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Protective Effects of Cucumeropsis Mannii (*Egusi* Seed) on Hematological Parameters in Diabetic Wistar Rats

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ABSTRACT

Background: Cucumeropsis mannii is rich in bioactive compounds, including phenolic compounds, flavonoids, and essential fatty acids, which contribute to their protective properties across multiple organ systems. Considering the adverse effects of hyperglycemia and its complications; this study is aimed at evaluating the protective properties of cucumeropsis mannii in diabetic Wistar rats' hematological parameters.
Methods: Twenty (20) Wistar rats were used in the study. The rats were grouped into five groups: Control group as Group 1, Group 11 (Alloxan induced diabetes), Group 111(Negative group that were induced with diabetes and treated with only metformin for two weeks), Group IV (Induced with diabetes and treated daily with only Cucumeropsis mannii oil 10ml/kg body weight for 2 weeks)., and Group V (Induced with diabetes and treated daily with metformin and Cucumeropsis mannii oil 10ml/kg body weight for 2 weeks). After which the Wistar rats were euthanized and the blood sample were collected into EDTA and Floride oxalate containers for the estimation of hematological parameters and blood glucose levels; using hematology analyzer and glucose oxidase method respectively.
Results: The combination of metformin and Cucumeropsis mannii show statistically significant difference of reduction of blood glucose levels $p<0.001$, and improved hematological parameters in group V, when compared with the other groups.
Conclusion: Cucumeropsis mannii when administered along with metformin demonstrates its positive protective effects on hematological parameters and reduction in blood glucose.

Keywords: Egusi seed, Diabetes, Hematological parameter, WBC, Metformin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease [1]; that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Diabetes mellitus is a global metabolic disorder [2]. Incidence pertaining diabetes mellitus continues to escalate with almost equal propensity in elderly and also in young age group due to adverse life style changes like excess calorie intake and sedentary life style [3].

Characterized by persistent hyperglycemia and increased risk of microvascular and macrovascular complications which are accountable for majority of morbidity and mortality associated with the disorder.

Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [2]. Diabetes symptoms include excessive urination, thirst, visual problems, and weight loss. In certain situations, diabetic ketoacidosis and hyperosmolarity are severe enough to cause stupor and coma. However, many symptoms are not severe, which may lead to damage or failure of multiple organs in the long term, causing irreversible disabilities such as blindness, amputation, stroke, and death [4].

Cucumeropsis mannii is rich in bioactive compounds, including phenolic compounds, flavonoids, and essential fatty acids, which contribute to their protective properties across multiple organ systems [5, 6]. One of the primary protective mechanisms of Egusi seeds lies in their antioxidant properties. These seeds contain compounds that help neutralize free radicals, thereby reducing oxidative stress within the body. Recent studies have investigated the protective effects of Cucumeropsis mannii seed oil against various forms of toxicity in Wistar rats. Cucumeropsis mannii demonstrated a significant reduction in antioxidant enzymes, glutathione, body weight, and testicular volume with elevation in the levels of reactive oxygen [7]. In cisplatin-induced nephrotoxicity, Cucumeropsis mannii attenuated kidney dysfunction and oxidative stress by decreasing serum creatinine, urea, and blood urea nitrogen levels while boosting antioxidant enzyme activities [8]. Furthermore, Cucumeropsis mannii showed Reno protective effects against Bisphenol A-induced testicular toxicity, reversing biochemical and histological alterations in male rats [9].

This study is aimed at evaluating Cucumeropsis mannii 's potential as a protective agent against various forms of toxicity, attributing its effects to its rich phenolic and flavonoid content. Overall, Egusi seed extract has demonstrated multiple protective effects in Wistar rat models, including antioxidant, anti-inflammatory, and lipid-lowering properties. These findings suggest significant potential for Egusi seeds to support health and manage conditions related to oxidative stress, metabolic dysfunction, and organ protection.

MATERIALS AND METHODS

Alloxan, Animal cages, Cannula, Cotton wool, Cucumeropsis mannii oil, dissecting kit, feeding bowl, feeding trough, Glucometer machine (Accu-check), water bath, Laboratory equipment, Masking tape Metformin, Methylated spirit, Personal protective equipment (PPE), Rat feed, Sample bottles, saw dust, Water bowl, Weighing scale and Wistar rats.

EXPERIMENTAL ANIMALS AND ETHICAL APPROVAL

Twenty (20) Wistar rats were procured from the Veterinary Research Institute, Vom, Plateau State, Nigeria. The rodents were maintained at Bingham University's Animal Care Unit in Nasarawa, Nigeria. The Animal Care Unit was adequately aired and cleaned daily, and the rats were kept in appropriately aerated plastic cages (4 per cage), with saw dust as bedding. The bedding for the animals was changed every three days. They were kept at a temperature of 27 degrees Celsius with 12 hours of light and 12 hours of darkness alternately. The rats were adequately fed during the period.

The study, rat handling, and treatment adhere to National Institute of Health recommendations (NH release 85-23, 1985). The rats were kept by the standards of animal care outlined in the 1964 Helsinki Declaration. The animal ethics committee at Bingham University in Karu, Nasarawa State, approved the study procedure, with number- BHU/FOAHS/DML 12.04.2024

CHEMICAL USED

Alloxan and Metformin.

CUCUMEROPSIS MANNII OIL

The oil was extracted from fresh Cucumeropsis mannii seeds and administered to the rats when needed.

ANIMAL GROUPING, AND EXPERIMENTAL DESIGN

The Wistar rats were allowed to acclimatize for two weeks before being supplied with essential feed and water. Administration began and lasted for 14 days (2 weeks). A 5% solution of Alloxan monohydrate was produced and used to induce type 2 diabetes in mice who had fasted for 16 hours with an intraperitoneal injection of 150mg/kg body weight. The rats' blood glucose levels were assessed before the trial began and 72 hours after alloxan administration, using a glucometer. Rats with blood glucose levels more than 11mm/L were assigned to test groups II-V. The rats were then grouped as follows:

GROUP I: Control group that was fed with normal feed and distilled water for 2 weeks with no induction of diabetes.

GROUP II: Alloxan-induced diabetic control group (not treated).

GROUP III: Negative group that was induced with diabetes and treated with metformin for two weeks

GROUP IV: Induced with diabetes and treated daily with only Cucumeropsis mannii oil 10ml/kg body weight for 2 weeks.

GROUP V: Induced with diabetes and treated daily with metformin and Cucumeropsis mannii oil 10ml/kg body weight for 2 weeks.

The animal's body weights were measured with a weighing scale following the final dosage. The animals were slaughtered and blood samples were taken individually via a heart puncture into well-labelled blood sample bottles (EDTA) and Floride oxalate. The hematological parameters were estimated using hematology analyzer, while the glucose was done using Glucose oxidase method.

STATISTICAL ANALYSIS

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 27. Measures of central tendency (mean) and variability (standard deviations) were computed. Pearson's correlation coefficient was used to determine the correlation between variables. The independent one-way Analysis of Variance (1-way ANOVA) and repeated measures ANOVA were employed to examine the differences in mean values across different groups. A p-value less than 0.001 was considered statistically significant.

RESULTS

The results of the study are presented in tables and figures as shown below.

Table 1 and Figure 1 presents the mean blood sugar levels before and after diabetes induction and treatment across the different groups. The results show that there is no significant variation in blood sugar levels among the Wistar rat groups before diabetes induction (p-value = 0.053). Post treatment show significant differences in blood sugar levels after induction (p-value = 0.001) and following treatment (p-value = 0.001) between the groups; as shown below.

Table 1: Evaluation of Blood Sugar Levels Pre- and Post-Diabetes Induction and Treatment Across Groups.

	G1	G2	G3	G4	G5	Total	F-stats	df	p-value
Blood sugar before alloxan (mmol/L)	3.50 (±0.55)	4.36 (±0.15)	4.23 (±0.25)	5.80 (±1.64)	4.76 (±0.15)	4.53 (±1.02)	3.40	4	0.053
Blood sugar after alloxan (mmol/L)	-	10.87 (±2.28)	22.37 (±5.73)	20.03 (±9.51)	24.03 (±5.57)	19.33 (±7.56)	2.59	3	0.001*
Blood sugar after treatment (mmol/L)	-	-	15.86 (±2.40)	18.30 (±9.46)	13.70 (±6.25)	15.95 (±6.13)	0.35	2	0.001*

*** One-way ANOVA; Significant at ≤0.001**

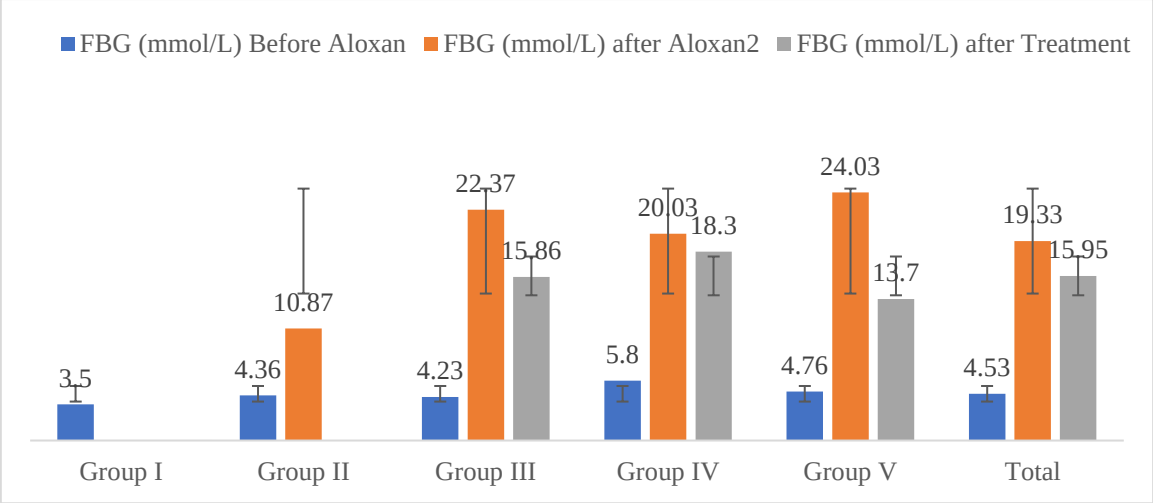

Fig. 1: Evaluation of Blood Sugar Levels Pre-Diabetes, Post-Diabetes Induction and Treatment Across Groups.

Table 2 and Figure 2 presents the comparison of blood sugar levels before and after treatment across the various drug groups. The results indicate that, there is a statistically significant interaction between time and treatment group (p = 0.007). There is reduction in blood glucose levels after treatment in all groups. Additionally, this shows that the combination of Cucumeropsis mannii oil and Metformin resulted in the lowest blood glucose levels (13.70 ±6.25), compared to Metformin alone (15.86 ±2.40) and Cucumeropsis mannii oil alone (18.30 ±9.46).

Table 2: Comparison of Blood Sugar Levels Before and After Treatment Across Different Drug Groups

	Time		
Treatment Groups	**Before Treatment**		**After treatment**
G3 (Metformin)	22.36 (±5.73)		15.86 (±2.40)
G4 (Cucumeropsis mannii oil)	20.03 (±9.51)		18.30 (±9.46)
G5 (Metformin + Cucumeropsis mannii oil)	24.03 (±5.57)		13.70 (±6.25)
	F-stat	df	p-value
Time*Treatment Group	12	2	0.007*

*** Repeated Measures ANOVA; Significant at ≤0.001**

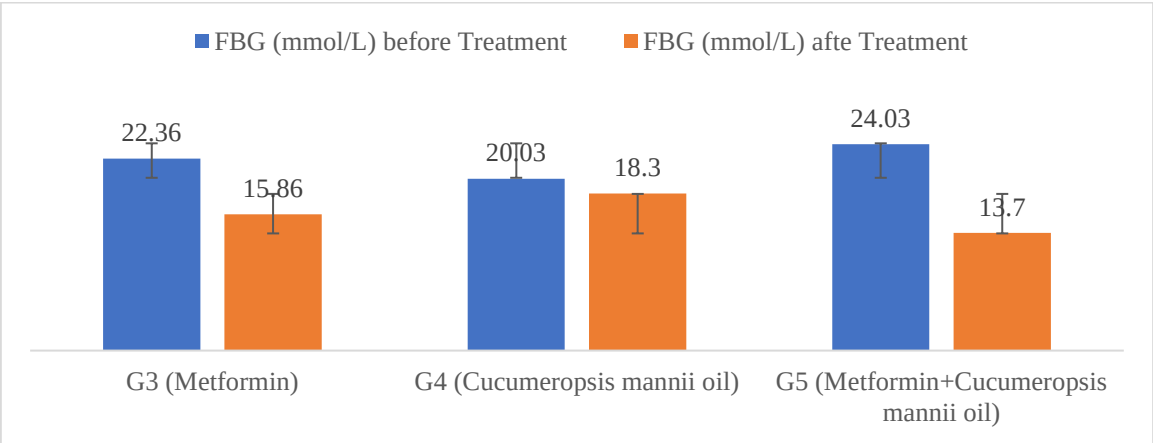

Fig. 2: Comparison of Blood Sugar Levels Before and After Treatment Across Different Drug Groups.

Table 3 and Figure 3, 4.4 provides an analysis of hematological parameters across pre-diabetes, post-diabetes induction, and treatment groups. The results indicate significant differences in WBC (p = 0.007), RBC (p = 0.011), HB (p = 0.015), PCV (p =

0.049), PLT (p = 0.006), NEU (p = 0.000), LYM (p = 0.000), EOS (p = 0.010), MCV (p = 0.030), MCH (p = 0.000), and MCHC (p = 0.000) among the groups.

WBC counts are highest in the alloxan-induced diabetic control group without treatment (G2) at 11.29 ±1.15, followed by the metformin-treated group (G3) at 9.33 ±1.98, and lowest in the group treated with Cucumeropsis mannii oil (G4) at 5.63 ±0.75. RBC, HB, and PCV levels are highest in the Cucumeropsis mannii oil-treated group (G4) at 9.26 ±0.75 (RBC), 16.90 ±1.21 (HB), and 49 ±8 (PCV), followed by the control group (G1) with 8.60 ±0.97 (RBC), 15.75 ±0.93 (HB), and 45 ±2 (PCV). The lowest values for RBC, HB, and PCV are found in the untreated diabetic control group (G2) at 4.72 ±0.65, 7.77 ±0.60, and 25 ±1, respectively.

PLT levels are highest in the group treated with both metformin and Cucumeropsis mannii oil (G5) at 489.66 ±145.74, while the lowest is observed in the control group (G1) at 215.79 ±3.86. NEU levels are elevated in the untreated diabetic group (G2) at 58.33 ±2.08, with the lowest NEU found in the metformin + oil treatment group (G5) at 19.00 ±3.60. In contrast, LYM is highest in the G5 group (73.33 ±3.78) and lowest in G2 (36.33 ±1.52). EOS levels peak in G5 at 4 ±1.00 and are lowest in G2 at 1.67 ±0.57.

Finally, MCV, MCH, and MCHC values are highest in G5 at 60.66 ±5.50 (MCV), 20.03 ±1.47 (MCH), and 33.03 ±1.01 (MCHC), while the lowest values are seen in G2 at 31.80 ±1.17, 12.91 ±0.86, and 27.71 ±1.16, respectively.

Table 3: Evaluation of Hematological Parameters across Pre- and Post-Diabetes Induction and Treatment Groups

	G1	G2	G3	G4	G5	Total	f-stats	df	p-value
WBC	6.62 (±1.08)	11.29 (±1.15)	9.33 (±1.98)	5.63 (±0.75)	7.36 (±2.11)	8.04 (±2.45)	6.69	4	0.007*
RBC	8.60 (±0.97)	4.72 (±0.65)	7.10 (±0.73)	9.26 (±0.75)	7.93 (±2.36)	7.52 (±1.95)	5.74	4	0.011*
HB	15.75 (±0.93)	7.77 (±0.60)	13.23 (±0.70)	16.90 (±1.21)	14.23 (±5.66)	13.57 (±3.97)	5.30	4	0.015*
PCV	45 (±2)	25 (±1)	42 (±3)	49 (±8)	43 (±17)	40 (±11)	3.50	4	0.049*
PLT	215.79 (±3.86)	350.75 (±18.94)	293.33 (±37.85)	247.66 (±40.41)	489.66 (±145.74)	319.44 (±116.17)	7.07	4	0.006*
NEU	45.33 (±0.57)	58.33 (±2.08)	37.67 (±1.52)	35.67 (±5.77)	19.00 (±3.60)	39.20 (±13.59)	58.11	4	0.000*
LYM	48.33 (±0.57)	36.33 (±1.52)	55.33 (±2.08)	60.33 (±4.04)	73.33 (±3.78)	54.73 (±12.94)	75.38	4	0.000*
MON	3.67 (±0.57)	3.67 (±0.57)	3.67 (±0.57)	1.67 (±1.15)	3.67 (±0.57)	3.27 (±1.16)	2.57	4	0.103
EOS	2.33 (±0.57)	1.67 (±0.57)	2.33 (±0.57)	2 (±0.00)	4 (±1.00)	2.47 (±0.99)	6.08	4	0.010*
BAS	0.33 (±0.57)	0.33 (±0.57)	1.00 (±0.00)	0.33 (±0.57)	-	0.40 (±0.50)	2.00	4	0.171
MCV	56.31 (±1.86)	31.80 (±1.17)	45.23 (±21.93)	58.66 (±1.15)	60.66 (±5.50)	50.53 (±14.08)	4.20	4	0.030*
MCH	17.46 (±0.66)	12.91 (±0.86)	18.70 (±1.03)	18.76 (±0.23)	20.03 (±1.47)	17.57 (±2.67)	25.41	4	0.000*
MCHC	31.68 (±0.92)	27.71 (±1.16)	31.56 (±0.47)	32.00 (±1.03)	33.03 (±1.01)	31.20 (±2.04)	13.66	4	0.000*

*** One-way ANOVA; Significant at ≤0.001**

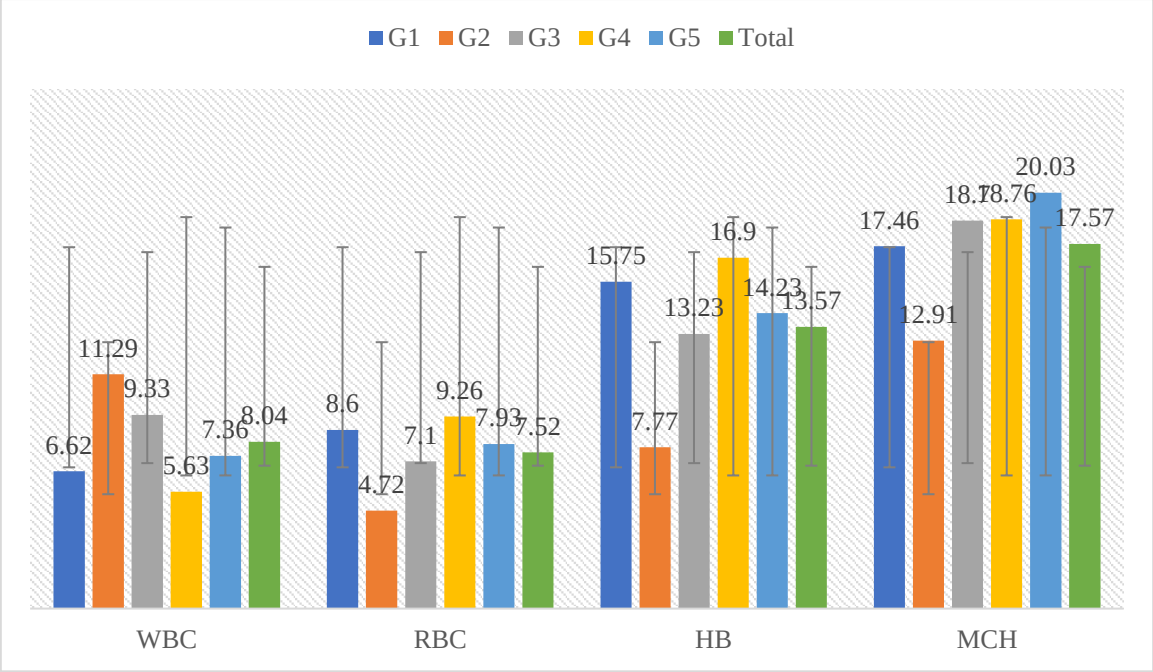

Fig. 3: Evaluation of WBC, RBC, HB and MCH Parameters across Pre-Diabetes, Post-Diabetes Induction and Treatment Groups

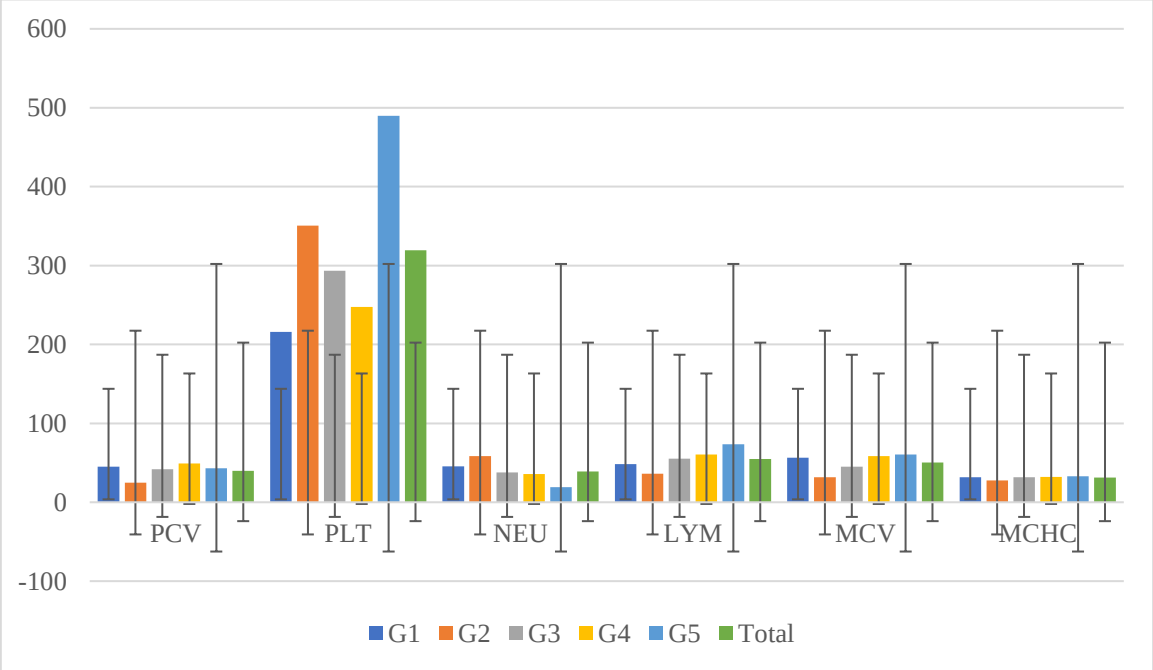

Fig. 4: Evaluation of PCV, PLT, NEY, LYM, MCV and MCHC Parameters across Pre-Diabetes, Post-Diabetes Induction and Treatment Groups.

Table 4 presents the bivariate correlation between pre-alloxan diabetes blood sugar levels and hematological parameters. The findings indicate that there is no significant association between blood glucose levels and hematological parameters in normal Wistar rats (those not subjected to Alloxan diabetes induction).

Table 4. Bivariate Correlation between Blood Sugar Levels before Alloxan-Diabetes Induction and Hematological Parameters. n = 13

Parameters	R-value	P-value
BSBA vs WBC	-0.154	0.583
BSBA vs RBC	-0.246	0.376
BSBA vs HB	0.114	0.685
BSBA vs PCV	0.018	0.950
BSBA vs PLT	0.060	0.832
BSBA vs NEU	-0.373	0.171
BSBA vs LYM	0.397	0.143
BSBA vs MON	-0.157	0.575
BSBA vs EOS	0.068	0.810
BSBA vs BAS	0.055	0.846
BSBA vs MCV	0.122	0.665
BSBA vs MCH	0.527	0.527
BSBA vs MCHC	0.042	0.882

*** Pearson Correlation; Significant at ≤0.05**

Table 5. presents the bivariate correlation between post-Alloxan diabetes induction blood glucose levels and hematological parameters. The findings indicate statistically significant correlations between blood glucose and RBC (R = 0.784, p = 0.003), Neutrophils (R = -0.576, p = 0.050), Lymphocytes (R = 0.603, p = 0.038), MCV (R = 0.613, p = 0.034), MCH (R = 0.676, p = 0.016), and MCHC (R = 0.633, p = 0.027).

Table 5.: Bivariate Correlation between Blood Sugar Levels after Alloxan-Diabetes Induction and Hematological Parameters. n=13

Parameters	R-value	P-value
BSAA vs WBC	-0.387	0.214
BSAA vs RBC	0.784	0.003*
BSAA vs HB	0.447	0.145
BSAA vs PCV	0.507	0.092
BSAA vs PLT	0.088	0.786
BSAA vs NEU	-0.576	0.050*
BSAA vs LYM	0.603	0.038*
BSAA vs MON	-0.164	0.612
BSAA vs EOS	0.256	0.422
BSAA vs BAS	-0.136	0.674
BSAA vs MCV	0.613	0.034*
BSAA vs MCH	0.676	0.016*
BSAA vs MCHC	0.633	0.027*

*** Pearson Correlation; Significant at ≤0.05**

Table 6 presents the bivariate correlation between blood sugar levels after treatments and hematological parameters. The findings suggests that there is no significant association between blood glucose levels and hematological parameters in diabetic Wistar rats after treatment.

Table 6: Bivariate Correlation between Blood Sugar Levels after Treatment and Hematological Parameters. n=13

Parameters	R-value	P-value
BSAT vs WBC	-0.138	0.723
BSAT vs RBC	0.371	0.326
BSAT vs HB	0.149	0.702
BSAT vs PCV	0.262	0.496
BSAT vs PLT	-0.222	0.565
BSAT vs NEU	0.437	0.239
BSAT vs LYM	-0.349	0.357
BSAT vs MON	-0.375	0.320
BSAT vs EOS	-0.513	0.158
BSAT vs BAS	-0.159	0.682
BSAT vs MCV	0.129	0.741
BSAT vs MCH	-0.134	0.732
BSAT vs MCHC	-0.208	0.592

*** Pearson Correlation; Significant at ≤0.05**

DISCUSSION

The results of this study demonstrate potential therapeutic benefits of Cucumeropsis mannii oil in combination with metformin. After diabetes was induced, the elevation in blood sugar was expected, as observed by other authors who worked on alloxan-induced diabetes [10].

The hematological parameters revealed significant differences between the groups, particularly in white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), and packed cell volume (PCV) levels. The untreated diabetic control group (G2) displayed the highest WBC count, indicative of heightened immune activation due to hyperglycemia, a pattern that is commonly reported in diabetic conditions [11, 12,13,

14]. Furthermore, the reduction in RBC, HB, and PCV in this group corresponds with the anemia frequently associated with diabetes [15,16] Notably, the Cucumeropsis mannii oil-treated group (G4) exhibited improved RBC, HB, and PCV levels, suggesting the potential of the oil in ameliorating anemia caused by diabetes, which aligned with previous studies on natural products used to counteract hematological disturbances [17].

The lack of significant correlations between blood glucose and hematological parameters in non-diabetic rats supports the understanding that normal blood sugar levels do not have a marked effect on blood parameters under healthy conditions [18]. This baseline data is crucial as it establishes a control for comparison with post-diabetes induction results.

In contrast, there were significant correlations between blood glucose levels and several hematological parameters after diabetes induction. The strong positive correlation between blood glucose and RBC ($R = 0.784$, $p = 0.003$) suggests a compensatory erythropoietic response to increased metabolic demands in hyperglycemic states, which has been reported by another authors [19]. The inverse relationship between glucose and neutrophil count ($R = -0.576$, $p = 0.050$) is indicative of immune suppression in prolonged hyperglycemia, a well-documented phenomenon in diabetic pathology [20].

No significant correlation was seen between post-treatment blood glucose and hematological parameters. This lack of association could indicate that the treatments were effective in restoring both blood glucose and hematological parameters to a state where no direct link between the two could be detected, this observation in seen in a study where significant difference was observed after treatment [21].

Finally, the current study reveals a significant interaction between treatment and time, with reductions in blood glucose levels in all groups post-treatment ($p = 0.007$). The combination of Cucumeropsis mannii oil and metformin was most effective in lowering blood glucose levels, outperforming either treatment alone. This finding is consistent with studies that have demonstrated the synergistic effects of combining plant-based therapies with conventional diabetes treatments like metformin [22, 23]. The ability of Cucumeropsis mannii oil to enhance the efficacy of metformin could be due to its bioactive compounds, which have been shown to have hypoglycemic effects [24]. The study emphasizes the need for further exploration of Cucumeropsis mannii oil as a complementary treatment in diabetes management, potentially opening doors for the use of such natural remedies alongside pharmacological interventions in managing hematological and metabolic disorders associated with diabetes. The study is limited to the protective effect of Cucumeropsis mannii on hematological parameter on diabetic Wistar rats. However, these allows further study on its effects on both histological and biochemical parameters of Wistar rats. Future research should also focus on elucidating the molecular mechanisms underlying these effects, as well as exploring the clinical applicability of these findings in human models.

CONCLUSION

The findings show that while diabetes induction leads to disruptions in various hematological markers, the administration of Cucumeropsis mannii oil, both alone and in combination with metformin, has a restorative effect on these disturbances. Specifically, the oil exhibited potential benefits in improving red blood cell counts, hemoglobin levels, and packed cell volume, suggesting its capacity to counteract anemia caused by hyperglycemia. Furthermore, the combination therapy of metformin and Cucumeropsis mannii oil proved most effective in reducing blood glucose levels, demonstrating a synergistic effect that surpasses the efficacy of either treatment alone.

Authors Involvement: ASP Involved in the conception of the study, data analysis, and writing of the manuscript. CFC involved in the collection of samples, analysis and collation of data. CE involved in analysis and statistical interpretation of data. OHB Involved in the proof reading of the manuscript. All authors agree to the publication of the manuscript.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare None

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