Original Research Article

ANTIOXIDANT AND NEPHROPROTECTIVE EFFECTS OF HONEY IN ALLOXAN-INDUCED DIABETIC RATS

ABSTRACT

Background and Purpose: Oxidative stress plays an essential role in the instigation of complications associated with diabetes. The present study evaluated honey's antioxidant and nephroprotective effects against oxidative stress in alloxan-induced Wistar rats.

Methods: Thirty-six (36) Wistar male rats (210-250g) were assigned to six (6) study groups with six (6) animals each (n=5). Group 1 was designated as positive control and received distilled water, group 2 was designated as negative control and received 120 mg/kg b.w of alloxan, group 3 was designated as diabetes-induced and received 5mg/kg b.w glibenclamide, groups 4, 5 and 6 were designated as diabetes-induced and treated groups which they received (0.2mls, 0.5mls and 0.8mls of honey) respectively. Treatment lasted for three weeks (21 days), after which rats were sacrificed by cervical dislocation under light ether anaesthesia. Blood was collected for biochemical evaluation using standard techniques (Randox kits).

Results: The results reveal that the actions of superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) were significantly increased ($p<0.05$) in honey treated diabetic rats. The activities of urea and creatinine in all the groups treated revealed a significant ($p<0.05$) decrease when compared with the negative control group, while the activity of creatinine was within the normal range.

Conclusions: The results obtained from all these assays justify the therapeutic efficacy of honey to ameliorate oxidative stress in alloxan-induced diabetic rats and has nephroprotective potential.

Keywords: *Alloxan monohydrate, Diabetes; Glibenclamide, Honey, Oxidative stress.*

ABBREVIATIONS
ANOVA: *Analysis of Variance*
CAT: *Catalase*
GSH: *Glutathione*
H_2O_2: *Hydrogen peroxide*
MDA: *Malondialdehyde*
ROS: *Reactive oxygen species*
SOD: *Superoxide Dismutase*

1.0 INTRODUCTION

Diabetes mellitus is one of the most common metabolic diseases, with over 285 million suffering in 2010 and 438 million suffering by 2030 [1,2]. The universality of diabetes may be determined genetically or developed at any age during an individual's lifetime, and studies have shown that diabetes is more prevalent in developing countries than in developed countries [2]. Demographic changes have been suspected of increasing incidence and undesirable risk factors such as for overweight (obesity) and sedentary life [3].

Diabetes mellitus is a metabolic malfunction with many factorial and diverse etiologies [3]. High blood glucose level is one prominent diagnostic feature of diabetes, although several other symptoms include unexplained fatigue, increased urination, increased hunger and thirst, unexpected weight loss, and blurred vision. The types of diabetes among humans include type 1 diabetes, resulting in the immune system wars against and overpowering insulin, leading to insulin destruction [4]. This type of diabetes is believed to be determined genetically, and also some environmental factors are essential in the disease. The symptom of this type of diabetes is noticed early within some weeks. The most common type of diabetes is type 2, which may arise due to numerous factors; this type of disease arises in several age brackets, and the symptoms are not always noticed because of this; many people are diagnosed with diabetes without specific or uncommon symptoms. Diabetes type 2 is attributed mainly to being overweight or in a state of obesity [3].

Current stream treatment modalities utilizing chemo-drugs such as metformin and sulfonylurea dissimulate multidrug resistance and other side effects, including; gastrointestinal effects, body fluid accumulation and heart disease [5]. This urges the quest for alternate options that will be efficaciously safe and harmless to reduce sugar levels and ameliorate other diabetic complications.

Natural products are considered a practical alternative [6], and recently honey has caught the interest of researchers as an alternative therapeutic agent [7]. Honey is composed of a minimum of 181 substances and majorly constitutes fructose (38%) and glucose (31%) as the predominant sugars. Honey's flavonoids and phenolic acid composition have been reported to be independently liable for honey's antioxidant and other medicinal activities [7]. However, there has been a revived interest in studying the potential health advantages of natural and unprocessed honey in managing numerous disorders. As a result, various

medical properties of honey have been discovered. Cardioprotective [8], hepatoprotective [9], hypoglycemic [9], and antihypertensive [10] actions are among them.

Oxidative stress has been suggested as the basis for ageing, synthesis of mutagens, atherosclerosis, cancer and degenerative diseases [7]. Cells always create a defence system against damages caused by oxidative stress. This defense system comprises free radical scavengers and other oxidative protective agents such as superoxide dismutase, catalase, peroxidase, tocopherol, ascorbic acid, and polyphenols [11]. These antioxidants stimulate biological molecules such as lipids, carbohydrates, nucleic acids and proteins. Cells are denatured by this triggering and intensely provoking an antioxidant response, and honey shows a robust antioxidant action [7]. Hence, this study investigates honey's antioxidant and nephroprotective effects in alloxan-induced diabetic rats.

2.0 MATERIALS AND METHODS

The fresh honey was bought from Fibers Global Farms, Isuochi, in the 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 male 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. The animals were randomly allocated into six groups and treated as follows:

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.)
3	Positive control rats	Alloxan (120 mg/kg, i.p.) + 5 mg/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

2.2 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 COLLECTION AND PREPARATION OF SERA SAMPLES

Blood samples were collected via cardiac puncture at the end of the experiment, which lasted for three weeks (21 days). The blood samples were stored in clean vacutainer tubes and centrifuged at 4000 g for 15 minutes. The serum was used for the estimation of biochemical markers such as MDA, SOD, CAT, UREA, and CREATINE using Randox Diagnostic kits

2.4 DETERMINATION OF ANTIOXIDANT ENZYMES

2.4.1 Determination of Malondialdehyde (MDA) level

Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA), as described by Onkawa et al. [12]. Malondialdehyde reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex that absorbs maximally in acid solution at 532 nm.

2.4.2 Determination of superoxide dismutase (SOD)

Superoxide dismutase was determined using the method Aebi [13]. Adrenaline (10 mg) was dissolved in 17 mL of distilled water to make adrenaline solution. Serum sample (0.1 mL) was added to 2.5 mL of

phosphate buffer (pH 7.8). Adrenaline solution (0.3 mL) was added, mixed well and absorbance was read at 450 nm at 30 seconds interval for 5 times

2.4.3 Determination of catalase activity (CAT)

Catalase activity was determined using the method of Aebi [14].

Estimation of serum urea level

This was done following the Bauer et al. [15] method.

Principle: Urea in serum was hydrolyzed to ammonia in the presence of urease. The NH_3 is then measured photometrically by Berthelot's reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

NH_3 + hypochlorite + phenol $\rightarrow$ indophenol (blue compound)

Estimation of serum creatinine

The method of Cockcroft and Gault [16] was employed for the estimation of serum creatinine

Principle: At alkaline pH values, creatinine reacts with picric acid to produce a coloured compound, creatinine alkaline picrate, which is photometrically read at 546 nm

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.

RESULTS

Table 1: Effect of honey on the levels of the antioxidant parameters

Groups	Treatment	Glutathione (µg/L)	SOD (U/mg Prot.)	MDA (nmol/ml)	CAT (U/mg Prot.)
1	Normal Control (non-diabetic rats)	0.04 ± 0.00	1.24 ± 0.00	7.44 ± 0.31	2.49 ± 1.67
2	Diabetic non-treated	0.02 ± 0.00	0.14 ± 0.20	32.96 ± 4.03	0.25 ± 0.06
3	Positive-control diabetic treated with 500mg glibenclamide	0.04± 0.01	1.19 ± 0.01	6.24± 0.85*	7.25 ± 0.15*
4	Diabetic treated with 0.2ml/kg of Honey	0.03 ± 0.02	2.12 ± 0.13*	7.61± 0.84*	4.25± 0.58*
5	Diabetic treated with 0.5ml/kg of Honey	0.04± 0.01	2.05 ± 0.03*	8.03± 0.42*	3.88± 0.04
6	Diabetic treated with 0.8ml/kg of Honey	0.06± 0.01*	2.15 ± 0.02*	7.00± 0.74*	2.22± 0.01

Values are expressed as Mean ± Standard Deviation (n=5). Values with an asterisk (*) are significantly increased ($p<0.05$) when compared with the negative control. SOD: Superoxide Dismutase, MDA: malondialdehyde, CAT: Catalase.

The result for the effect of honey on Glutathione, SOD, MDA and CAT of diabetic animals, as presented in Table 1, showed that glutathione was significantly ($P<0.05$) reduced in the diabetic control animals compared to the normal control animals. Glutathione concentration significantly increased back to normal in the honey treated groups compared to the diabetic control animals. Glutathione depletion was also reversed in the Glibenclamide group. The diabetic control group showed a significant increase in SOD and CAT expression compared to the normal animals. SOD and CAT also increased significantly in the Honey group at 0.2mls, 0.5mls and 0.8mls compared to the diabetic control and normal animals. Expression of the MDA was significantly elevated in the diabetic control animals compared to the normal animals, and honey treatment was able to reverse the expression of the MDA to normal significantly.

Table 2: Effect of honey on levels improved parameters of kidney function

Group	Treatment	UREA (mg/dL)	CREATININE (mg/dL)
1.	Normal Control (non-diabetic rats)	25.43± 5.69	0.96 ± 0.02
2.	Negative Control (diabetic non-treated)	97.86 ± 2.04	2.90 ± 0.03
3.	Positive Control (diabetic treated with 500mg Glibenclamide)	53.77± 5.12*	0.81 ± 0.40*
4.	Diabetic treated with) 0.2ml/kg of Honey	51.59± 0.82*	1.12± 0.04*
5.	Diabetic treated with 0.5ml/kg of Honey	54.96 ± 0.22*	1.14± 0.04*
6.	Diabetic treated with 0.8ml/kg of Honey	55.99 ± 1.31*	1.22 ± 0.02*

Values are Mean ± SD: (n=5); values are statistically significant *p<0.05 when compared with the negative control group.

The effect of honey and glibenclamide on urea and creatinine in diabetic animals is presented in Table 2. Diabetic animals showed a significant increase ($P < 0.05$) in creatinine concentration compared to normal animals. Honey at 0.2mls, 0.5mls and 0.8mls and Glibenclamide significantly ($P < 0.05$) lowered creatinine levels compared to the diabetic control animals. The diabetic control animals showed significant elevation of urea when compared to the normal animals, while the honey and Glibenclamide significantly lowered urea levels compared to the normal animals.

4.0 DISCUSSION

Diabetes mellitus is a metabolic disorder considered a significant health problem and affects millions of people worldwide. Diabetics can be treated with natural products, and honey has recently piqued the attention of researchers as an alternative therapeutic agent. This study focused on honey's positive antioxidant benefits and nephroprotective effect on alloxan-induced rats.

Alloxan has been shown to cause hyperglycemia due to partial or complete destruction of the beta cells. Increasing evidence indicates that oxidative stress plays a significant role in diabetes mellitus pathogenesis, both in experimental and clinical studies.

Glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins are all responsible for the formation of free radicals in diabetes. Abnormally high free

radical levels and the loss of antioxidant defense mechanisms can harm cellular organelles and enzymes, increase lipid peroxidation, and increase insulin resistance. These effects of oxidative stress can increase the risk of diabetes mellitus complications [17]. This study has investigated how changes in oxidative stress biomarkers, such as superoxide dismutase, catalase, glutathione levels, and lipid peroxidation (MDA), are analyzed.

One of the features of chronic diabetes is peroxidation, and the production of more free radicals may cause them to react with polyunsaturated fatty acids. The peroxidation of Lipid will cause the elevated production of free radicals. Our results showed a significant ($P < 0.05$) MDA elevation in the diabetic control animals (32.96 ± 4.03) compared to the normal animals (7.44 ± 0.31). Administration of honey at doses of 0.2mls, 0.5mls and 0.8mls was able to reverse the expression of the MDA to normal significantly. The result of this study is in line with the findings of Akana et al. [18], which indicated an increased level of MDA and reduced activity of superoxide dismutase in alloxan-induced diabetic rats.

The result of the Glutathione concentration revealed a significant ($P<0.05$) reduction in the diabetic control animals compared to the normal control animals. Glutathione concentration significantly increased back to normal in the honey treated groups compared to the diabetic control animals. Glutathione depletion was also reversed in the Glibenclamide group. These findings were in agreement with the report of Erejuwa [19].

Superoxide anion is best defended by Superoxide dismutase (SOD), which converts the superoxide anion into hydrogen peroxide and water. SOD overexpression is detrimental to cells, according to some studies. The study results showed that SOD's activity was lower in diabetic control rats. SOD activity may be attributed to inactivation by H_2O_2 or glycation of the enzyme, which is documented in diabetes. The activities of SOD significantly increased in the Honey group at 0.2mls, 0.5mls and 0.8mls compared to the diabetic control and normal animals.

The CAT hemoprotein achieved the reduction of hydrogen peroxides and protected the tissues of the hydroxyl radical. The decrease in CAT activity could result from the glycation of the enzyme, and an increase in the SOD activity may protect CAT against the effects of superoxide radicals [20]. The reduction in creatinine concentration after treatment can be attributed to the ability of the honey to reduce glucose concentration. Honey significantly ($P<0.05$) reduced the creatinine level compared with the

negative control. This result agreed with the previous studies of Obia [21]. The present study results showed that honey administration could exert an antioxidant effect in alloxan-induced diabetic rats.

5.0 CONCLUSION

Based on the result obtained from this study, it is concluded that the best dose level of honey that yields the best result with good efficacy and less oxidative stress biomarker enzymes is 0.8mls. Therefore, it is recommended to synthesize a standard antidiabetic drug if further purified.

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care (NIH publication No. 85- 23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the College of Natural Sciences, Michael Okpara University of Agriculture (MOUAU) Research and Ethics Committee.

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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