

## Original Research Article

### HISTOLOGICAL ASSESSMENT AND HEMATOLOGICAL PARAMETERS OF HONEY ON ALLOXAN INDUCED DIABETIC MALE ALBINO RATS

#### 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 0.2mls of honey) group 5, (treated with 0.5mls of honey and group 6 (treated with 0.8mls 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 (21 days), after which rats were sacrificed by cervical dislocation under light ether anaesthesia. Blood samples were collected by ocular puncture method from the animal at baseline for haematological analysis using an automated haematology analyzer. Kidney and liver were extracted, weighed and fixed immediately in 10% formal saline, transported to the laboratory, processed to paraffin wax, cut at 5 microns, stained using Hematoxylin and Eosin technique and observed histopathologically under a light microscope. **Results:** Administration of honey to the diabetic rats significantly reduced ( $p < 0.05$ ) RBC count, HB concentration and packed cell volume (PCV) compared to the control. White blood cell counts showed a reduced level in the test group at a dose-dependent concentration compared to the control. Histopathological results showed no pathological changes observed in experimental animals' tissue sections compared with tissue sections of the same organs in control animals. **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

#### 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 insulin [4]. Environmental factors are important in determining type 1 diabetes, although type 1 diabetes is genetically related. The symptoms of this type of diabetes usually start in a few weeks. The most crucial

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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]. Reactive oxygen species (ROS) produced by protein glycation and glucose oxidation mediates the pathogenic effects of high glucose. Reactive oxygen species (ROS) can directly impose molecular damage and cellular damage by activating many cellular stress-sensitive pathways, which direct to late complications of diabetes. Moreover,  $\beta$ -cell dysfunction and insulin resistance also link to the same pathways [7]. Studies have shown that individuals with diabetes have a higher incidence of liver and kidney function abnormalities and formation of free radicals due to glucose oxidation and non-enzymatic glycosylation of proteins [4]. Subsequent oxidative degradation of glycosylated proteins leads to a decline in antioxidant defence mechanisms and damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance [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 advances in the management of DM, the mortality from macro-vascular complications, particularly coronary heart disease (CHD), is still high [4].

In recent years, there has been a renewed interest in establishing an efficient and appropriate alternative medicine to treat diabetes [7]. Pure honey has been shown to benefit health-related effects, making it a perfect remedy for diabetes, heart disease, kidney disease, and high blood pressure. The use of honey has been controversially discussed and not well accepted in modern medicine [8]. The sugar content of honey has led to uncertainty about the risks of diabetes. 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].

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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 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 blood glucose levels higher than 150mg/dl were considered diabetic after 3 days of induction using the fasting blood sugar method 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; Alloxan induced negative control, positive control was induced by alloxan and treated with glibenclamide, while groups 4,5 and 6 were induced by alloxan,

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confirmed diabetic and were treated with 0.2mls 0.5mls and 0.8mls of honey respectively after 72 hours of induction. Examination of blood glucose level was performed on days 0, 7, 14 and 21.

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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.) + 500mg/kg/day 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

## 2.4 PREPARATION OF DRUGS

Glibenclamide (500mg) manufactured by Vee Excel Drugs, and Pharmaceuticals Private Limited, India, purchased from Ludino Pharmacy shop, Ahiaeke, Umuahia Abia State, was made ready by mashing the tablet in a glass mortar and dissolved in distilled water (1ml) to produce a 500mg/ml solution. Glibenclamide was given orally to the animals at 500 mg/kg.

## 2.5 COLLECTION AND PREPARATION OF SERA SAMPLES

The study period lasted three (3) weeks, after which the rats fasted for 8hrs. Then rats were sacrificed by exposing them to an overdose of chloroform soaked in cotton wool placed in an anaesthetic box covered with a lid. Blood samples were drawn from the heart of each sacrificed rat from all groups by puncture, and blood samples were collected in EDTA specimen bottles. The clear serum was obtained by centrifuging the whole blood and used to estimate biochemical analysis.

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## 2.6 HEMATOLOGICAL STUDIES

### a. Erythrocytic profile

#### i. 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]

#### ii. 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.

#### iii. 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.

$$\text{RBC count (million}/\mu\text{L}) = \text{total number of RBC counted in 5 squares} \times \frac{10000}{1000000}$$

#### iv. 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:

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

#### v. 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.

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

#### vi. 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].

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

## **b. LEUCOCYTIC PROFILE**

### **i. 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].

### **ii. Differential leucocyte count**

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

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

## **2.7 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.

## **3. RESULTS**

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

	HB (g/dL)	PCV (%)	RBC ( $\times 10^6/\mu\text{L}$ )	MCV (f)	MCH	MCHC
1	16.80 $\pm$ 0.42	44.67 $\pm$ 1.45	7.27 $\pm$ 0.25	61.42 $\pm$ 0.13*	23.11 $\pm$ 0.23*	37.63 $\pm$ 0.328
2	18.33 $\pm$ 0.88	45.00 $\pm$ 1.15	7.22 $\pm$ 0.20	62.34 $\pm$ 0.23	25.37 $\pm$ 0.56	40.70 $\pm$ 0.94
3	17.20 $\pm$ 0.12	46.00 $\pm$ 0.00	7.52 $\pm$ 0.02	61.21 $\pm$ 0.16*	22.89 $\pm$ 0.09*	37.39 $\pm$ 0.25*
4	14.60 $\pm$ 0.12*	40.50 $\pm$ 0.29*	6.56 $\pm$ 0.03*	61.78 $\pm$ 0.14	22.27 $\pm$ 0.07*	36.05 $\pm$ 0.03*
5	15.90 $\pm$ 0.75*	44.50 $\pm$ 2.02	7.16 $\pm$ 0.33	62.20 $\pm$ 0.06	22.22 $\pm$ 0.02*	35.72 $\pm$ 0.06*
6	15.67 $\pm$ 0.18*	42.67 $\pm$ 0.33	6.86 $\pm$ 0.02	62.17 $\pm$ 0.48	22.83 $\pm$ 0.30*	36.72 $\pm$ 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

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.

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**Comment [S16]:** Please change it to MCHC (g/dl)

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

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

Tables 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.



### 3.1 Histopathology of Kidney

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

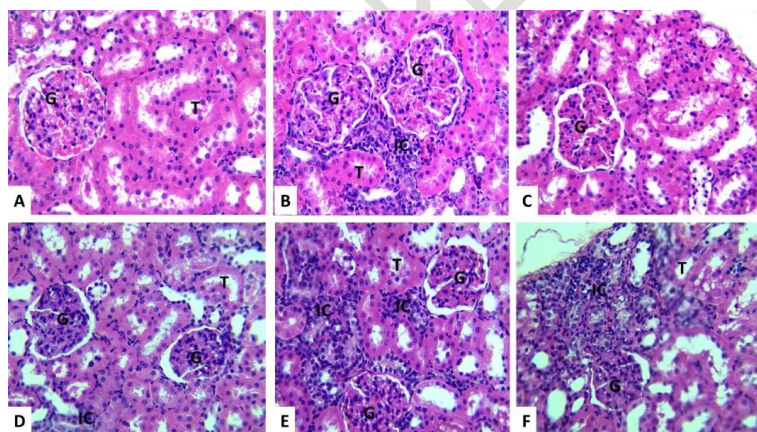
**Plate B:** Diabetic control (DC) of Kidney tissue induced with 120mg/kg of alloxan showed cellular abnormalities with an area of vascular degeneration, tubular necrosis, glomerular inflammation, epithelial lining degeneration and desquamation as compared with the normal control group.

**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.



**Fig. 1: Histological effect of honey on the kidney of alloxan-induced Wistar rat**

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.

### 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.

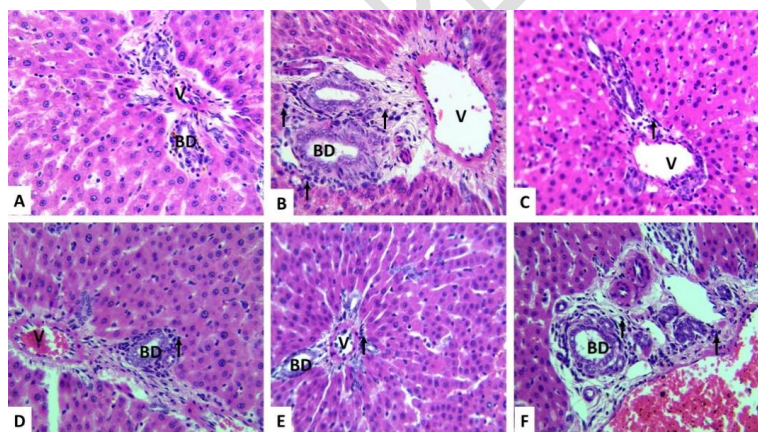
**PLATE B:** Diabetic control of Liver tissue induced with 120mg/kg of alloxan showed cellular abnormalities with vascular degeneration, necrosis, vascular congestion, and cellular degeneration compared with the normal control group.

**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.



**Fig. 2: Histological Effect of Honey on Liver alloxan-induced wistar rat**

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

#### 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 concentration in rats compared with the negative control. This reduction in haemoglobin 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 production at high doses. Failure to produce haemoglobin occurs in many diseases, including iron deficiency anaemia, thalassemia (an inherited disease in which globin chain production is deficient), and anaemias associated with chronic infection or disease. Iron is an essential component of many enzymes in cells and is also part of the heme group in haemoglobin (which consists of a porphyrin ring containing iron). Much of the body's iron stores are within red blood cells, critical for haemoglobin synthesis. Iron deficiency could be due to inadequate intake or absorption of iron, excessive loss with external haemorrhage, or interference with iron metabolism [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. The observed decrease in RBC count, Hb and PCV may therefore be assumed to be associated with retarded haemopoiesis, destruction and shrinkage of RBC. Also, the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues could be affected following the extract administration since RBC and Hb are essential in transferring respiratory gases [18]. These results suggest that chronic administration of honey may induce some level of anaemia.

A decrease in white blood cells (WBC) was also observed in rats after administration of honey, although the decrease was not statistically significant. The crucial role of WBC in defending the body against infection and tissue damage is well known. This suggests that honey may have an immune-boosting effect on the animals, and such effects may also be due 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 usually recommended to strengthen and harmonize degenerative body systems and assist the immune system to fight invading agents such as bacteria and viruses [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.

Histopathological changes in the kidney showed that the normal control (NC) animals in the kidney tissues 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. It is also established that alloxan administration to experimental rats selectively causes pancreatic  $\beta$ -cell membrane disruption and cytotoxicity after intracellular accumulation [20].

This study observed that administration of pure honey to alloxan-induced diabetic rats revealed preserving cellular architecture, the appearance of glomerular capillaries, squamous lining cell of the bowman capsules, proper distribution of afferent and efferent arterioles, arrangement of convoluted tubules and collecting ducts in fig.1D as mild restoration, in fig. 1E as moderate restoration and in fig. 1E as complete regeneration and restoration. These findings suggest a possible nephroprotective role played by the preparation of honey in a single administration.

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. It is well established that alloxan administration to experimental rats selectively causes

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pancreatic  $\beta$ -cell membrane disruption and cytotoxicity after its intracellular accumulation [21]. The increase in serum AST and ALT activities indicated diabetes-induced hepatic dysfunction. Supporting the finding by [22], liver cells were necrotized in diabetic patients. 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 concluded that complications caused by alloxan in the liver could be overcome by restoring the liver functions to normal through effective control over hyperglycemic conditions [23]. The present study showed that honey treatment displayed noticeable capacities to reverse renal and hepatic tissue degeneration and disarrangement.

## 5. CONCLUSION

Based on the above findings, 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 pancreas has successfully shown that honey possesses anti-inflammatory properties that hugely affect the kidney and pancreas 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.

## 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.

## REFERENCES

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