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Histological Assessment and Haematological Parameters of Honey on Alloxan Induced Diabetic Male Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Purpose: The study investigated honey's histological assessment and haematological parameters on alloxan-induced diabetic male albino rats.
Methods: Thirty-six (36) male Wistar rats were assigned into six (6) groups with six (6) animals each, group 1 (Normal control), group 2 (Negative control), group 3 (Glibenclamide), group 4 (treated with 0.2ml of honey) group 5, (treated with 0.5ml of honey and group 6 (treated with 0.8ml of honey). The rats were fed with standard feed and drinking water ad libitum. The diabetic control, diabetic glibenclamide and the treated groups (0.2 mL/kg, 0.5 mL/kg and 0.8 mL/kg) were induced with diabetes by intraperitoneal injection of 120 mg/kg bodyweight alloxan monohydrate, and confirmation was done using a glucometer. Treatment lasted for three weeks, and blood samples for haematology [red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration

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(MCHC)] analyses were collected on day 21. On the 21st day, two rats per group were humanely sacrificed, and the vital organs (kidney and liver) were collected for histopathology.
Results: Haematologic results obtained showed marked reduction ($p < 0.05$) in RBC count, PCV, Hb and MCHC, a significant increase ($p < 0.05$) in MCV compared to the positive control. White blood cell counts showed a reduced level in the test group at a dose-dependent concentration compared to the positive control. Histopathological investigations of diabetic rats' liver and kidney indicated degradation of normal tissue architecture as well as a variety of other problems; however, after treatment with honey, reparative alterations were seen.
Conclusions: Overall, the findings suggest that honey could ameliorate metabolic disorders caused by diabetes as no pathological changes were elicited in the organs of rats exposed to honey.

Keywords: Alloxan monohydrate; diabetes; glibenclamide; histopathological changes; haematology.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most common diseases in modern times, with more than 285 million people diagnosed in 2010 and about 438 million people expected by 2030 in all parts of the world [1]. It is a metabolic disorder classified as a non-communicable disease characterized by chronic hyperglycemia due to a lack of insulin resistance [2]. Diabetes prevalence can be genetically determined or inherited at any age during life [3]. Although high blood sugar levels are the "symptom" of diabetes, other symptoms should not be ignored, including increased thirst and hunger, unexplained exhaustion, increased urination, blurred vision, and unexpected weight loss [3]. Among humans, two types of diabetes are common (Typ1 and Type 2). Type 1 diabetes is characterized by an immune system attack and destroys the cells in the pancreas that make insulin [4]. Environmental factors are important in determining type 1 diabetes, although type 1 diabetes is genetically related [5]. The symptoms of this type of diabetes usually start in a few weeks. The most crucial factor in determining type 2 diabetes is lifestyle, but different genes may also determine it. The symptoms of this disease are not noticeable, and many people find themselves with diabetes without specific or unusual symptoms. Most of the time, type 2 diabetes is related to being overweight or obese [5].

For decades, scientists have been trying to solve the mysteries surrounding diabetes's pathogenesis and its complications, aiming at achieving the primary motif: prevention or remission of diabetes, or at least prevention of its complications, particularly those related to the heart and pancreas. According to the amount of evidence, it is now known that free radicals make an essential contribution to the progression and complications of diabetes [6]. The pathogenic consequences of excessive glucose are mediated by reactive oxygen species (ROS) generated by protein glycation and glucose oxidation. By activating several cellular stress-sensitive pathways, reactive oxygen species (ROS) can directly cause molecular and cellular damage, leading to late complications of diabetes. Furthermore, -cell dysfunction and insulin resistance share common mechanisms [7]. Diabetes patients are more likely to have impaired liver and renal function, as well as the generation of free radicals from glucose oxidation and non-enzymatic glycosylation of proteins [4]. Following oxidative breakdown of glycated proteins, antioxidant defense systems are depleted, cellular organelles and enzymes are damaged, lipid peroxidation increases, and insulin resistance develops [6]. The heart and the pancreas are among the body's major organs contributing to the onset and morbidity associated with diabetes mellitus. The pancreas is the chief organ primarily affected by the genetic alterations that lead to diabetes mellitus. On the other hand, the heart is among the major organs affected by diabetes mellitus [4]. Despite recent improvements in DM care, mortality from macro-vascular complications, particularly coronary heart disease (CHD), remains high [4].

There has been a resurgence of interest in developing an effective and appropriate diabetes alternative therapy [7]. Pure honey contains health-promoting properties, making it an ideal treatment for diabetes, heart disease, kidney illness, and high blood pressure. Honey's use has been hotly debated and is not widely acknowledged in modern medicine [8]. Honey's high sugar content has raised concerns about diabetes risks. Reduced haemoglobin has been reported in diabetes [9,10]. Reduction in haemoglobin may lead to a fall in red blood cell count and packed cell volume [11], anaemia

could be indicated by low hematocrit readings [11].

Honey has been used for several years to manage and treat diabetes mellitus, and many works have laid diverse claims to its efficacy [12]. However, although several works elucidate the therapeutic roles of honey in the management of diabetes mellitus [13], data is limited, and findings are inconclusive about the valuable role of honey in attenuating the structural distortions that occur in the heart and pancreatic tissues as a result of diabetes mellitus. Hence, this work investigated honey's histological assessment and haematological parameters on alloxan-induced diabetic male albino rats.

2. MATERIALS AND METHODS

The fresh honey was bought from Fibers Global Farms, Isuochi in Umunneochi Local Government Area of Abia State. It was evaluated at the Beekeeping Extension Society, Umuahia, Abia state, to have a moisture content of 18.7% certifying it to be pure, unadulterated honey.

2.1 Experimental Animals

Thirty-six (36) male Wistar rats (210-250g) purchased from Dr Daniel of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, were used for this study. The animals were acclimatized for two weeks and kept under natural conditions, including 12 h light and 12 h dark throughout the investigation, with free access to pellet feed and water ad libitum.

2.2 Induction of Diabetes

At the end of acclimatization, the animals of groups (2-6) were allowed to fast for 8 hours, and then diabetes was induced by intraperitoneal (IP) injection of 120mg/kg body weight of alloxan monohydrate solution. Animals with fasting blood glucose levels higher than 150mg/dl were considered diabetic after 3 days of induction and were selected for the study.

2.3 Experimental Design

Rats were divided into six groups of six rats each: Group 1 (Normal control), group 2 (Negative control), group 3 (Positive/Glibenclamide), group 4 (animals treated with 0.2mls of honey), group 5 (animals treated with 0.5mls of honey), group 6 (animals treated with 0.8mls of honey) respectively. Glucose level was observed on days 0, 7th, 14th and 21st. Honey was administered orally twice for the three weeks treatment. Normal control received only food and water; Negative control was induced by alloxan, positive control was induced by alloxan and treated with glibenclamide, while groups 4,5 and 6 were induced by alloxan, confirmed diabetic and were treated with 0.2mls 0.5mls and 0.8mls of honey, respectively after 72 hours of induction.

2.5 Hematological Studies

2.5.1 Erythrocytic profile

2.5.1.1 Haemoglobin (Hb) concentration

The haemoglobin concentration in the blood was determined by cyanomethemoglobin method. The blood sample (0.2 mL) was mixed with 4 mL of Drabkin's solution in a test tube and allowed to stand for 15 minutes at room temperature. The absorbance of the mixture was read at 540 nm against a reagent blank using a spectrophotometer. The Hb concentration was obtained by multiplying the absorbance sample with a calibration factor (36.8) derived from the absorbance and concentration of the standard [14].

2.5.1.2 Packed cell volume (PCV)

This was done using the standard technique of Coles as described in Brar et al. [14]. Briefly, blood samples were collected into heparinized capillary tubes. A hematocrit centrifuge sealed one end of the tubes with plasticine and centrifuged for 5 minutes at 2500 rpm. The levels of the packed red blood cells in the capillary tubes were read utilizing a PCV hematocrit reader.

Chart 1. Treatments Details

Groups	Descriptions	Treatments
1	Normal control rats	Normal saline and feed only
2	Negative control rats	Alloxan (120 mg/kg, i.p.) untreated
3	Positive control rats	Alloxan (120 mg/kg, i.p.) + 5 mg/kg bw glibenclamide
4	Diabetic treated rats	Alloxan (120 mg/kg, i.p.) + 0.2 mL/kg/day honey
5	Diabetic treated rats	Alloxan (120 mg/kg, i.p.) + 0.5 mL/kg/day honey
6	Diabetic treated rats	Alloxan (120 mg/kg, i.p.) + 0.8 mL/kg/day honey

2.5.1.3 Red blood cell (RBC) count

The RBC count was determined using the Hematocrit method [15]. A blood sample (0.02 mL) was collected with a pipette and added to 4 mL of red blood cell diluting fluid in a clean test tube to make 1:200 dilution of the blood sample. The diluted blood sample was loaded into a Neubauer counting chamber and counted with the aid of a light microscope.

$$\text{RBC count (million/µL)} = \textit{t}\text{otal number of RBC counted in 5 squares} \times \frac{10000}{1000000}$$

2.5.1.4 Mean Corpuscular Volume (MCV)

The MCV was calculated by dividing the PCV by erythrocyte count then multiplied by 10. The values obtained were expressed in femtolitres:

$$\text{MCV (fl)} = \frac{\text{PCV (\%)}}{\text{RBC (million/µL)}} \times \frac{10}{1}$$

2.5.1.4 Mean Corpuscular Haemoglobin (MCH)

The MCH was calculated by dividing the haemoglobin concentration by the erythrocyte count, already determined and then multiplied by a factor of 10. The values were expressed in a picogram.

$$\text{MCH (pg/dl)} = \frac{\text{Hemoglobin (g/dl)}}{\text{RBC (million/µL)}} \times \frac{10}{1}$$

2.5.1.5 Mean corpuscular haemoglobin concentration (MCHC)

The MCHC was calculated by dividing haemoglobin concentration by the obtained PCV value and then multiplied by 100. The values were expressed in grams per litre [14].

$$\text{MCHC (g/dl)} = \frac{\text{hemoglobin (g/dl)}}{\text{PCV (\%)}} \times \frac{100}{1}$$

2.5.2 Leucocytic profile

2.5.2.1 Total White Blood Cell (WBC) count

The white blood cell count was determined by the hemocytometer method. The blood sample was diluted (1:20) using Turk's solution (2% glacial acetic acid). The diluted sample was loaded into a Neubauer counting chamber with a Pasteur pipette. The WBC was determined by counting the required number in the appropriate squares on the counting chamber under a microscope. The number of cells counted for each blood sample was multiplied by 50 to obtain the total white blood cell count per microlitre of blood [15].

2.5.2.2 Differential leucocyte count

Leucocyte count was determined as described in Brar et al. [14].

$$\text{Absolute number} = (\text{relative number} \times \text{total WBC})/100$$

2.6 Histopathological Studies

Tissue samples (liver and kidney) collected after the sacrifice of the rats at the end of the 21 days treatment with drug or extract were fixed in 10% formalin saline for a minimum of 24 h. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome, stained with hematoxylin and eosin (H and E) and mounted on Canada balsam. All the sections were examined under a light microscope under different (x10, x20 and x40) magnifications. Photomicrographs of the lesions were taken with an Olympus photo microscope for observations and documentation of histopathological lesions.

2.7 Statistical Analysis

Data obtained was expressed as mean ± SD and statistically analyzed using one-way analysis of variance (ANOVA) with Turkey's multiple comparison post hoc tests to compare the level of significance between the test groups. The values of $p<0.05$ were considered significant.

3. RESULTS

The data for the Erythrocytic profile presented in Table 1. showed that honey supplementation in diabetic Wistar rats resulted in a significant ($p<0.05$) decrease in red blood cell count, packed cell volume, haemoglobin concentration, mean cell haemoglobin concentration when compared to the negative control.

Table 2 also illustrated that honey treated diabetic produced decreased white blood count, lymphocytes, eosinophil, basophil and a significant increase ($P<0.05$) in neutrophil and monocytes compared to the control. Overall, all honey treated groups demonstrated a significant lowering of platelet count.

Table 1. Effect of honey on Erythrocytic profile of Alloxan-induced Wistar rat

	HB (g/dL)	PCV (%)	RBC (x10^6/µL)	MCV (fl)	MCH (pg/dl)	MCHC (g/dl)
1	16.80 ± 0.42	44.67 ± 1.45	7.27 ± 0.25	61.42 ± 0.13*	23.11 ± 0.23*	37.63 ± 0.328
2	18.33 ± 0.88	45.00 ± 1.15	7.22 ± 0.20	62.34 ± 0.23	25.37 ± 0.56	40.70 ± 0.94
3	17.20 ± 0.12	46.00 ± 0.00	7.52 ± 0.02	61.21 ± 0.16*	22.89 ± 0.09*	37.39 ± 0.25*
4	14.60 ± 0.12*	40.50 ± 0.29*	6.56 ± 0.03*	61.78 ± 0.14	22.27 ± 0.07*	36.05 ± 0.03*
5	15.90 ± 0.75*	44.50 ± 2.02	7.16 ± 0.33	62.20 ± 0.06	22.22 ± 0.02*	35.72 ± 0.06*
6	15.67 ± 0.18*	42.67 ± 0.33	6.86 ± 0.02	62.17 ± 0.48	22.83 ± 0.30*	36.72 ± 0.27*

*HB: Haemoglobin, PCV: Packed cell volume, RBC: Red blood cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, 1: Normal control, 2: Negative control, 3: positive control, 4-5: Experimental group *p<0.05 when compared with the negative control*

Table 2. Effect of Honey on Leucocytic profile Alloxan-induced Wistar rat

	1	2	3	4	5	6
TWBC (x10^3/µL)	9.52 ± 0.56*	11.73 ± 0.93	11.80 ± 0.12*	9.40 ± 0.12	11.50 ± 0.29	11.10 ± 1.29
RE LYMP (%)	62.00 ± 1.53	65.33 ± 2.03	62.00 ± 0.58	57.00 ± 0.58*	61.00 ± 0.58*	61.33 ± 1.33*
RE NEUT (%)	28.33 ± 1.86*	23.67 ± 1.20	29.00 ± 0.00*	32.50 ± 0.29*	29.50 ± 0.87*	28.33 ± 2.19*
RE MONO (%)	6.67 ± 0.33	5.67 ± 1.20	7.00 ± 0.58	6.00 ± 0.58	6.00 ± 0.00	7.67 ± 0.33*
RE EOSINO (%)	3.00 ± 0.58*	4.67 ± 0.67	2.00 ± 0.00*	4.00 ± 0.58	3.00 ± 0.00*	2.67 ± 0.67*
RE BASO (%)	0.00 ± 0.00*	0.67 ± 0.33	0.00 ± 0.00*	0.50 ± 0.29	0.00 ± 0.00*	0.00 ± 0.00*
AB LYMP (x10^3/µL)	5.90 ± 0.35*	7.65 ± 0.58	7.31 ± 0.00	5.36 ± 0.12*	7.02 ± 0.24	6.80 ± 0.75
AB Neutro (x10^3/µL)	2.70 ± 0.27	2.76 ± 0.14	3.42 ± 0.03	3.05 ± 0.01	3.39 ± 0.01	3.15 ± 0.48
AB MONO (x10^3/µL)	0.64 ± 0.07	0.68 ± 0.19	0.83 ± 0.08	0.57 ± 0.06	0.69 ± 0.02	0.86 ± 0.13
AB EOSINO (x10^3/µL)	0.28 ± 0.04*	0.56 ± 0.12	0.24 ± 0.00*	0.37 ± 0.05	0.35 ± 0.01*	0.29 ± 0.07*
AB BASO (x10^3/µL)	0.00 ± 0.00*	0.08 ± 0.04	0.00 ± 0.00*	0.05 ± 0.03	0.00 ± 0.00*	0.00 ± 0.00*

*TWBC: Total white blood cell, LYMP: Lymphocytes, NEUT: Neutrophils, MONO: Monocytes, EOSINO: Eosinophils, BASO: Basophils, 1: Normal control, 2: Negative control, 3: positive control, 4-5: Experimental group *p<0.05 when compared with the negative control*

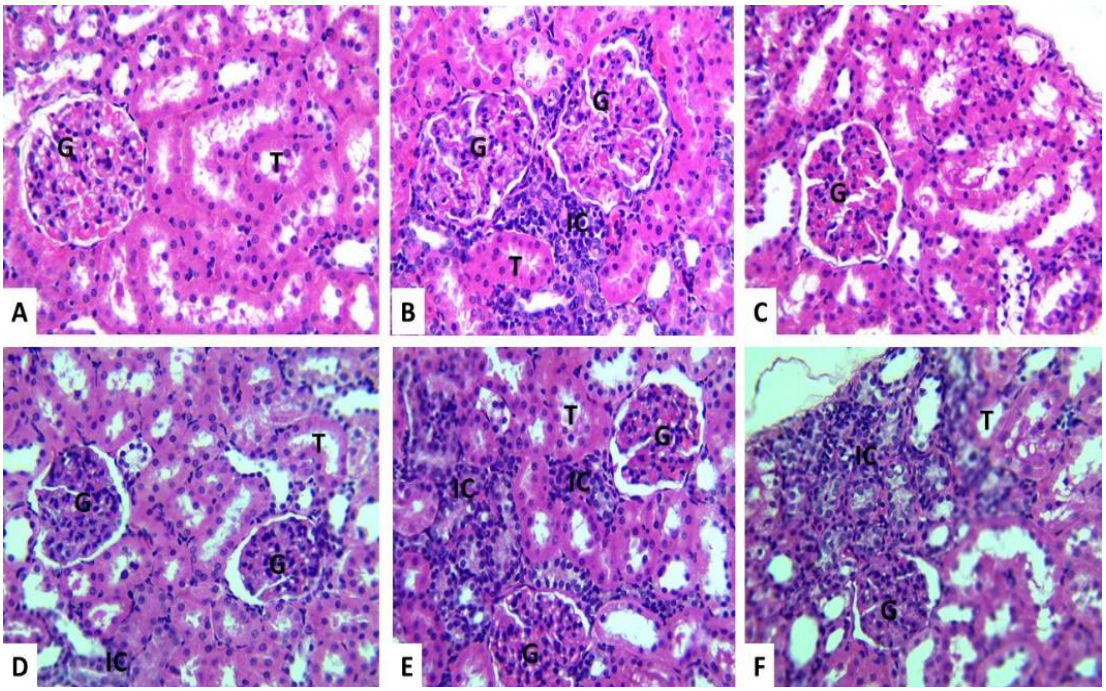

Fig. 1. Histopathological examination of the kidney of alloxan-induced Wistar rat treated with honey

A = normal control group; B = negative control group; C = positive control group; D = low dose honey; E = mid dose honey; F = high dose honey; G = glomerulus; IC = inflammatory cells in the peritubular spaces; T = tubules

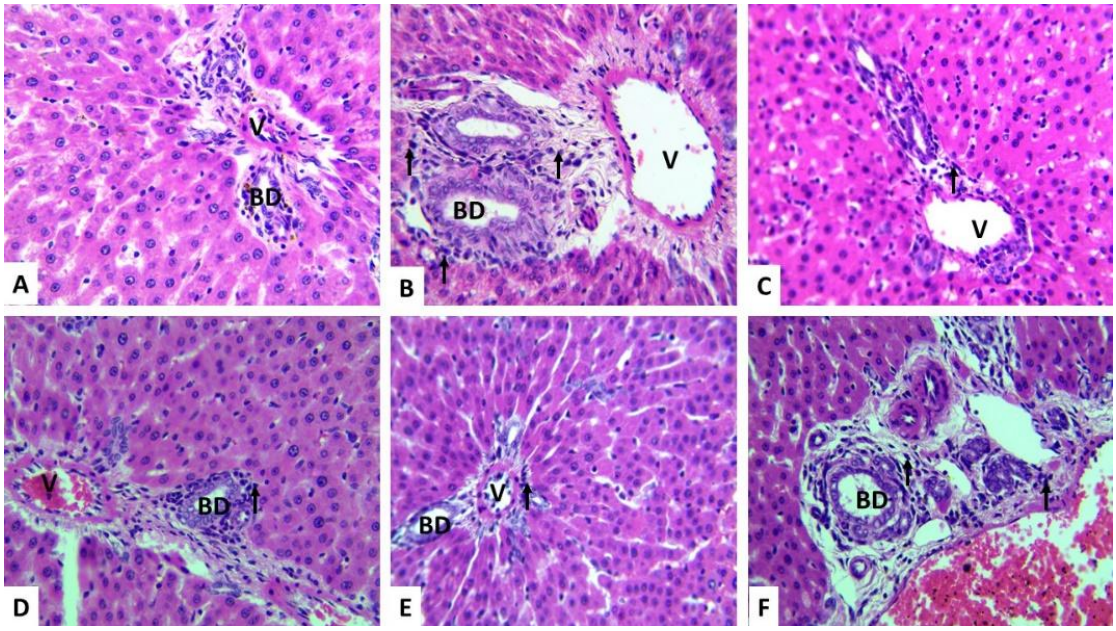

Fig. 2. Histopathological examination of the liver of alloxan-induced Wistar rat treated with honey

A = normal control group; B = negative control group; C = positive control group; D = low dose honey; E = mid dose honey; F = high dose honey; BD = bile duct; V = portal vein; arrow = inflammatory cells

3.1 Histopathology of Kidney

Plate A: Normal control (NC) of Kidney tissue showing normal cellular architecture.

Plate B: Diabetic control (DC) of Kidney tissue induced with 120mg/kg of alloxan showed cellular abnormalities with an area of vascular degeneration, tubular necrosis, glomerular

inflammation, epithelial lining degeneration and desquamation as compared with the normal control group.

Plate C: Positive control (PC) of the kidney tissues induced with 120mg/kg of alloxan and cotreated with glibenclamide showed increased cellular regeneration compared with the nondiabetic control group.

Plate D: Kidney tissue treated with pure honey at a dose of 0.2mg/kg and standard pellets for 21days showed cellular regeneration with prominent nuclear rearrangement compared with the diabetic and nondiabetic control group.

Plate E: Kidney tissues treated with pure honey at a dose of 0.5mg/kg and standard pellets for 21days technique showed cellular regeneration with prominent nuclear rearrangement compared with the diabetic and nondiabetic control group.

Plate F: Kidney tissue treated with pure honey at a dose of 0.8mg/kg and standard pellets for 21days showed increased cellular regeneration with prominent nuclear rearrangement compared with the diabetic and nondiabetic control group.

3.2 Histopathology of Liver

Plate A: Normal control of Liver tissue showed normal cellular architecture with the portal triad, central vein, numerous hepatocytes and sinusoidal lining.

Plate B: Diabetic control of Liver tissue induced with 120mg/kg of alloxan showed cellular abnormalities with vascular degeneration, necrosis, vascular congestion, and cellular degeneration compared with the normal control group.

Plate C: Positive control of liver tissue induced with 120mg/kg of alloxan co-treated with glibenclamide showed moderate cellular restoration vascular area comparable with the control (plate A).

Plate D: Liver tissue treated with pure honey at a dose of 0.2mg/kg and standard pellets for 21 days showed a slight area of cellular restoration with marked vascular congestion and cellular degeneration with pyknotic nuclei compared with the normal diabetic control group.

Plate E: Liver tissue treated with pure honey at a dose of 0.5mg/kg and standard pellets for 21 days showed a moderate area of cellular restoration vascular congestion and pyknotic nuclei compared with normal and diabetic control groups.

Plate F: Liver tissue treated with pure honey at a dose of 0.8mg/kg and standard pellets for 21 days showed complete restoration compared with normal and diabetic control groups.

4. DISCUSSION

Diabetes mellitus (DM) has been described as a complex metabolic disorder characterized by a deficit of blood glucose concentration homeostasis and an abnormal metabolic pattern of carbohydrates and lipids [16]. Thus, this study investigated honey's histological assessment and haematological parameters on alloxan-induced diabetic male albino rats.

In this study, the administration of honey showed a significant reduction in haemoglobin (Hb) concentration in rats compared with the negative control. This reduction in haemoglobin (Hb) concentration was concentration-dependent and statistically significant ($p<0.05$) in rats administered honey at doses of 0.2mg/kg, 0.5mg/kg and 0.8mg/kg, respectively. This implies that administered honey could disrupt haemoglobin (Hb) production at high doses. Many disorders, such as iron deficiency anaemia, thalassemia (an hereditary disease in which globin chain production is insufficient), and anemias linked with chronic infection or disease, are caused by a failure to produce haemoglobin (Hb). Iron is a component of numerous enzymes in cells, as well as a member of the heme group in haemoglobin (which consists of a porphyrin ring containing iron). Red blood cells store a large portion of the body's iron, which is necessary for haemoglobin synthesis. Inadequate iron intake or absorption, excessive loss via external bleeding, or interaction with iron metabolism can all cause iron insufficiency [17].

Packed cell volume (PCV) and red blood cell (RBC) were significantly ($p<0.05$) reduced in honey-treated rats. PCV and RBC had a reduction at dose 0.2mg/kg compared to the control, and there was no significant change in both at doses of 0.5mg/kg and 0.8mg/kg in the diabetic treatment group compared with the control group seen in Table 1. The reduction may have occurred due to the lysis of blood cells. This implies that honey could cause disturbances in the osmoregulatory system of the blood cells and

oxidative injury to the cell membrane and could suppress the haemopoietic system. The observed decrease in PCV is believed to result from the decreased RBC. As a result, the observed decreases in RBC count, Hb, and PCV can be linked to slowed haemopoiesis, RBC destruction, and shrinking. Also, because RBC and Hb are required for the transfer of respiratory gases, the oxygen-carrying capacity of the blood and the amount of oxygen given to the tissues may be changed after honey administration [18]. These findings imply that consuming honey on a regular basis may cause anaemia. The administration of honey to rats resulted in an increase in white blood cells (WBC), however the drop was not statistically significant. WBC plays a critical part in the body's defence against infection and tissue damage. This shows that honey may have immune-boosting properties in animals, which could be attributed to an increase in vascular permeability. The administration of honey appears to exhibit a stimulatory effect on the immune system's effectors cells. Immune boosters are commonly used to help the immune system resist invading agents such as germs and viruses by strengthening and harmonising deteriorating bodily systems [19].

There was a statistically significant ($p<0.05$) increase in the differential white blood cell counts (Neutrophils and monocyte) and a decrease in eosinophils and Lymphocytes at doses of 0.2mg/kg, 0.5mg/kg and 0.8mg/kg. Basophil had a reduction at dose 0.2mg/kg compared to the control, and there was no significant change in basophil at a dose of 0.5mg/kg and 0.8mg/kg in the diabetic treatment group compared with the control group, as seen in Table 2.

The histopathological examination showed that kidney tissue of rats in normal control groups were stable. In contrast, the diabetic control group showed high cellular abnormalities, including tubular necrosis, thickening of the basement membrane, glomerular damages and edematous convoluted tubules, atrophy, and disarrangement cytoarchitectural component.

This study found that giving pure honey to alloxan-induced diabetic rats preserved cellular architecture, the appearance of glomerular capillaries, the squamous lining cell of the bowman capsules, proper distribution of afferent and efferent arterioles, and the arrangement of convoluted tubules and collecting ducts in Fig. 1D as mild restoration, Fig. 1E as moderate restoration, and Fig. 1E as complete regeneration and restoration (Fig. These results imply that honey preparation in a single dosage may have a nephroprotective effect [20]. The histology of the liver demonstrated that the normal control (NC) animals' group were found to be stable. In contrast, the diabetic control group showed a high level of cellular abnormalities, including necrosis, cellular and vascular degeneration, vascular congestion, hyperplasia of the hepatocytes and vacuolation. This study observed that the administration of honey to alloxan-induced diabetic rats revealed preserving cellular architecture, reappearance and cellular restoration, vascular congestion, and reappearance of hepatocytes with pyknotic nuclei migrating from the sinusoidal lining layer in Fig. 2D as mild, Fig. 2E as moderate restoration and Fig. 2F as complete regeneration in the liver tissues compared to nondiabetic and diabetic control groups. Findings indicate the possible hepatoprotective and anti-diabetic role the honey plays in a single administration. Various researchers found that alloxan-related liver problems might be avoided by restoring normal liver functions by appropriate treatment of hyperglycemic situations [21]. Honey treatment was found to have significant abilities to reverse renal and hepatic tissue degradation and disarray in the current study.

5. CONCLUSION

Based on the results, it can be concluded that this study has demonstrated considerable evidence that honey supplementation can exert haematopoietic, thrombopoietin, immune-stimulatory and glycosylated haemoglobin in alloxan diabetic rats. Also, the histo-examination of the kidney and liver has successfully shown that honey possesses anti-inflammatory properties that hugely affect the kidney and liver of patients with diabetes. Honey is therefore said to have nephroprotective and anti-inflammatory effects on diabetic patients. However, it is recommended that further study is needed to evaluate honey as a potential candidate as a natural alternative for the management of nephrotic diseases and the need to perform well-designed random clinical trials.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for

any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was conducted following the National Institute of Health guidelines, the USA, as approved by the College of veterinary medicine, Michael Okpara University of Agriculture, Umudike. The ethical committee's reference number is: MOUAU/CVM/REC/202015.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diab. Res. and Clin. Pract. 2018;87(1):4–14.
2. Prince OA, Chinwe EO, Chiemeziem AO, Chimaraoke O, Peter, OE. and George C.N. Hypoglycemic effect of methanol extract of pawpaw (Carica papaya) leaves in alloxan-induced diabetic rats. Intl. J Innovat. Sci. Res. Tech. 2022;7(1): 627-631.
3. Roglic G, Unwin N. Mortality attributable to diabetes: estimates for the year 2010. Diabetic Res. and Clin. Practice. 2017; 87(1):15–19.
4. Arkkila PE, Koskinen PJ, Kantola IM, Rönnemaa T, Seppänen E, Viikari JS. Diabetic complications are associated with liver enzyme activities in type 1 diabetes. Diabetes Res. Clin. Pract. 2001;52:113–8.
5. Otilia B, Daniel S, Adela R. Honey and Diabetes: The Importance of Natural Simple Sugars in Diet for Preventing and Treating Different Type of Diabetes. J. Oxida. Medi. Cellu. Long. 2019;23: 121-130.
6. Yim DG, Jang KH, Chung KY. Effect of GdL addition on physico-chemical properties of fermented sausages during ripening. Korean J. Food Sci. Ani. Reso. 2015;35:322–329.
7. Ahmed ABA, Rao AS, Rao MV. Role ofin vivo and in vitro callus of Gymnema sylvestre (Retz) R.Br. Ex. Roemer & Schultes in maintaining the normal levels of blood glucose and lipid profile in diabetic Wistar rats. Biomedicine. 2008;28:134–138.
8. Pitocco D, Martini F, Zaccardi F, Ghirlanda G. Antioxidants, healthydiet, and diabetes: oxidative stress and nutrition in diabetes. In: BagchiD, Sreejayan N (eds.). Nutritional and therapeutic interventions for diabetes and metabolic syndrome. Academic Press, San Diego. California. 2012;7:299-313.
9. Ajagbonna OP, Onifade KI, Suleiman U. Haematological and biochemical changes in rats given extract of Calotropis procera. Sokoto J. of Vet. Sci. 2009;1:36–42.
10. Mansi KMS. Effects of administration of alpha-melanocyte stimulating hormone (α-MSH) on some hematological values of alloxan-induced diabetic rats. American J. Pharmacol. Toxi. 2006;1:5–10.
11. Muhammad NO, Oloyede OB. Haematological parameters of broiler chicks fed Aspergillus niger - fermented Terminalia catappa seed meal-based diet. Global J. Biotech. Biochem. 2009; 4:179–183.
12. Erejuwa OO, Sulaiman SA, Wahab MS. Honey: A novel antioxidant, ǁ Mol. 2012;17(12):4400– 4423.
13. Gheldof N, Wang X, Engeseth N. Identification and quantification of antioxidant components of honey from various floral sources. J Agri. Food Chemi. 2002;50:5870-7.
14. Brar RS, Sandhu HS, Singh A. Veterinary clinical diagnosis by laboratory methods. New Delhi: Kalyani Publishers. 2000; 2:10-50.
15. Schalm, OW, Jain NC, Carrol E. Veterinary haematology, 3rd edition. Lea and Febieyer, Philadelphia. 1975;2:55–77.
16. Mcanuff M, Omoruyia F, Morrison E, Asemote H. Hepatic function enzymes and lipid peroxidation in STZ-induced diabetic rats fed bitter yam (Dioscorea polygonoides) steroidal sapogenin extract. Diabet. Croat. 2003;32:17–23.
17. Kee JL. Handbook of Laboratory and Diagnostic Tests, 4th Ed. Upper Saddle River, NJ: *Prentice-Hall.* 2001; 20-101.
18. De Gruchy GC. Clinical haematology in Medical Practice. Blackwell Scientific Publication. *Oxford, London.* 1976;33-57.

19. Al- Mamary MA. Antioxidant activity of commonly consumed vegetables in Yemen. Malays. J. Nutri. 2002; 8:179-189.
20. Larcan A, Lambert H, Laprevote-Heully MC, Delorme N. Light and electron microscopic study of hepatic lesions in the course of hyperlactatemia in diabetic patients. Diab. Meta. 1979;5:103–112.
21. Kind PRN, King FJ. Alkaline Phosphatase determination. Clin. Patho. 1972;7:322.

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