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Assessment of the Haematological Profile of Streptozotocin-induced diabetic Wistar Rat Administered with Water Extracts of a commercial and two wild strains of Ganoderma lucidum via oral gavage

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Abstract

The prevalence of diabetes mellitus is increasing globally and poor countries are increasingly experiencing difficulties in the procurement of conventional medications. This has prompted research in alternative remedies. This study assessed the effects of aqueous extracts of *Ganoderma lucidum* on the blood profile of streptozotocin-induced diabetic Wistar rats. Sixty male rats of 11-12 weeks old weighing 120-150 g were used for the study. The rats were streptozotocin-induced to become diabetic and administered with 500 g/kg and 1000g/kg water extract of commercial and wild strains of *Ganoderma lucidum* and metformin. Their blood profile was analyzed at the end of the 28th days experiment. The results show that there were no significant differences in majority of the parameters tested, irrespective of the diabetic status of the rats, and the strain and dose of the mushroom extracts used. There were no significant differences in RBC, WBC, PCV, platelets, MCH, MCHC, RDW-CV, HB, granulocytes (neutrophils, eosinophils, and basophils), monocytes, lymphocytes, LIC, MPV and PCT ($p > 0.05$). Significant differences were however reported for RDW-SD, MCV, ALY, PDW ($p > 0.05$). The MCV, PDW and RDW-SD were slightly different across the treatments, without exhibiting any discernible pattern. ALY was slightly higher in the negative control than the other treatments ($p < 0.05$), but was however not significantly different between the different *Ganoderma* strains and metformin treatment ($p > 0.05$). We conclude that the water extract of the mushroom does not cause significant alterations in the major blood parameters of streptozotocin-induced diabetic rats.

Key words: *Blood markers, Blood profile, Cardiovascular health, Chronic conditions, Herbal medicine, Medicinal mushroom*

1. Introduction

Ganoderma lucidum is a mushroom belonging to the class Agaricomycetes. This fungus is important in traditional Chinese medicine and in the Orients, where it has been used for centuries in the treatment of diverse chronic health conditions (Dudhgaonkar *et al.*, 2009; Wu *et al.*, 2019). The mushroom has been increasingly used as complementary medicine for cardiovascular health even in some western countries (Klupp *et al.*, 2015). The

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mushroom is known to contain hundreds of bioactive substances of which the most studied especially for their pharmacological effects are the triterpenes and polysaccharides particularly beta-glucans (Boh *et al.*, 2007; Hu *et al.*, 2018). Extracts from the mushroom have been demonstrated to exhibit biological activities that can contribute to cardiovascular health, boost immunity and enhance the circulatory system in general.

Extracts from the mushroom have been used to treat diabetes (Teng *et al.*, 2012), obesity (Guo *et al.*, 2019) and hypertension (Tran *et al.*, 2014). For instance, Ganoderic acids from the mushroom have been reported to lower blood pressure, reduce platelet stickiness and could significantly decrease plasma lipids particularly LDL-cholesterol (Meschino, 2002; Chu *et al.*, 2012). Elkhateeb *et al.* (2019) mentioned some of the cardioprotective effect of extracts from the mushroom, which includes the prevention of atherosclerosis and lowering of cholesterol and blood pressure. The antithrombotic properties of the mushroom can guide against several cardiovascular conditions such as deep vein thrombosis, unstable angina, pulmonary embolism, acute myocardial infarction and ischemic stroke. Johra *et al.* (2023) reported that the fungus is used for the treatment of diverse ailments in the Orients including hypertension, coronary diseases, asthma, bronchitis, arteriosclerosis, hepatitis, nephritis, arthritis, gastric ulcer and cancer.

Extracts from the mushroom have been shown to exhibit anti-inflammatory effects through the suppression of proinflammatory reactions (Stijlemans *et al.*, 2018; Wu *et al.*, 2019) especially the secretion of inflammatory cytokine including TGF-β, TNF-α, IL-1 β and IL-6 (Dudhgaonkar *et al.*, 2009; Johra *et al.*, 2023). The mushroom has been shown to boost immunity in several ways. It has been reported that beta-glucans from the fungus can stimulate white blood cells by binding to receptors on the outer membranes of macrophages, neutrophils, natural killer cells, and cytotoxic T cells, which enhances immunity, which can neutralize pathogens, cancer cells and other foreign bodies (Murray and Pizzorno, 2012). Besides, the antimicrobial activities of the mushroom are well documented both in-vitro and in-vivo (Ellan *et al.*, 2019; Naveen Kumar *et al.*, 2018; Djide, 2014). Extracts of the mushroom have been reported to exhibit immunomodulatory effects through the activation of immune cells such as macrophages, cytotoxic T or B lymphocytes, dendritic cells, natural killer cells and their secretory products (Bulam *et al.*, 2019). Besides, the antioxidant property of polysaccharides from the mushroom can scavenge for free radicals and reduce cell damage caused by mutagens, which has a protective effect on several organs in the body (Boh *et al.*, 2007).

Blood markers especially the various types of white blood and red blood cells are used to assess the health conditions of animals. They can be used to diagnose disease conditions, assess immunity and toxicity. Hence, this study was designed to assess the effect of administration of water extracts of *Ganoderma* mushroom on the blood profile of streptozotocin-induced diabetic Wistar rats.

2. Materials and methods

The study and methodology were reviewed and approved by the Ethics Committee of the Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The experimentation followed standard techniques for animal experiments.

2.1. Source and handling of experimental Wister rats

Sixty male Wistar rats were purchased from the Animal Farm of the University of Benin, Edo State, Nigeria and used for the study. The weight of the rats, which ranged from 120-150g, were 11-12 weeks at the beginning of the study. The rats were acclimatized for 1 week and housed in cages having the following dimensions; 25 × 30 × 30 cm^3, which was stocked at the rate of five rats per cage. The rats were maintained at room temperature with 40–60% relative humidity and where exposed to 12 hours light and dark cycle. During the study, all the experimental rats were allowed access to drinking water and standard pelleted laboratory rodent feed, rodent chow, produced from flour mill of Nigeria.

2.2. Source of mushroom

Three strains of Ganoderma mushroom, obtained from different locations in Nigeria was used for the study. A commercial strain (W1) was obtained from the mushroom production facilities of Rohi Biotechnologies Ltd, while two wilds strains were harvested from wood stump at a rainforest in Benin-City, Nigeria. All three strains were identified to be *Ganoderma lucidum* (Poyeri and Ohimain, 2024) and characterized to contain phytochemicals (Poyeri *et al.*, 2024). The aqueous extraction of Ganoderma was done according to the procedure adopted by Ding *et al.* (2022). 20g of dried mushroom fruiting bodies were milled using hammer mill and

extracted in 1000 mL of distilled water. The aqueous extract was collected by centrifugation, concentrated to 100 mL by vacuum drying and stored at -20 °C until use.

2.3. Animal experimental design

The blood glucose of the rats was measured before they were induced to become diabetic by a single dose of streptozotocin (prepared by dissolving 01 g of streptozotocin in 1 mL of 0.1 M sodium citrate buffer at a pH of 4.5) injection given at a dose of 50 mg/kg body weight. The diabetic status of the rat where confirmed by a blood glucose level > 200 mg/dl within three days (Ratnaningtyas *et al.*, 2018).

The streptozotocin-induced diabetic Wistar rats were allocated to 12 groups, A1-A12 and treatment was administered as indicated (Table 1). The treatments were administered once daily via oral gavage. The experiment lasted for 28 days, after which blood samples were collected via orbital vena using capillary tube and dispensed into EDTA and non-EDTA bottles, and made ready for blood analysis. The negative control was not induced to become diabetic (A11), while the positive control was induced but not treated (A12). Note that the positive control, was sampled at day 7, because they all died before day fourteen (14). The rats were fasted for 12 hours prior to blood sampling.

Table 1: Experimental design of *Ganoderma* and other treatment of Streptozotocin-induced diabetic rats

Treatment Groups	Dose	Health Condition	Treatment Group
Ganoderma lucidum Wild strain (W3)	500 mg/kg	Diabetic	A1
	1000 mg/kg	Diabetic	A2
	500 mg/kg	Non-Diabetic	A3
Ganoderma lucidum Wild strain (W2)	500 mg/kg	Diabetic	A4
	1000 mg/kg	Diabetic	A5
	500 mg/kg	Non-Diabetic	A6
Ganoderma lucidum Commercial strains (W1)	500 mg/kg	Diabetic	A7
	1000 mg/kg	Diabetic	A8
	500 mg/kg	Non-Diabetic	A9
Metformin (Glucophage XR produced by Merck)	50 mg/kg	Diabetic	A10
Negative Control	No treatment	Non-Diabetic	A11
Positive Control	No treatment	Diabetic	A12

2.4. Blood analysis

Standard analytical methods described by Baker and Silverton (2014) was used for the analysis of the blood samples. The microhematocrit method was used for the determination of packed cell volume (PCV). The procedure involved using plain capillary tubes and microhematocrit centrifuge (Hawksley, England) and PCV reader. Colorimetric method was used for the determination of hemoglobin (Hb). The procedure involved using cyanmet Hb standard to measure absorbance at 540 nm wavelength using a colorimeter (Gulfex, England). Red blood cell count (RBC) was determined using Neubauer counting chamber. White blood cell (WBC) count was determined using a counting chamber. The mean cell haemoglobin concentration was calculated from Hb concentration and the RBC. Mean cell volume was computed from PCV and RBC. Differential leucocyte counts were determined using a thin blood film prepared on slides by the spread technique, which involved staining with Leishmann's stain and viewing and counting at ×100 objective on a binocular microscope (Olympus, Japan).

3. Statistical analysis

Results from the study were compiled using Microsoft Excel and analysed with Minitab version 21. Results were presented as mean ± standard deviation (n=3). Turkey HSD test statistics was used for multiple comparison.

4. Results and discussion

The results of the blood analysis of the rats following the administration of different concentrations of the aqueous extracts of *Ganoderma lucidum* is presented in Tables 2-4. There were no significant differences in the RBC, WBC, PCV and platelets ($p > 0.05$) irrespective of the diabetic status of the rats, the strain and dose of the mushroom extracts. There were similarly no significant differences in MCH, MCHC, RDW-CV, HB, granulocytes (neutrophils, eosinophils, and basophils), monocytes, lymphocytes, LIC, MPV and PCT ($p > 0.05$). Significant differences were however reported for RDW-SD, MCV, ALY, PDW ($p < 0.05$). The MCV, PDW and RDW-SD were slightly different across the treatments, without exhibiting any discernible pattern. ALY, which was 0.22 cell 10^9/L, was slightly higher in the negative control than the other treatments ($p < 0.05$), but was however not significantly different between the different Ganoderma strains and metformin treatment ($p > 0.05$).

Table 2: Effects of aqueous extract of Ganoderma mushroom on Red Blood Cell parameters on experimental rats

Treatment	RBC(x10^{12}/L)	MCV(fL)	MCH(pg)	MCHCg/L	RDW-SD (Fl)	RDW-CV (%)	HB(g/L)
A1	6.42^{a}	65.47^{abcde}	19.37^{a}	295^{a}	46.43^{abc}	0.17^{a}	124^{a}
A2	7.17^{a}	61.20^{bcde}	18.80^{a}	307^{a}	38.7^{abc}	0.15^{a}	133^{a}
A3	6.70^{a}	61.33^{bcde}	18.53^{a}	303^{a}	40.83^{abc}	0.16^{a}	124^{a}
A4	6.50^{a}	61.57^{bcde}	19.28^{a}	313^{a}	39.73^{abc}	0.16^{a}	125^{a}
A5	7.02^{a}	56.43^{e}	17.83^{a}	315^{a}	36.83^{bc}	0.16^{a}	125^{a}
A6	7.42^{a}	59.97^{cde}	19.27^{a}	271^{a}	39.20^{abc}	0.16^{a}	130^{a}
A7	7.51^{a}	57.07^{de}	17.83^{a}	313^{a}	36.20^{c}	0.15^{a}	137^{a}
A8	7.22^{a}	61.93^{bcde}	17.70^{a}	308^{a}	44.00^{abc}	0.17^{a}	137^{a}
A9	7.94^{a}	59.93^{cde}	18.23^{a}	308^{a}	38.63^{abc}	0.16^{a}	149^{a}
A10	6.36^{a}	69.13^{abc}	20.03^{a}	290^{a}	51.93^{a}	1.80^{a}	127^{a}
A11	7.20^{a}	66.13^{abcd}	18.87^{a}	286^{a}	47.53^{abc}	1.18^{a}	135^{a}
A12	7.66^{a}	57.47^{de}	18.20^{a}	318^{a}	40.17^{abc}	0.17^{a}	139^{a}
p-value	0.304	0.000	0.224	0.188	0.000	0.482	0.462

Note: Results are presented as mean (n = 3); Different superscript alphabet along the column indicates significant difference ($p < 0.05$); * MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; MCV = Mean Corpuscular Volume; RDW-CV = Red Cell Distribution Coefficient of Variation; RDW-SD = Red Cell Distribution Standard Deviation.

Ahmed and Aslam (2018) studied the effect on the blood profile of Wistar rats that was given by oral gavage 150 mg/kg and 300 mg/kg body weight of *Ganoderma lucidum* supplements. They found most of the haematological parameters studied including hematocrit level, RBC count, MCV, MCH, and MCHC except leukocyte count and haemoglobin level increased significantly in both doses, but platelet count increased only at 150 mg/kg. In our study, various types of white blood cells and their subsets including lymphocytes, monocytes and granulocytes such as neutrophils, basophils and eosinophils, that help to fight infections and other disease conditions, where not significantly different among the treatments, except atypical lymphocytes. In human trials, Chu *et al.* (2012) observed no significant differences in lymphocyte subsets across various treatments when twenty-six patients were treated with 1.44 g Ganoderma daily for 12 weeks. Mohammed *et al.* (2009) studied the effect of aqueous extract of *Ganoderma lucidum* on some haematological parameters of

Table 3: Effects of aqueous extract of Ganoderma mushroom on White Blood Cell parameters on experimental rats

Treatment	WBCx10⁹/L	NUETx10⁹/L	EOSIx10⁹/L)	BASOx10⁹/L	MONOx10⁹/L	LYMPx10⁹/L	ALY(10⁹/L)	LIC(10⁹/L)
A1	5.16[a]	0.28[a]	0.001[a]	0.011[a]	0.106[a]	0.68[a]	0.11[ab]	0.01[a]
A2	12.25[a]	0.29[a]	0.004[a]	0.012[a]	0.007[a]	0.70[a]	0.05[ab]	0.01[a]
A3	9.03[a]	0.37[a]	0.011[a]	0.011[a]	0.010[a]	0.68[a]	0.11[ab]	0.00[a]
A4	9.76[a]	0.29[a]	0.006[a]	0.008[a]	0.080[a]	0.64[a]	0.19[ab]	0.02[a]
A5	7.23[a]	0.303[a]	0.020[a]	0.010[a]	0.016[a]	0.65[a]	0.05[ab]	0.03[bc]
A6	6.05[a]	0.27[a]	0.008[a]	0.005[a]	0.035[a]	0.68[a]	0.14[ab]	0.00[a]
A7	10.53[a]	0.22[a]	0.020[a]	0.013[a]	0.006[a]	0.74[a]	0.06[ab]	0.01[c]
A8	10.33[a]	0.37[a]	0.016[a]	0.011[a]	0.020[a]	0.62[a]	0.06[ab]	0.00[a]
A9	13.12[a]	0.29[a]	0.014[a]	0.008[a]	0.025[a]	0.66[a]	0.10[ab]	0.02[a]
A10	5.75[a]	0.54[a]	0.018[a]	0.005[a]	0.019[a]	0.44[a]	0.16[ab]	0.00[a]
A11	6.91[a]	0.28[a]	0.006[a]	0.008[a]	0.002[a]	0.69[a]	0.22[a]	0.01[a]
A12	5.22[a]	0.57[a]	0.002[a]	0.013[a]	0.023[a]	0.37[a]	0.00[b]	0.00[a]
p-value	0.084	0.649	0.166	0.488	0.190	0.355	0.010	0.813

Note: Results are presented as mean (n = 3); Different superscript alphabet along the column indicates significant deviation/difference ($p < 0.05$) according to Turkey HSD test statistics; * ALY = Atypical lymphocyte; BASO = Basophil; EOSI = Eosinophil; LIC = Large Immature Cells; LYMP = Lymphocyte; MONO = Monocyte; NUET = Neutrophil; WBC = White Blood Cell.

Table 4: Effect of Aqueous Extract of Ganoderma on PCV and platelet parameters of experimental rats

Treatment	Plateletx10⁹/L	MPVFl	PCT(ml/L)	PDW(Fl)	PCV(L/L)
A1	846[a]	8.50[a]	7.14[a]	15.33[bc]	0.42[a]
A2	801[a]	7.67[a]	5.96[a]	15.30[c]	0.44[a]
A3	893[a]	7.67[a]	6.85[a]	15.37[abc]	0.41[a]
A4	728[a]	8.03[a]	5.81[a]	15.43[abc]	0.40[a]
A5	875[a]	7.60[a]	6.65[a]	15.27[c]	0.39[a]
A6	899[a]	7.83[a]	7.03[a]	15.37[abc]	0.47[a]
A7	765[a]	7.78[a]	5.90[a]	15.30[c]	0.46[a]
A8	695[a]	7.83[a]	5.42[a]	15.30[c]	0.45[a]
A9	660[a]	7.63[a]	5.00[a]	15.40[abc]	0.48[a]
A10	602[a]	8.00[a]	4.82[a]	15.43[abc]	0.43[a]
A11	787[a]	8.06[a]	6.26[a]	15.43[abc]	0.44[a]
A12	995[a]	7.10[a]	7.04[a]	15.43[abc]	0.44[a]
p-value	0.036	0.484	0.269	0.002	0.587

Note: Results are presented as mean (n = 3); Different superscript alphabet along the column indicates significant deviation/difference ($p < 0.05$) according to Turkey HSD test statistics; * MPV = Mean Platelet Volume, PCT = Platelet Criticality Test; PDW = Platelet Distribution Width.

alloxan-induced diabetic Wistar rats and found insignificant differences in HB, RBC, PCV, MCV, MCHC and MCH in the ethyl acetate fraction of the mushroom.

Conclusion

The study assessed the effect of water extract of three strains of *Ganoderma lucidum* and metformin administered to streptozotocin-induced diabetic male Wistar rat via oral gavage. The study found no significant differences in the major haematological parameters that was assessed. We therefore conclude that the water extract of the mushroom does not pose any negative impact on the blood health of the tested rats.

Conflicts of interest

The authors declared no conflict of interest

Author Contributions

This work was based on the Ph.D. research of the first author supervised by the second author. The second author wrote the initial draft, while all authors reviewed and okayed the final manuscript.

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