAT DIET-STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The effect of ethanol extract of the fruiting bodies of organically cultivated Pleurotusostreatus on the haematologicalindicesof HS-HFD-Streptozotocin induced diabetic rats was determined using standard methods. The pharmacological model was 20% High Sucrose (HS) + 20% High Fat Diet (HFD) + 35mg/kg body weight (via intraperitoneal) Streptozotocin (STZ) induced diabetic rat model, with the fruiting body ethanol extracts administered orally at 50, 150 and 300mg/kgb.w.The level of RBC indices, (count, Hb, HCT, MCH, MCHC and MCV in all the groups treated with ethanol extracts of P.ostreatus, the reference group and the control group were significantly($p< 0.05$) higher compared to the diabetic control group while the WBC,PLT, MPV, PDW , PCTRDW-CV and RDW-SD levels in the P.ostreatus ethanol extract groups, metformin reference treatment group and the normal control were significantly (($p< 0.05$) lower compared to the diabetic control groupafter the 3^{rd}, 6^{th} and 9^{th} week of treatment respectively.The results of the study revealed that the RBC , hemoglobin, HCT, MCH, MCHC and MCV levels were significantly raised within normal ranges by the extract in a time and dose dependent manner in the treated diabetic rats. The extracts also showed positive effects on WBC, PLT, MPV, PDW, PCT, RDW-CV and RDW-SD levels by lowering them towards the normal ranges, time and dose dependently. These results therefore suggest that ethanol extract of the fruiting bodies of organically cultivated Pleurotusostreatus may be employed in the management of anaemia, prevent bleeding or disorder of platelet function and improve immune function in diabetics.

Keywords: Haematological indices, *Pleurotusostreatus*, Diabetes mellitus, hemoglobin, HS-HFD-Streptozotocin induced diabetic rats.

INTRODUCTION

One vital physiological process that occurs in mammals is hematopoiesis. It is responsible for the formation of all the cellular components of blood which include erythrocytes (RBCs), leucocytes (WBCs) and platelets (PLTs). These blood cells possesses life sustaining functions. The red blood cells help to deliver oxygen and nutrients to the body tissues through the flow of blood via the circulatory system. Red blood cells have

secondary function which is the release of Adenosine Triphosphate Phosphate(ATP) as a result of shear stress in constricted vessels there by making the walls of the vessel to relax and dilate so as to promote normal blood flow (Diesen et al; 2008). The white blood cells are cells of the immune system involved in protecting the body against both infectious diseases and foreign invaders. The platelets work along with the coagulation factors to stop bleeding by clumping and clogging blood vessel injuries. Platelets also play hemostasis role (Essiet et al.,2020). One of the factors that may affect the process of hematopoiesisis exposure to acute and chronic diseases (Joly et al., 2017; Mansi&Lahham, 2008).

In the world today, diabetes mellitus has been reported as a burden of disorder in the structure and function of biological systems (Lozano *et al*., 2010). According to Okoroh*et al*., (2021), diabetes mellitus is a non-communicable disease which has been singled out as a major factor in the endocrine region of the bio system responsible for the crisis in the metabolism of biomolecules suchas fats, carbohydrates and proteins. Diabetes mellitus is a contributory factor to impaired vision, stroke, kidney failure, cardiovascular diseases (WHO, 2016), Its prevalence has been rapidly on the increase particularly among middle- and low- income nations like Nigeria. Infact, World Health Organization, WHO(2016) highlighted diabetes mellitus to be the 7th leading cause of deaths by 2030.The implication is that diabetes mellitus presents a major challenge to researchers and health care systems around the globe .

Diabetes mellitus is defined as a group of metabolic diseases of endocrine origin indicated when there is high glucose level in the blood over a prolonged period and large amount of sugar in urine detected because of complete or relative lack of insulin resulting from the impairment of insulin secretion ,insulin action or both (WHO,2014). Its symptoms include osmotic diuresis which eventually causes excessive loss of water from tissues, increased thirst, hunger, and high concentration of lipids in the blood (WHO, 2013).

Diabetes mellitus can affect the hematopoietic system by alterations in blood indices (Alamgeer et al., 2012; Helal et al., 2005; Mansi & Lahham, 2008). An increase in free radical production coupled with non-enzymatic glycation of biomolecules induced by high blood sugar level may play a major role. These processes alter cellular structure and function, leading to the formation of advanced glycation end products (AGEs) which bind to specific receptors for AGEs to form ligand receptor complexes. This will enhance metabolic disturbances and further increase free radical production, resulting to changes in the structure and biophysical features of vascular endothelium (Meza et al.,2019). These structural alterations brings about vasodilatation, increased permeability and damage to RBCs with consequent decrease in RBC indices (Diederich et al., 2018). Agrawal et al., (2016) recorded that the erythrocytes of patients suffering from retinopathy linked to diabetes suffer membrane deformation because of peroxidation of red blood cell membrane. High blood sugar level in addition results to the activation of the coagulation cascade thereby contributing to the vascular complications of diabetes mellitus (Papatheodorou et al.,2018). Diabetes mellitus also promotes differentiation and maturation of leucocytes (Demirtas et al., 2015).Hasslacher (2007) reported that diabetic patients suffer anaemiaand this may be due to the impairment of erythropoietin production because of pathologic conditions that affect renal function.

Today, Insulin is mostly used in the treatment of diabetics and this is supported using a lot of anti-diabetic compounds including sulfonylurea, biguanides, and thiazolidinediones. These medications are costly, and difficult to access by the poor. Synthetic drugs are expensive and have side effects (Lee *et al.,* 2012).

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Pleurotusostreatus belongs to the family of mushrooms known as pleurotaceae (Kuo, 2005).*P.ostraetus* is also called tree oyster mushroom (Stamets, 2000) or grey oyster mushroom and this name marks it out from the other species in the genus. Some people call it straw mushroom. The people from Japan call *Pleurotusostreatus*Hiratake which impliesflat mushroom (Hall,2010). The Igbo-speaking people of South-East, Nigeria, call it Eroatakata because it has very tough texture on mastication (Akpaja et al., 2003). *Pleurotusostreatus is* edible, medicinal and also very common. The people of Germany cultivated this macrofungi just to sustain life during the world war. However, in the globe today, people grow Pleurotusostretus in large quantity and trade it as means of generating revenue and for consumption too (Hall, 2010). The mushroom has quality nutritional value, numerous medicinal properties and many other beneficial effects. It has been used as food and as means of treating ailments by numerous people all over the world for many years (Finimundy et al., 2013). The mycochemical composition of this mushroom has made it a special dietary substance for the prevention and treatment of medical conditions associated with high level of cholesterol in the blood (Hossain etal., 2003). It has also been reported that the macro fungi, due to the presence of mychochemicals in them coupled with their antioxidative properties may be used to cure ailments associated with virus, bacteria, high cholesterol level in blood.It has been reported to possessheamatological characteristics as well as the capacity to enhance immune functions (Finimundy et al., 2013; Makropoulou*et*al., 2012). It also provides important mineral nutrients such as selenium, potassium, magnesium, copper, calcium, vitamins like riboflavin, niacin, vitamin D, tocopherol, vitamin C, folic acid, vitamin K and dietary fiber to humans (Maria et al., 2014).

The changes that occur in the hematological indices could be used to monitor whether the administration of extracts at different doses and time intervals to experimental animal models may have either therapeutic or toxicological effect (Essiet et al ., 2020). The present scientific research was conducted with the aim of investigating the effect of ethanol extract of the fruiting bodies of organically cultivated *Pleurotusostreatus* on the haematological indices and heart histology of HS-HFD-Streptozotocin induced diabetic rats. The therapeutic information highlighted in this work would help to educate the people on the medicinal benefits of organically cultivated mushrooms their environment.

MATERIALS AND METHODS

Preparation of High Calorie Density Diet

High calorie density diet was prepared according formulation by Okoroh et al.,(2021)The high calorie density diet was prepared using normal animal diet, sucrose and lard in the combination ratio of 3:1:1. This is shown in table1 below

Table1 : High Sucrose-High Fat Diet (HS-HFD%)

Composition	Proportion (%)
Normal diet	60.0
Sucrose	20.0
Lard	20.0
Total	**100.0**

*Diet was prepared daily to avoid microbial contamination and fed to the animals ad libitum, throughout the period of the experiment.

Collection of Mushroom Materials and Preparation of Mushroom Ethanol Extracts

Pleurotusostreatus fruiting bodies were obtained from the samples cultivated using organic supplements at the Research Unit Demonstration Farm of the University of Port Harcourt, Rivers State, Nigeria. Ethanol extract of *P.ostreatus* was prepared according to the method reported by Okoroh et al., (2021).

Figure 1: The resoursefungi :*Pleurotusostreatus*

Collection of Experimental Animals, Induction of Diabetes and Determination of Blood Glucose and Body Weight

Animal handling was conducted in accordance with the International Guidelines for the Care and Handling of Experimental Animals (National Institute of Health , 2011), and the study protocol was duly obtained from the Faculty of Basic Medical Sciences Research and Ethical committee, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria. A total of 54 normoglycemic female Wister albino rats were used for this research and what informed the use of female rats is because diabetic complications could result to iron deficiency related anemia which may affect pregnancy. The animals were purchased from the Animal House, Department of Biochemistry, Faculty of Basic Medical Sciences, Gregory University, Uturu, kept and maintained in a house that is well-ventilated, having a 12hour light / 12hour dark cycle in propylene cages, at room temperature. Food and water were adequately given to the animals ad libitum till the experimental research commenced. The animals were acclimatized to laboratory conditions, 7days prior to starting of experiment. After acclimatization of theanimals , the nine animals in Normal control group were placed on normal diet of guinea growers mash diet while the other rats in the remaining five groups (n=9) were fed with High Sucrose-High Fat Diet (HS-HFD) throughout the experimental period. The forty-five rats (n=9 rats/group) in the other five groups were placed on HS – HFD for 21 days, fasted overnight and induced diabetes using a single intraperitoneal injection of streptozotocin (35mg/kg bw). Stroptozotocin (Sigma, USA) at a dose of 35mg/kg bw was prepared in fresh and cold normal saline solution and administered immediately to the animals. The animals were first weighed using an electronic scale (TH 500) and their base line fasting blood glucose level taken using Fine Test Auto-coding™ Premium Blood Glucose Monitoring System and Blood Glucose Strips via tail vein cut before they were injected with streptozotocin (Okoroh et al.,2021).

Experimental Design

The experimental model was 20% High Sucrose (HS) + 20% High Fat Diet (HFD) + 35mg/kg body weight (via intraperitoneal) streptozotocin (STZ) induced diabetic rat model .The Metformin HCl and ethanol extract were given once daily(1ml per animal) by intragastric gavage to the reference and experimental groups respectively at doses 150mg/Kg b.w., 50mg/Kg b.w, 150mg/Kg b.w. and 300mg/Kg b.w. respectively while the normal control received saline solution for 88days. The rats (3 from each group) were sacrificed after 3, 6 and 9 weeks of treatment.

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Blood samples and major organs were collected for analysis. The extracts and metformin HCl (reference drug) were kept in plastic bottles with cap tightly sealed before and after each use, stored in the refrigerator, protected from direct sunlight to prevent spoilage throughout the time of animal treatment.(Okoroh et al., 2021).

Evaluation of Hematological Parameters and Indices

Haematological parameters and indices were evaluated using Vet - automated haematology analyzer (Abacus Junior, Italy).The principle of automated haematology analyzer was according to Coulter which is based on the fact that the flow of the blood cells within charged fluid will generate pulses according to the sizes of these cells. Based on this principle, number of the cells were measured depending on its characteristic like size, surface area, and the granules within and haemoglobin estimated by reacting with a specific reagent (Thrall et al., 2004).

Statistical Analysis

Data were statistically analyzed by a one way analysis of varience (ANOVA) using SPSS/PC + package. Multiple comparisms of differences between means were conducted using Fisher's Least Significance Difference(LSD). Significance was accepted at a p-value of less than 0.05 ($p<0.05$).

RESULTS AND DISCUSSIONS

Hematological indices provide vital information on the physiological states of blood cells, and these biomarkers may suffer distortions in Diabetes Mellitus. In this investigation, the hematological biomarkers (RBC, WBC and platelet indices) were altered in diabetic rats as compared to normal control rats, a finding that is in consonance with findings by earlier researchers (Alamgeer et al., 2012; Helal et al., 2005; Mansi&Lahham, 2008).

Administration of extracts to experimental animals at different doses and time may have therapeutic or toxicological effect and this could be monitored using the changes that occur in their haematological indices. According to Hasslacher (2007), anaemia suffered by diabetic patients may be because the production of erythropoietin may have been impaired because of pathologic conditions that affect renal function. High level of blood sugar may lead to increase in peroxidation of erythrocyte membrane bringing about malfunction on the cell membrane (Jain, 1989). This causes the membrane to become deformed (Brown *et al.*, 2005). This has been noticed in the erythrocytes of patients suffering from retinopathy linked to diabetes mellitus (Agrawalet al., 2016). The consequence is reduction in blood flow in capillaries and micro vessels as reported by Cho et al., (2008) and Shin et al., (2008).

The results in this scientific study are shown in Tables 2, 3 and 4 respectively. The study revealed that the level of the RBC indices, (count, Hb, HCT, MCH, MCHC and MCV in all the groups treated with ethanol extracts of P.ostreatus, the reference group and the control group were significantly($p< 0.05$) higher compared to the diabetic control group after the 3rd, 6th and 9th week of treatment respectively. Red blood cell indices are used to evaluate erythropoiesis. Alterations in the process may be in the direction of a decrease, indicated by anaemia or an increase, indicated by polycythemia. Findings of reduced RBC indices (count, Hb, HCT, MCH, MCHC and MCV) in diabetic control rats compared to the normal control group may suggest anaemia, consistent with previous reports (Alamgeer et al., 2012; Francis et al .,2013). There has been a report by Pandey and Rizvi (2011) that erythrocytes are chemically essential in haematologic cells and specially observed as one of the early blood cells affected when there is diabetic problems. In addition, glycosylation of hemoglobin known to occur in diabetic states (Gerner et al., 2013), may contribute to the low Hb concentration. The revelation in the study that the RBC ,haemoglobin, HCT, MCH, MCHC and MCV levels were significantly raised within normal ranges by

the extract in a time and dose dependent manner in the treated diabetic rats could be adduced to the fact that the mushroom ethanol extract may have improved the haemopoietic system. Frankel et al., (1998) equally highlighted that *Pleurotusostreatus* extracts administered to experimental animals improved most haematological indices. This could be attributed to the reduction of the level of lipid peroxidationin cell membrane which resulted to a decrease in haemolysis of red blood cells. The observed increase in red blood cell indices in diabetic rats following treatment with the mushroom extracts also suggests enhanced erythropoiesis. The antioxidant activity of the mycochemicals in the extract could be another reason why damages likely to be caused by free radicals were prevented (Frankel et al., 1998). For example, flavonoids stimulate the production of erythropoietin, the key hormonal stimulant of erythropoiesis, and prevent free radicals-induced hemolysis of RBCs (Sheela& Augusti,1992; Zheng et al., 2010). The results from this study therefore suggests that ethanol extract of the fruiting bodies of organically cultivated *Pleurotusostreatus* may be employed in the management of anaemia

The results in the the 3rd, 6th and 9th weeks of the study revealed that the values of red blood cell distribution width (RDW: RDW-CV and RDW-SD) levels of the diabetic control group was significantly (($p< 0.05$) higher compared to all the mushroom extract treated groups, the reference treated group and the normal control group. The RDW isa measure of the range of variation of RBC volume that is reported as part of standard complete blood count [Nah et al.,2018)].RDW and MCV values are used to determine the possible causes of anemia as they can be employed to differentiate an anemia of mixed causes from anemia of a single cause. Elevated RDW is a hallmark of iron deficiency anemia. Elevated RBC of unequal sizes is called anisocytosis.Anemia of chronic disease and acute blood loss may present normal RDW. High RDW and normal MCV is presented in hemorrhages (Kjeldsberg and Perkins, 2010)]. The high values of RDW-CV andRDW-SD observed in the diabetic rats could be attributed to presence of fragments, groups of agglutination, and / or abnormal shape of RBCs. (Curry, 2017). The reduced values of RDW in the mushroom-treated rats and reference treated group could be attributed to the mushroom extract and the metformin.

This study also showed that the level of WBC in all the groups of diabetic rats treated with the ethanol extracts of P.ostreatus , the reference treatment group and the normal control group were significantly ($p< 0.05$) lower compared to the diabetic control group after the 3rd, 6th and 9th week of experimental treatment. Elevated WBC count is a classical marker of inflammation and is associated with type 2 diabetes mellitus as well as other diseases (Asgary et al., 2005). The increase in the diabetic state is due to hyperglycemia-induced oxidative stress, advanced glycation end products and the activities of cytokines and angiotensin II (Demirtas et al., 2015). Reduction in the levels of leukocytes could be a reflection of changes in the functions of the immune system while elevated levels of white blood cells above normal portend infection. The WBCs levels of the treated rats were lowered to normal by the extract, time and dose dependently. The reduction in WBC count upon treatment of diabetic rats with the mushroom extracts may be attributable to its ant-inflammatory action (Okokon et al., 2013), antioxidant effect (Atiko et al.,2016) and free radical scavenging activity (Baba et al., 2018). The other effects of chronic hyperglycemia mentioned above may have been countered by the hypoglycemic effect of the mushroom (Inyang et al.,2015). The results from this study therefore reveals that extracts from *Pleurotusostreatus* samples may be used to improve immune function against infection.

The result in this study also indicated that in the 3rd, 6th and 9th weeks of treatment, the PLT, MPV, PDW and PCT levels in the *P.ostreatus* ethanol extract groups, metformin reference treatment group and the normal control were significantly (($p< 0.05$) lower compared to the

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diabetic control group.Platelets play a major role in hemostasis, wound healing, angiogenesis as well as pathogenesis of many inflammatory diseases, and its indices are indicators of diabeticmicrovascular complications (Aladodo et al., 2013). The significant (($p< 0.05$) increase in platelet count is in accord with earlier findings (Aladodo et al.,2013; Papatheodorouet al., 2018 ;Pogorzelska et al., 2020)and may have resulted from activation of the megakaryocyte-platelet system which occurs in diabetes resulting in increased turnover of platelets (Akinsegun et al., 2014). The observed increase in plateletcrit (PCT) is naturally explainable by the raised platelet count since it represents the percentage of blood volume occupied by platelets and is calculated from the formula, platelet count × MPV / 10,000 (Tschoepe, 1995). It has been shown that increased MPV depicts larger platelet diameters, which can be used as a marker of platelet production and activation rates (Chandrashekar,2013). It can thus be opined that the significant rise of MPV in diabetic control rats compared with the normal control group results from increased platelet turnover and activation which are common features of DM as earlier mentioned.

The rise in the Platelet Distribution Width (PDW) is still linkable to the activated megakaryocyte-platelet system because of platelet anisocytosis resulting from the process (Thomas et al., 2012). Treatment of diabetic rats with different preparations of the mushroom extract yielded reduced platelet indices compared to DC; implying that the effects of DM-induced activation of the megakaryocyte- platelet pathway was attenuated.

Since hyperglycemia is behind the activation (Papatheodorou et al., 2018), the mushroom extract may have produced the observed effects through reduction in blood glucose since it has hypoglycemic activity (Inyang et al., 2015).

The decrease in platelet count could be because the activities in the bone bone marrow may have been blocked. It could also be because the blood platelets may have been reduced or there is increase in platelet consumption. Clumping of platelets could also be a congent reason (Moncada*et al.*, 1991). The extracts showed positive effects on PLT, MPV, PDW and PCT levels by lowering them to the normal range, time and dose dependently. The extracts may therefore be used as agents to prevent bleeding and can equally contribute to hemostasis.

Table2: Effect of ethanol extract of the fruiting bodies of *Pleurotusostreatus* on the haematological indices of HS-HFD-streptozotocin induced diabetic rats after three weeks of treatment

			Level			
Parameter	NC	DC	$D+POE_{50}$	$D+POE_{150}$	$D+POE_{300}$	$D+MET_{150}$
WBC (10^9/l)	4.00±0.02	5.28±0.22	5.40±0.39	4.95±0.15	3.24±0.04	3.99±0.11
RBC (10^9/l)	$6.60±0.00^{a}$	$3.65±0.01^{b}$	$5.83±0.24^{c}$	$6.66±0.06^{a}$	$6.81±0.11^{a}$	$12.50±0.30^{ac}$
HGB(g/dl)	$11.63±1.45^{ac}$	$10.77±0.05^{ac}$	$12.63±1.55^{ac}$	$11.23±1.52^{ac}$	$11.00±1.71^{bc}$	$12.50±0.30^{ac}$
PLT (10^9/l)	6.26±0.08	8.54±0.00	6.54±0.11	4.90±0.01	4.75±0.30	6.84±0.01
HCT (%)	$43.70±0.00^{a}$	$29.80±2.86^{b}$	$38.23±3.84^{cf}$	$40.50±3.38^{af}$	$36.80±0.53^{df}$	$37.50±0.10^{ef}$
MCV (fl)	$66.17±0.06^{a}$	$58.20±0.10^{b}$	$61.10±5.98^{cf}$	$64.03±0.06^{af}$	$60.03±0.06^{da}$	$62.47±0.40^{eg}$
MCH(pg)	$17.47±0.06^{a}$	$16.20±0.10^{b}$	$20.20±2.00^{c}$	$17.20±0.10^{a}$	$17.43±0.12^{a}$	$18.17±0.15^{a}$
MCHC(g/l)	$26.47±0.06^{a}$	$25.97±0.06^{b}$	$34.00 ±0.40^{c}$	$27.03±0.06^{d}$	$32.43±0.06^{eg}$	$32.53±0.15^{fg}$
RDW-CV(%)	$16.47±0.06^{a}$	$39.03±0.06^{b}$	$24.87±7.65^{c}$	$17.10±0.10^{a}$	$17.43±0.12^{a}$	$18.17±0.15^{a}$

RDW-SD	45.57±0.12^{a}	110.07±0.06^{b}	59.67±20.95^{ac}	45.30±0.10^{a}	38.80±0.10^{a}	38.37±0.42^{a}
MPV (fl)	5.50±0.10	6.03±0.06	5.77±0.55	5.53±0.06	5.80±0.70	5.77±0.15
PDW(%)	15.23±0.06	15.37±0.06	15.27±0.47	15.40±0.10	15.57±0.21	15.33±0.25
PCT(%)	0.44±0.00^{a}	0.27±0.00^{cd}	0.35±0.02^{ad}	0.27±0.00^{cd}	0.37±0.17^{ad}	0.41±0.00^{a}

Means ± SD, n= 9 ,per group. Values in the same row with different superscripts letters are significantly different at p<0.05, NC = normal control, DC = diabetic control, D + POE50 , D+ POE150 , D+ POE300 ,D+MET150 are diabetic groups treated with mushroom extracts and metformin at different dose levels.

Table3: Effect of ethanol extract of the fruiting bodies of *Pleurotusostreatus* on the haematological indices of HS-HFD-streptozotocin induced diabetic rats after six weeks of treatment.

			Level			
Parameter	NC	DC	D+POE$_{50}$	D+POE$_{150}$	D+POE$_{300}$	D+MET$_{150}$
WBC (10^9/l)	4.31±1.05^{a}	5.64±0.78^{bd}	5.10±0.94^{cd}	5.49±0.83^{ad}	5.20±0.76^{ade}	5.12±1.90^{ad}
RBC (10^9/l)	7.43±0.52^{a}	3.97±0.73^{bd}	6.74±0.82^{a}	5.07±0.85^{cde}	6.31±0.97^{ae}	6.28±0.11^{ae}
HGB(g/dl)	12.43±0.89^{ac}	11.00±1.31^{a}	12.90±0.75^{bc}	11.40±1.18^{ac}	11.90±0.20^{ac}	11.70±0.90^{ac}
PLT (10^9/l)	6.33±0.81^{a}	7.95±0.39^{ad}	5.82±0.45^{ae}	4.55±0.77^{bde}	4.48±1.48^{cde}	6.57±0.95^{a}
HCT (%)	46.47±2.38^{a}	36.77±1.10^{bg}	38.70±2.69^{cg}	36.97±4.74^{dg}	37.57±2.60^{eg}	39.37±1.02^{fg}
MCV (fl)	65.47±1.45^{a}	60.17±2.35^{b}	63.07±4.11^{ad}	66.17±4.10^{ae}	61.00±1.82^{af}	62.33±1.11^{cdf}
MCH(pg)	18.10±0.36^{a}	17.97±1.62^{be}	19.70±0.75^{ae}	22.57±2.85^{ce}	20.23±2.15^{de}	18.10±0.40^{ae}
MCHC(g/l)	28.80±0.46^{a}	27.03±3.11^{bf}	29.83±0.15^{af}	36.60±5.02^{cg}	32.17±1.46^{dfg}	30.60±0.95^{ef}
RDWCV(%)	15.93±0.35^{a}	27.33±6.29^{b}	17.97±1.68^{a}	18.37±1.12^{a}	16.93±0.15^{a}	17.60±0.87^{a}
RDW-SD	43.10±0.45^{a}	72.07±3.54^{b}	56.93±14.32^{cd}	51.90±6.65^{ad}	41.63±1.78^{a}	43.97±3.06^{a}
MPV (fl)	5.53±0.06^{a}	6.33±0.06^{be}	5.60±0.30^{af}	5.40±0.10^{a}	6.20±0.66^{cef}	5.80±0.42^{be}
PDW(%)	15.53±0.15^{ad}	16.13±0.75^{a}	15.37±0.35^{bd}	15.43±0.15^{cd}	16.00±0.10^{ad}	15.63±0.15^{ad}
PCT(%)	0.42±0.00^{a}	0.36±0.41^{ad}	0.33±0.03^{bde}	0.27±0.02^{ce}	0.41±0.09^{a}	0.44±0.06^{a}

Means ± SD, n= 9 ,per group. Values in the same row with different superscripts letters are significantly different at p<0.05, NC = normal control, DC = diabetic control, D + POE50 , D+ POE150 , D+ POE300 ,D+MET150 are diabetic groups treated with mushroom extracts and metformin at different dose levels.

Table4: Effect of ethanol extract of the fruiting bodies of *Pleurotusostreatus* on the haematological indices of HS-HFD-streptozotocin induced diabetic rats after 9 weeks of treatment.

			Level			
Parameter	NC	DC	D+POE$_{50}$	D+POE$_{150}$	D+POE$_{300}$	D+MET$_{150}$
WBC (10^9/l)	6.89±0.63^{a}	7.41±0.65^{be}	5.41±0.82^{ce}	6.06±1.28^{de}	7.20±0.97$_{ae}$	7.11±3.48^{ae}
RBC (10^9/l)	7.31±0.32^{a}	5.87±0.18^{bf}	6.63±0.71^{af}	6.51±0.23^{c}	6.12±0.82^{df}	6.20±0.02^{ef}
HGB(g/dl)	12.30±0.56^{ac}	11.00±0.40^{a}	11.40±0.90^{a}	13.30±2.10^{bcd}	12.43±0.06^{ad}	11.50±0.10^{a}
PLT (10^9/l)	6.17±0.00^{a}	6.92±0.12^{a}	6.45±0.05^{ac}	4.40±0.00^{ad}	4.19±0.05^{bd}	5.39±0.20^{a}
HCT (%)	47.27±3.25^{a}	40.90±2.70^{be}	42.40±4.30^{ae}	41.27±1.15^{c}	41.77±3.95^{de}	41.80±2.00^{ae}
MCV (fl)	64.60±1.70^{ad}	61.70±2.60^{a}	64.00±0.40^{bd}	67.37±5.95^{ad}	63.90±2.10^{cd}	67.40±2.00^{ae}
MCH(pg)	19.47±0.25^{a}	18.80±1.30^{ad}	20.50±-.50^{a}	29.30±3.10^{b}	21.87±2.85^{cde}	22.90±0.10^{ae}

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MCHC(g/l)	25.50±1.00[a]	23.17±3.00[a]	26.87±0.55[a]	44.30±8.50[b]	32.27±3.35[a]	26.57±1.05[a]
RDWCV(%)	15.60±0.80[a]	25.00±1.90[a]	16.10±1.30[a]	19.37±1.45[b]	19.50±0.30+	16.70±0.30[a]
RDW-SD	41.86±0.85[a]	47.97±7.35[ac]	42.80±4.00[a]	42.90±8.40[bcd]	43.80±0.20[a]	46.67±1.35[ad]
MPV (fl)	5.70±0.00a	6.60±0.20[bd]	5.67±0.25[a]	5.30±0.10[a]	6.40±0.60[cd]	6.10±0.10[ad]
PDW(%)	15.60±0.30[a]	16.57±0.25[bc]	15.70±0.30[a]	15.50±0.30[a]	16.27±0.06[ac]	16.00±0.30[ac]
PCT(%)	0.36±0.00[a]	0.41±0.07[ac]	0.36±0.01+	0.23±0.01+	0.26±0.04[ad]	0.42±0.13[a]

Means ± SD, n = 9 ,per group. Values in the same row with different superscripts letters are significantly different at $p<0.05$, NC = normal control, DC = diabetic control, D + POE50 , D+ POE150 , D+ POE300 ,D+MET150 are diabetic groups treated with mushroom extracts and metformin at different dose levels.

CONCLUSION

These results therefore suggest that the crude ethanol extract of the fruiting bodies of organically cultivated *Pleurotusostreatus*can reverse the hematological anomalies associated with diabetes mellitus induced with HS-HFD-STZ in female Wistar albino rats. The effects were evidencedby the results of the study revealing that the RBC , haemoglobin, HCT, MCH, MCHC and MCV levels were significantly raised within normal ranges by the extract in a time and dose dependent manner in the treated diabetic rats. The extracts also showed positive effects on WBC, PLT, MPV, PDW, PCT, RDW-CV and RDW-SD levels by lowering them towards the normal ranges, time and dose dependently. The crude ethanol extract from the macro fungi may therefore be employed in the management of anaemia , prevent bleeding or disorder of platelet function and improve immune function in diabetics.

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Competing Interests

The authors hereby declare that there was no conflict of interest or financial inducement which may have negatively influenced them in writing this scholarly article

Authors' Contributions

PNO and DO designed the study, drafted the manuscript, carried out the experiments, collected resource materials, DO conducted statistical analysis. UAA supervised laboratory work, revised and edited the manuscript. The authors read and approved the manuscript.

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