Original Research Article

EFFECTS OF BEE BREAD ON SERUM LIPID PROFILE IN ALLOXAN INDUCED DIABETIC FEMALE RATS.

ABSTRACT

Introduction: Diabetes Mellitus (DM) represents one of the most frequent public health problems with increasing prevalence worldwide. This condition is caused by increased hepatic glucose production, lower insulin secretion, and impaired insulin action. Subsequently, the lipid is used as an alternative resource of cellular energy, resulting in abnormalities of lipid metabolism.

Aim: To determine effects of Bee Bread on Serum Lipid Profile in Alloxan Induced Diabetic rats

Methodology: A total thirty female Sprague-Dawley rats were grouped in to five. Gp1: Non-DM, Gp2: DM Naïve, Gp3: DM + 200BB, Gp4: DM + 400BB and Gp5: DM + Metformin. Animals were induced with DM following single injection of freshly prepared alloxan in normal saline. Animals were treated for three weeks before they were sacrificed using chloroform sedation. Blood samples were collected for Serum Lipid Profile analysis.

Results: When compared, Serum TC, TG, and LDL-C were significantly higher in the DM group compared to Non-DM, BB administered and DM + Metformin groups.

Reduction in these lipids was achieved with the administration of Bee Bread. HDL-C was significantly lower in the DM group, compared to other groups. The study revealed improvements in serum HDL-C with the administration of Bee Bread.

Conclusion: Bee Bread causes reduction in lipids and improves HDL-C in Diabetic Rats.

Key word: BEE BREAD ,LIPID PROFILE ,ALLOXAN INDUCED ,RATS, HDL-C

1.0 INTRODUCTION

"Diabetes Mellitus (DM) represents one of the most frequent public health problems with increasing prevalence worldwide. The World Health Organization states that the number of people with diabetes will double in the next ten years" (1). Generally, DM is a multifactorial chronic endocrine disorder (2) manifested by hyperglycaemia. "This condition is caused by increased hepatic glucose production, lower insulin secretion, and impaired insulin action" (3). "Subsequently, the lipid is used as an alternative resource of cellular energy, resulting in abnormalities of lipid metabolism. Hyperlipidaemia is characterized by serum lipids alterations, especially triglyceride (TG) increase and high-density lipoprotein (HDL) cholesterol decrease" (4). Therapy for T2DM can be supported by various biological substances. Bee products are considered to be well-known functional foods. They contain a lot of proteins, sugars, essential amino acids, fatty acids, macro and microelements, and vitamins (5), which are responsible for their high nutritional value (6). "Because of a presence of other bioactive compounds also having a beneficial effect on human health, they are useful tools for therapeutical approach as well" (7). "In general, Bee Bread (BB), not a very well-known bee product, is the result of bee pollen fermentation in hives" (8). The bees (*Apis mellifera*) mix plant pollen with nectar or honey and their salivary enzymes (9) and fill the cells in hives with this mixture. Afterwards, anaerobic lactic fermentation begins (10) that decreases pH and enhances the bioavailability of nutrients (11, 12). "Depending on geographical region, botanical origin, and storage conditions, the chemical composition of BB may differ. Salutary health impacts of BB have been rarely investigated thus far and they are conditioned by the presence of phytosterols, fatty acids, polyphenols and Polysaccharides with anticancer properties, and antioxidant and immunological properties" (13).

2.0 MATERIALS AND METHODS

STUDY AREA

The study was carried out at Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Teaching Hospital, Sokoto (UDUTHS) Nigeria. **UDUTHS** is a tertiary health institution located in Sokoto State, North-west Nigeria. The metropolitan city of Sokoto State lies between longitude 05^0.11 to 13^0.03 east and Latitude 13^0.00 to 13^0.06 north and covers an area of 60.33 km^2.

3.0 EXPERIMENTAL ANIMALS

Thirty females Sprague-Dawley with ten proven Male rats (8-10) Weeks and weighing between 180g to 200g were purchased from the animal house, of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were maintained as described by Aniagu *et al*., (14) in a well-ventilated animal house where they will be allowed to acclimatize at 25 ± 2^{oC} with 12hours light and 12hours dark cycle for one week prior to experimentation. Diabetes was induced following 16hours fasting by single intraperitoneal injection of freshly prepared alloxan (70mg/kg body weight) in normal saline 0.9%. Negative controls Non-Diabetic Mellitus (Non-DM) group received an equivalent of amount of normal saline.

The animals were maintained on a pellet feeds (vital), obtained from Grand cereals oil mills limited, Jos, Nigeria, and were supplied with drinking water *ad libitum*. Cleaning of the animal cages was carried out daily. All the experimental protocols were in compliance with the

Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National Institute of Health, 1992), and also in accordance with the recommendation of the International Association for the study of pain (IASP) (15).

4.0 EXPERIMENTAL DESIGN

The animals were randomly divided in to 5 groups with six (6) rats per group as follows:

Non-DM: Healthy rats on distilled water only (1.0ml) daily (Negative control) group one.

DM: Diabetic rats on distilled water only (1.0ml) daily (Positive control) group two

DM + 200BB: Diabetic rats on 200mg/kg body weight BB (Low dose treatment) group three

DM + 400BB: Diabetic rats on 400mg/kg body weight BB (High dose treatment) group four

DM + Metformin: Diabetic rats on 100mg/kg body weight metformin (Standard treatment) group five.

All supplementation and treatments were given by oral gavage daily between 8:00 to 10:00am.

5.0 ANIMALS INDUCTION AND SACRIFICE

The animals were induced using alloxan (150mg/kg body weight) intraperitoneally. Those with FBG above 200mg/dl were considered as DM. Animals were given one week to acclimatize and 3 weeks for treatments. Animals were sacrificed using chloroform sedation prior to collection of blood samples before complete demise of the experimental animals and occurrences of blood clots which usually makes blood sample collection extremely difficult or sometimes impossible when animal is finally dead.

6.0 BLOOD SAMPLE COLLECTION AND PROCESSING

After three weeks of treatments, animals were subjected to active euthanasia using chloroform sedation, after which blood samples were collected, blood for FBG was collected in Fluoride oxalate container while blood samples for Insulin was collected in Plain container. Samples in plain containers were centrifuged at 5000rpm for 10minutes, Serum was harvested and stored in cryovials which was refrigerated at $-4^{O}C$ Until assayed.

7.0 ESTIMATION OF SERUM TC, TG, HDL AND LDL

Serum lipids were estimated by colorimetric methods for TC, TG and HDL, while LDL and VLDL were determined as follows.

LDL= TC- (TG/5) - HDL

VLDL= (TG/5)

8.0 RESULTS

The TC of Non-DM group was significantly lower when compared to TC of DM, Bee Bread Administered and DM + Metformin groups ($P<0.05$). The result of the present study showed that TC of DM group was significantly higher compared to Non-DM, Bee Bread administered and DM + Metformin groups ($P<0.05$), Similarly when compared, between Bee Bread administered

groups for TC, no statistically significant differences were observed (P>0.05), similarly when compared between Bee Bread administered groups and DM + Metformin groups, no statistically significant differences were observed for TC (P>0.05). TG was significantly lower in the Non-DM group compared with DM, Bee Bread administered and DM + Metformin groups (P<0.05). Similarly, TG levels of DM group was significantly higher when compared to other groups (P<0.05). No statistically significant differences were observed for TG when compared between Bee Bread administered groups and between Bee Bread administered groups and DM + Metformin group. HDL levels were significantly higher in Non-DM group when compared with DM group, Bee Bread administered groups and DM + Metformin group (P<0.05). HDL levels were significantly lower in DM group compared to other groups (P<0.05). No statistically significant differences were observed for HDL levels when compared between Bee Bread administered groups and DM + Metformin groups (P>0.05). LDL levels were significantly lower in the Non-DM group compared with DM group, Bee Bread administered groups and DM + Metformin group (P<0.05). LDL levels were significantly higher in the DM group compared to other groups (P>0.05). No statistically significant differences were observed for LDL when compared between Bee Bread administered groups and DM + Metformin (P>0.05). VLDL levels were significantly lower in the Non-DM group compared to DM group, Bee Bread administered groups and DM + Metformin groups (P<0.05). VLDL levels were higher in the DM group, but not statistically significant when compared to Bee Bread administered groups and DM + Metformin groups (P>0.05). furthermore, no significant differences were found for VLDL when compared between Bee Bread administered groups and DM + Metformin group (P>0.05).

TABLE 1: EFFECTS OF BEE BREAD ON SERUM LIPIDS IN ALLOXAN INDUCED DIABETIC FEMALE RATS

GROUP	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NON-DM	94.50 ± 1.98	42.50 ± 2.07	58.83 ± 1.60	27.25 ± 0.63	8.41 ± 0.21
DM	172.67 ± 4.33 [a]	104.50 ± 4.01 [a]	28.33 ± 3.39 [a]	121.49 ± 4.45[a]	20.85 ± 2.90 [a]
DM +200BB	151.67 ± 1.97 [a, b]	84.33 ± 5.09 [a, b]	47.50 ± 4.14[a, b]	87.32 ±2.60 [a, b]	16.88 ± 2.77 [a]
DM +400BB	148.33 ± 3.88 [a, b]	82.17 ±2.79 [a, b]	48.33 ± 3.56 [a, b]	83.56 ± 2.20 [a, b]	16.43 ± 2.61 [a]
DM +Metformin	147.33 ± 2.34 [a, b]	80.67 ± 2.16 [a, b]	48.50 ± 3.55 [a, b]	82.71 ± 2.01 [a, b]	16.12 ± 2.52 [a]

[a] P<0.05 when Compared with Non-DM group, [b] P<0.05 when Compared with DM group.

10.0 DISCUSSION

Bee products have been used as alternative medicine to treat various serious disorders including diabetes mellitus. Our study, for the first time, examined the protective impacts of BB (formed by mono floral rape bee pollen) against T2DM-related complications consistent with lipid and

bone disorders. Therefore, comparison with other studies using either identical bee product, animal model, or both, was not possible in this field of research. Only our findings related to Blood Glucose, Blood Insulin levels, and total BW of ZDF rats were discussed with published researches focused on BB and obese Zucker diabetic rats.

Capcarova et al. (16) state that "ZDF rats receiving BB at a dose of 700 mg/kg BW (for 10 weeks) had significantly lower BG levels only in the pre-diabetic state versus the diabetic control group. At the end of the treatment, Blood Glucose did not change significantly between treated and control groups".

"In numerous types of organisms, it is quite evident by intensive research that free radical cause transformations in molecules and DNA modifications. Various diseases are caused by oxidative stress", according to Atanasov *et al.* (17) and experts from different disciplines turn out to be more engrossed towards natural sources, which possibly will offer proactive constituents to inhibit or decrease oxidation effects on cells (18). Pure BB encloses "numerous flavonoids such as (hesperetin, pinocembrin, apigenin, quercetin, kaempferol, galangin, and chrysin), phenolic acids (caffeic, p-coumaric, ellagic and ferulic acids), Maillard reaction products, catalase, tocopherols, ascorbic acid, superoxide dismutase, reduced glutathione, and peptides" (19).

"Based on above findings, numerous flavonoids (including quercetin) found in Bee Bread scavenge reactive oxygen species (ROS) and this might offer protection of β cells from oxidative damage, this could also increase the amount of circulating Blood Insulin levels. To support this hypothesis, quercetin (15 mg/kg/d intraperitoneally injected for 4 weeks) was determine to partially restore the streptozotocin (STZ)-induced insulin deficiency in rats" (20).

The current study showed that levels of TC, TG, VLDL-C, and LDL Cholesterol (LDL-C) were reduced in the diabetic with administration versus the lean one, thus supporting the findings of Pang et al. (21) and Zhou et al. (22). "Our results revealed that a higher dose of BB reduces TG and TC levels, presumably due to hypotriglyceridaemic and hypocholesterolemic properties of Bee Bread. Significantly decreased TC and TG values were also observed in alloxan-induced diabetic rats supplemented with Nigerian honey (1.0 or 2.0 g/kg for 3 weeks)" (23).

10.0 CONCLUSION

Bee Bread causes significant reduction in Lipids Profile parameters and improves HDL-C in Alloxon induced Diabetic Rats.

ETHICAL APPROVAL

The ethical clearance was collected from Ethics and Research Committee on the use of Animal in Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto.

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