Original Research Article

HEPATO-PROTECTIVE EFFECT AND LIPID PROFILE OF HONEY ON ALLOXAN-INDUCED DIABETIC RATS

ABSTRACT

Purpose: The study investigated honey's hepatoprotective effect and lipid profile on alloxan-induced diabetic rats.

Methods: Thirty-six (36) Wistar rats weighing (210-250g) 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 induction of diabetes in rats was done by an intraperitoneal injection of 120mg/kg/body weight of alloxan for 21 days; honey was administered orally to diabetic rats. Blood glucose level was monitored on days 0, 7, 14, and 21.

Results: Administration of honey to the diabetic rats significantly reduced ($p < 0.05$) glucose level (119.50 mg/dl), total cholesterol (TC), triglyceride (49.5 mg/dl) and low-density lipoprotein cholesterol (LDL-C), while significantly increasing ($p < 0.05$) high-density lipoprotein cholesterol (HDL-C) when compared to the diabetic untreated rats. Liver enzyme parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (T.Bil.) were found to be within the normal range.

Conclusions: Overall, the findings suggest that honey could ameliorate metabolic disorders caused by diabetes.

Keywords: *Diabetes, Lipid profile, Liver function, Bilirubin, Alloxan monohydrate*

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder that has emerged as a significant challenge in the 21st century which its incidence is on the rise globally, particularly in Africa (Ime *et al.,* 2011). This metabolic disorder affects protein, lipid, and carbohydrate metabolism, which are essential biochemical pathways of the body. Diabetic status can result in additional metabolic abnormalities and complications, including dyslipidemia, hepatomegaly, liver disease, weight loss, renal disease, and coma (Scott *et al.,* 2000). Excess glycogen in the hepatic tissue may cause diseases in patients with diabetes. Patients who only have excessive glycogen deposition can suffer from hepatomegaly and liver enzyme abnormality, which can be improved with sustained glucose control. In the untreated diabetic population, the activities of liver damage markers, including serum alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), are increased (Arkkila *et al.,* 2001). Diabetes-related complications include

diabetic nephropathy and renal dysfunction, and about 20-40% of patients with diabetes (type 1 and type 2) develop nephropathy.

Serum lipids abnormalities are also associated with diabetes. Previous studies have shown that elevated levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and low concentration of high-density lipoprotein cholesterol (HDL-C) in diabetes are associated with coronary heart disease (Lotfy *et al.,* 2013). Current treatment modalities utilizing drugs dissimulate multidrug resistance and other side effects (Castro *et al.,* 2006). Natural products are considered a practical alternative, and recently honey has caught the interest of researchers as an alternative therapeutic agent (Ahmed and Othman 2013).

Honey is one of the oldest known medicines and sweeteners. It is a natural product formed from the nectar of flowers by honeybees (Family: Apidae). It is considered one of the last untreated natural food substances (Wang and Li 2011). Honey, which comprises monosaccharides and oligosaccharides predominantly, contains 181 constituents (Gheldof *et al.*, 2002). Several studies have shown that honey can be used as an antioxidant anti-inflammatory; wound healing, and recent research showed that honey has anti-diabetic effects.

It has been reported that honey has about 200 components, such as glucose, fructose, amino acids, flavonoid, vitamins and minerals. Flavonoids are components that act as antioxidants and anti-inflammatory in damaged cells. Honey is said to be made up of high flavonoids might have a good potential in lowering blood glucose levels due to lack of insulin secretion and insulin action. Evidence indicates that some honey varieties contain kynurenic acid (a tryptophan metabolite with neuroactive activity), contributing to its antinociceptive and antimicrobial properties (Beretta *et al.,* 2007). Several enzymes such as glucose oxidase, diastase, invertase, phosphatase, catalase and peroxidase have also been documented in honey (Crane, 2015). The use of honey in folk medicine dates back to 2100-2000 BC 4 (Beretta *et al.,* 2007).

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) Wistar rats (210-250g) purchased from 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 EXPERIMENTAL DESIGN

The wistar rats were randomly allocated into six groups and treated as follows:

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

2.3 INDUCTION OF DIABETES

Some groups 2-6 of Wistar rats fasted overnight for a minimum of 8 hours. Diabetes was induced in the fasted rats by administering alloxan monohydrate (120 mg/kg intraperitoneally) in normal saline. The control groups were given normal saline intraperitoneally, while Groups 4-6 received oral doses of honey at 0.2ml, 0.5ml and 0.8ml three times daily for 21 days, respectively. Blood glucose levels were determined by the glucose oxidase method using a glucometer (Accu-check Advantage, Roche Diagnostic, Germany) at baseline at days 7, 14, and 21 post-administrations of alloxan.

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.

2.6 EVALUATION OF EFFECT ON BIOCHEMICAL PARAMETERS

The clear serum was obtained by centrifugation of the whole blood and used to estimate AST, ALT, ALP, Total Bilirubin and Total protein. The following parameters were analyzed:

2.7 DETERMINATION OF LIVER FUNCTION MARKERS

2.7.1 Determination of Aspartate Aminotransferase Activity

The method of Reitman and Frankel (1957) described by Randox laboratories, the United Kingdom using Randox kits, was used for this study.

Principle: The activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

2.7.2 Determination of Alanine Aminotransferase Activity

This was also done using the method of Reitman and Frankel (1957).

Principle: The activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, -dinitrophenylhydrazine.

2.7.3 Determination of Alkaline Phosphatase Activity

The activities of alkaline phosphatase were evaluated using the methods of Kind and King (1972).

Principle: Serum alkaline phosphatase hydrolysis yields a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein, which at alkaline pH, turns pink and can be determined photometrically.

2.7.4 Determination of Total Protein Estimation Activity

Total protein estimation was assayed using the direct Biuret method (Gornall *et al*., 1948).

Principle: At alkaline pH value, proteins form a blue coloured complex with copper II ions which is photometrically measured.

2.7.5 Determination of Total and Conjugated Bilirubin Activity

Total and conjugated bilirubin levels were determined according to the method of Jendrasik and Grof (1938). Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in an alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin-bound bilirubin by the reaction with diazotized sulphanilic acid.

2.8 DETERMINATION OF LIPID PROFILE BIOMARKERS

2.8.1 Determination Of Total Cholesterol Concentration

Total cholesterol was determined using the enzymatic colourimetricchod-pad test method described by Allain *et al*. (1974) with Randox laboratory test kits.

Principle:

The sample's free and esterified cholesterol originates utilizing a coupled reaction where serum cholesterol reacts with enzymes to produce a coloured complex whose intensity is proportional to the serum cholesterol concentration and is measured spectrophotometrically.

2.8.2 Determination of Triglycerides Concentration

This was also determined spectrophotometrically using the method of Tietz (1990).

Principle: The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinonemine formed from hydrogen peroxidases, 4-amino phenazone and 4-chlorophenol under the catalytic influence of peroxidase.

2.8.3 Determination of High-density Lipoprotein Concentration

This was evaluated by the method of Grove (1979) as described in the Randox Laboratory test kit

Principle: It involves a precipitation reaction with phosphotungstate and magnesium ion where the supernatant contains HDL, which is measured spectrophotometrically.

2.8.4 Determination Of Low-Density Lipoprotein Cholesterol Concentration

Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation (Friedewald*et al*., 1972). LDL-C = [TC – {HDL-C+ (TG/5)}] where VLDL-C = (TG/5) (Bhandari *et al*., 2013).

2.8.5 Determination of Very Low-Density Lipoprotein Cholesterol Concentration

This was calculated according to the method of Wilson et al. (1981) as VLDL = 0.2 x TG (where TG is total glycerides)

2.9 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. RESULT

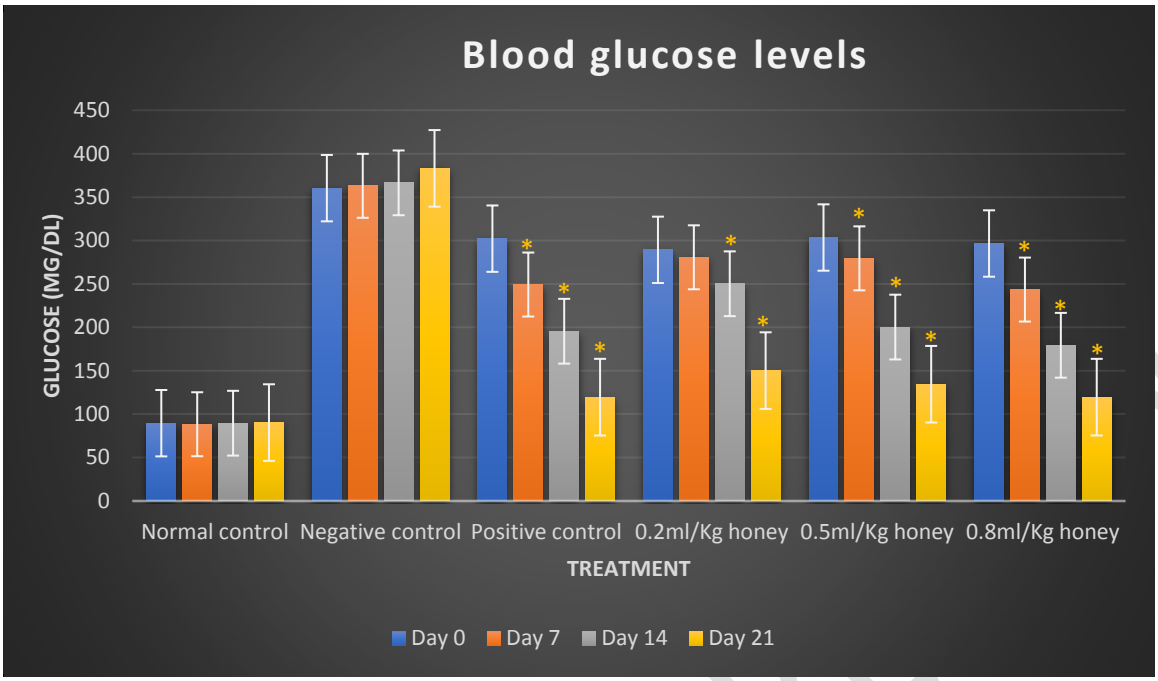

Fig. 1: Effect of Honey on blood glucose levels on day 0, 7th, 14th and 21st

Values are expressed as Mean ± Standard Deviation (n=5). **p<0.05* when compared with the negative control

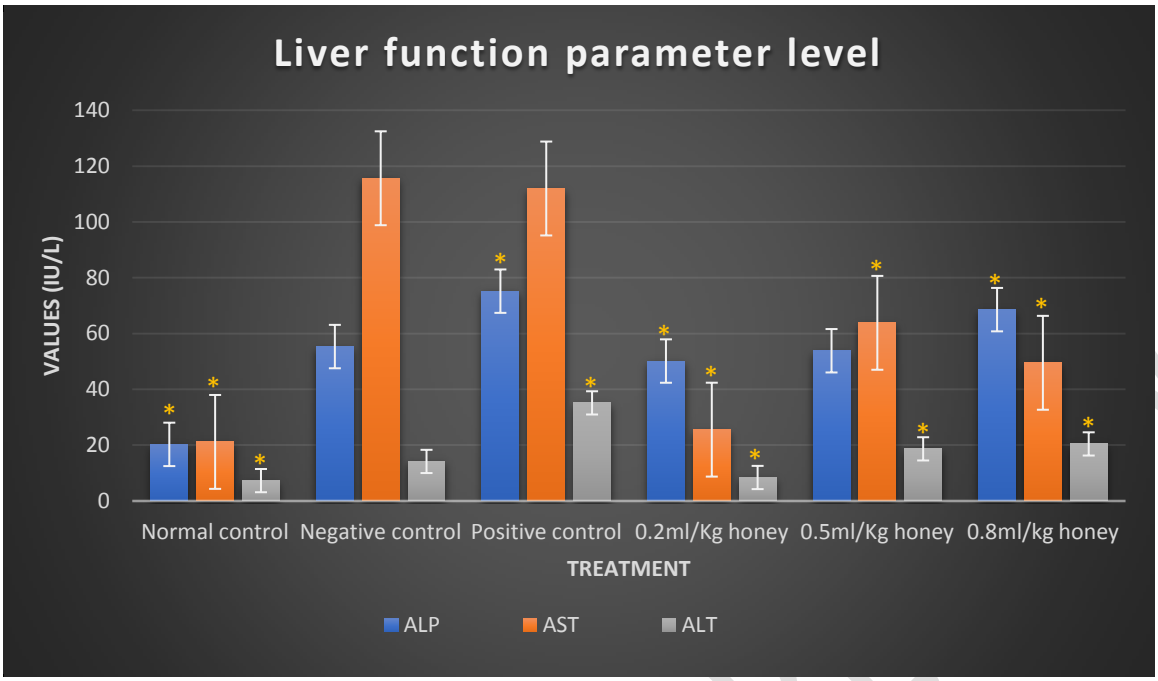

Fig. 2: Effect of Honey on Liver function parameter level

Values are expressed as Mean ± Standard Deviation (n=5). *$p<0.05$* when compared with the negative control

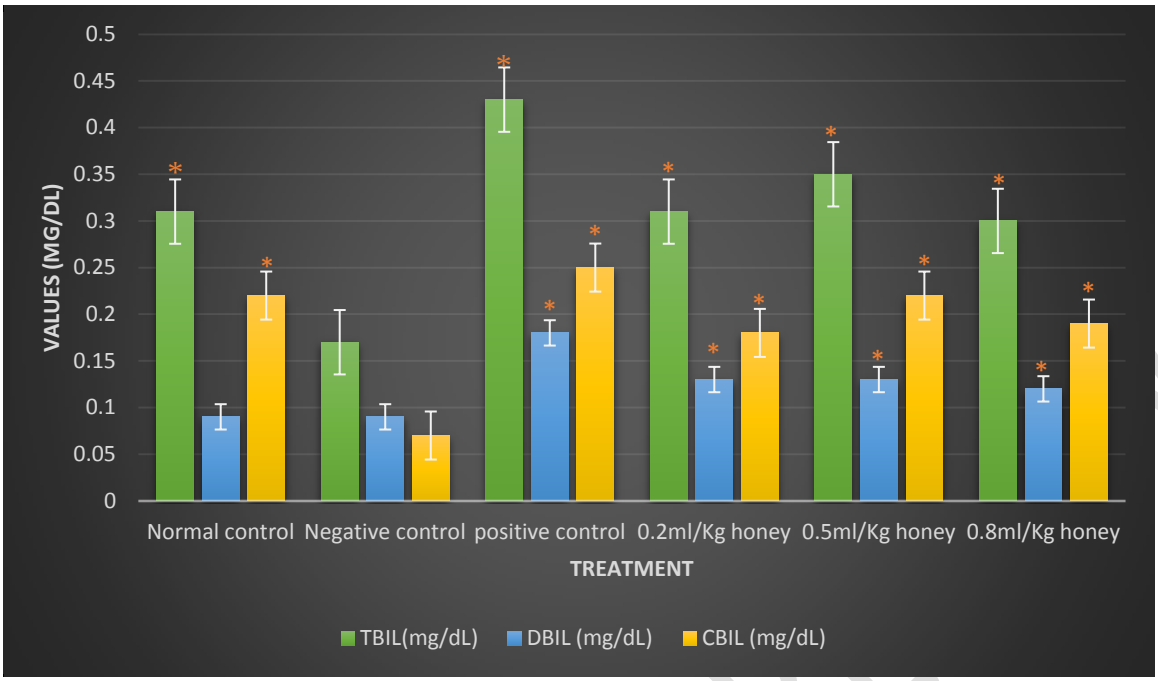

Fig 3: Effect of Honey on Bilirubin level

Values are expressed as Mean ± Standard Deviation (n=5). *$p<0.05$* when compared with the negative control

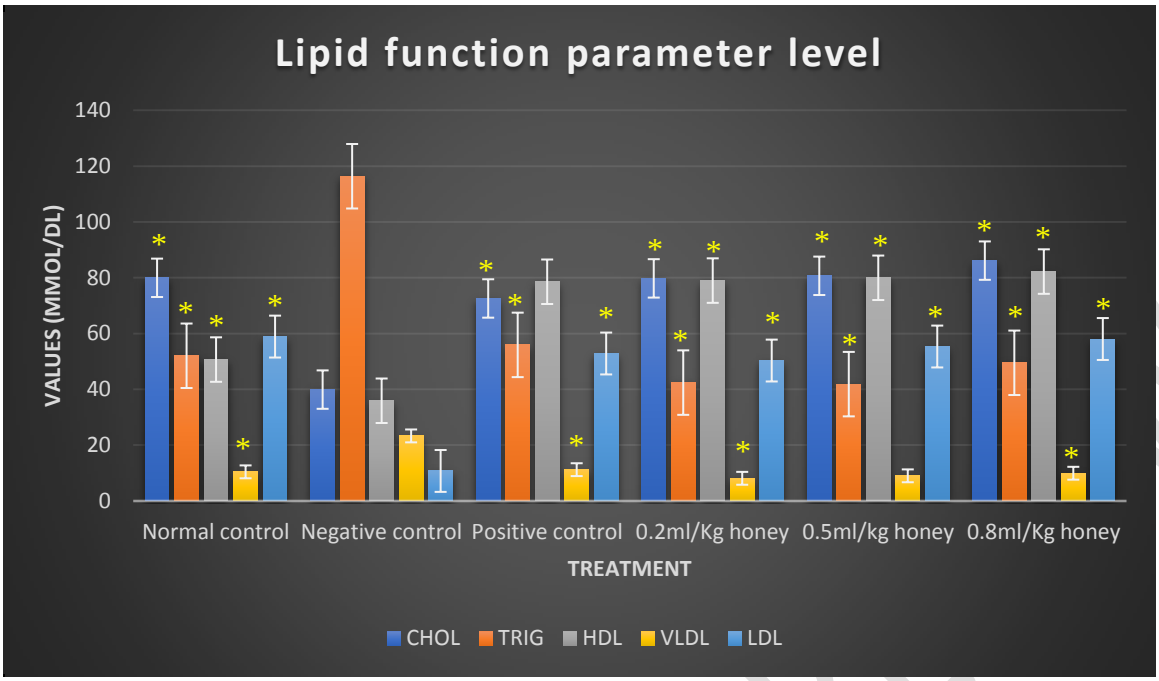

Fig 4: Effect of Honey on Lipid function parameter level

Values are expressed as Mean ± Standard Deviation (n=5). *$p<0.05$ when compared with the negative control.

4. DISCUSSION

Diabetes mellitus are chronic diseases that occur due to inadequate insulin supply, which results in multiple changes in the body's metabolism. This chronic disease has drastically increased in searching for natural remedies to manage the disease. Thus, this study investigated honey's hepatoprotective and lipid profile effects in alloxan-induced diabetic rats.

In the present study, honey treatment significantly ($P < 0.05$) decreased blood glucose levels in diabetic rats. These findings concur with the previous results of Song et al. (2009), which demonstrated the glucose-lowering effect of honey in diabetic rats and diabetic patients. Honey's anti-hyperglycemic activity is due to several nutritious ingredients such as fructose and flavonoids, which increase insulin secretion in people with diabetes and reduce blood glucose levels (Amalia, 2015). Fructose in honey can catalyze the conversion of glucose into glucose-6 phosphate, lowering blood glucose levels. Moreover, fructose can stimulate insulin secretion from pancreatic cells. Blood glucose and fructose levels have increased hepatic glucose phosphorylation by activating glucokinase and inhibiting glycogenolysis by emphasizing phosphorylase (Erejuwa *et al.,* 2012).

Elevated serum lipids such as triglycerides were noticed in diabetic rats. This is because insulin activates lipoprotein lipase, hydrolyzes triglycerides (Reitman and Frankel, 1957), and inhibits lipolysis. However, in

diabetes, there is increased lipolysis which finally leads to hyperlipidemia. From our study, the administration of honey significantly decreased ($p < 0.05$) the serum triglyceride and serum very-low-density lipoprotein levels and significant increase ($p < 0.05$) total serum cholesterol and the serum high-density lipoprotein in groups treated with honey when compared to the untreated diabetic control group. It is noteworthy that honey administration significantly reduced elevated TGs and HDL cholesterol VLDL, LDL and cholesterol fractions). This is important because increased non-HDL cholesterol level and hypertriglyceridemia in the presence of abnormal glucose metabolism increases the risk of CVD (Negre-Salvayre *et al*., 2010). Therefore, the marked ameliorative effects of honey on TGs and non-HDL cholesterol indicate honey can reduce CVD risk.

Liver disease in diabetes mellitus is caused by overworking the liver, which is responsible for maintaining normal glucose levels by storing excess glucose as glycogen. Because the cells are resistant to insulin, the liver is overworked by producing more glucose (Brain *et al.,* 2004). The toxic effect of alloxan on the liver could cause it to malfunction.

In the present study, the serum elevation of liver damage biomarkers occurred due to the deleterious effect of hyperglycemia in the liver of diabetic rats. The increase in activities of these enzymes can be attributed to alloxan toxicity which leads to liver damage. However, the dose-dependent (0.2, 0.5 and 0.8mg/kg) treatment with honey for 21 consecutive days significantly ($P < 0.05$) decreases the above enzymes' activities. Thapa and Anuj (2007) reported that ALT (10 – 55 µ/L), AST (10 – 40 µ/L), and ALP (45 – 115 µ/L) are the standard range of accepted values for liver function tests, beyond which liver disease can be suspected. Kamal and Hessah (2015) confirmed this by stating that increases in AST, ALT and ALP values above these thresholds indicate early hepatotoxicity and tissue damage detection. From our findings, liver enzyme parameters differentials (AST, ALT, ALP, TBIL. CBIL and DBIL) were found to remain within normal range after the administration of honey in all the treated groups, which could suggest the hepatoprotective effects of the alloxan-induced Wistar rats, which is in tandem with Al-waili, (2003).

5. CONCLUSION

The findings in this study indicate that honey administration to alloxan-induced diabetic rats reduced elevated levels of liver function parameters and lipid profile. However, we recommend that further studies be carried out to determine the biomolecules that cause this effect and their mechanism of action.

ETHICAL APPROVAL

The study was conducted by following the guidelines set by the National Institute of Health, 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 DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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