Effect of the Golden Liquid from Honeybees and Refined Sugar on the Blood Glucose and Serum Iron Levels of Albino Rats

ABSTRACT

This study investigated the effect of the golden liquid from honeybees (natural honey) and refined granulated sugar on the blood glucose and serum iron levels of Wistar albino rats. The experimental animals used in this study were grouped into five treatments based on the dose of natural honey and granulated sugar administered namely: T1 (1.02g of honey /kg BW), T2 (1.40g of honey /kg BW), T3 (1.02g of granulated sugar /kg BW), T4 (1.40g of granulated sugar /kg BW) while rats in T5 were not administered with honey and granulated sugar hence, served as the control. The results of the weekly glucose level of albino rat, orally administered natural honey and refined granulated sugar showed that the mean glucose level was highest in T3 (112.95mg/dl), followed by T5 (92.20mg/dl) while the least value was recorded in T2 (74.86mg/dl). The blood glucose concentration of the albino rats was measured using the glucose strips with glucometer while the serum iron analysis was conducted using Atomic Absorption Spectrophotometer. The result showed that the highest serum iron level was recorded in T2 (1.22^{a}±0.115) while the least glucose concentration was recorded in T2 (74.86^{a} ±24.457) though the differences were not statistically significant ($P > 0.05$). The use of natural honey is recommended since, albino rats orally administered honey at varying doses had lower blood glucose level and also the consumption of natural honey increases the serum iron level in the blood.

Keywords: Blood Glucose, Serum Iron Levels Albino Rats, Natural Honey, Refined Sugar

INTRODUCTION

Honey is a sweet natural food made by bees (an insect) using water, pollen, and nectar from flowers (El Sohaimy *et al*., 2015). Honey is produced by bees, In Nigeria; honey is commonly produced by honey bees from the Genus *Apis melifera*. The colour, aroma and consistency of honey all depend upon which flowers the bees have been foraging. Honey is collected by most beekeepers and consumed by people (de Sousa *et al*., 2016; Famuyide *et al*., 2014). Nectar is reduced to honey containing predominantly carbohydrates with very little protein, vitamins, minerals, enzymes, amino acids, and as well several other compounds like phenol compounds thought to function as antioxidants (Wrigh *et al*., 2018).

These chemical components are of great importance as they contribute to the quality, granulation, texture, nutritional and medicinal properties of honey (Kadirvelu and Gurtu, 2013). The major constituents of honey are nearly the same in all honey samples; however, the biochemical composition and physical properties of natural kinds of honey vary significantly according to the plant species on which the bees forage (Formicki *et al*., 2013). Furthermore, the properties of natural kinds of honey also vary depending on the differences in climatic conditions and vegetation of the areas (Ononye and Akunne, 2019).

Natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties, which are attributed to the influence of the different groups of substances it contains (Khan *et al.*,2018). Honey as an antioxidant has been used by physicians throughout history and is utilized in both disease and health conditions, because of its anti-microbial and antioxidant properties (Adenekan *et al.*, 2012). Honey is full of enzymatic and non-enzymatic antioxidants such as catalase, alkaloids, ascorbate, and flavonoids that act against oxidative agents to prevent the body from combing with non-saturated fatty acids and lipoprotein oxidation (Okpokiri *et al.*, 2015). Honey is rich in fructose, glucose, and minerals such as magnesium, potassium, calcium, phosphate, sulphur, ferrous, and sodium chloride as well as vitamins such as B1, B2, B5, B6, and C (Adekanmbi *et al.*, 2019). However, natural honey is used as a replacement for many other sweeteners such as refined sugar/granulated sugar.

Refined granulated sugar commonly is widely used as a sweetener in meals in Nigeria such as breakfast cereals, beverages, and snacks, and many other food products (National Sugar Development Council (NSDC, 2020). Refined granulated sugar is typically refers to a category of simple carbohydrates that includes monosaccharides like fructose and glucose, and disaccharides, like sucrose and lactose, which have different effects on the body (Green *et al.*, 2019). Notwithstanding the vast use of this product, there has been marked growing awareness of the metabolic and associated health effects of excessive sugar consumption in the past several years (Ohilebo, and Odudu, 2020). As of 2019, the World Health Organization recommends reducing the level of sugar consumption to less than 5% of daily caloric intake to lower the risk of unhealthy weight gain and obesity (Green *et al.*, 2019). The American Academy of Paediatrics recommended that parents should not feed fruit juice containing high amount of refined granulated sugar to infants younger than one year because of its high content (Foterek *et al.*, 2016). There are continued awareness and a growing body of research investigating the adverse effect of refined granulated sugar as one of the causative agents of obesity and metabolic syndrome (a combination of risk factors like high blood pressure, high triglycerides, high fasting blood glucose, etc.) that increase the likelihood of cardiovascular disease, type 2 diabetes mellitus, and also non-alcoholic fatty liver disease (Stanhope, 2016).

The lifestyle of modern-day persons involves a high intake of refined granulated sugar either directly or indirectly from foodstuffs (Green *et al.*, 2019). The consumption of large amount of refined granulated sugar alters haematological and physiological changes in human body system (Pries *et al.*, 2017). Although, both honey and refined sugar are food components containing mainly fructose as sugars. Their dietary effects have been extensively investigated in adult animal models such as albino Wistar rats. Several scientific findings have revealed the use of honey as an alternative to the dangers and health risks associated with the consumption of refined granulated sugar (Samat *et al.*, 2017; Atangwho *et al.*, 2020). Many reports encourage consumption and the use of natural honey in food due to in nutritive and medicinal properties in human body (Oyeyemi *et al.*, 2017; Solayman *et al.*, 2016). The use of commercial sugar has been long documented (Rhee *et al.*, 2012). When food materials are ingested, the blood level changes from its normal state depending on the food substance consumed. Many micro nutrients in honey such as iron, calcium and potassium enhance the circulation of blood in human body. It is known that iron found in honey is a precursor for haemoglobin hence consumption of natural honey increases the red blood cell count and serum iron level in the body (Kadri *et al.*, 2017).

According to Erejuwa, (2014), administration of natural honey to albino rats was found to increase serum levels of insulin while it reduced serum concentrations of glucose and fructosamine in diabetic rats. Nevertheless, consumption of honey and refined granulated sugar also increases the blood glucose level at varying levels. In a study conducted by Atangwho *et al*. (2020) noted that in comparison with refined sugar, honey may reduce weight gain and adiposity, presumably due to lower food intake and also promote lower serum triglycerides (Nemoseck *et al*., 2011). Hence, this study was carried out to determine the effect of natural honey and refined granulated sugar on blood glucose and serum iron level on albino rats in Awka, Anambra State.

Refined sugar is one of the well-known sweeteners which have become a favourite sweetener for centuries. It is in the form of crystallized sucrose (a combination of fructose and glucose) which is extracted from sugar beets or sugarcane. Refined granulated sugar has been a part of the daily diet for literally hundreds of years. Research is now suggesting that sugar intake can be detrimental to our health (Moseley, 2012). A growing body of scientific evidence now links long-term overconsumption of added sugars to diabetes, cavities, liver disease, and heart disease. Much of this evidence focuses on a cluster of metabolic issues, known collectively as metabolic syndrome (MetS), that raises people's risk of developing chronic diseases (Ekawati *et al*, 2019). Atangwho *et al*. (2020), revealed that replacing sugar with honey could lower blood sugar levels, improve the growth performance and prevent weight gain or aid in weight loss.

There has been an increasing demand of natural honey and refined granulated sugar by consumers in both developed and developing countries, as well as renewed interest in prospecting for sweeteners with high nutritional quality and pharmaceutical relevance. Interestingly, the outcomes of many of these research exercises on health effect of sweeteners such as diabetic, obesity, increased weight gain in the human body. Addition of excess sugar ultimately affects arteries and circulation and may increase the risk of coronary diseases and also negatively affects the body's organs in human body (Moeller *et al*., 2017). Hence, natural honey as a sweetener has immense therapeutic, nutrition value and economic importance, which could extend beyond local boundaries of healthcare systems and national markets (Solayman *et al*., 2016). It is also been recognized as having several beneficial health properties, including slower uptake into the bloodstream, pharmacological action of reducing blood glucose levels, and a high level of bioavailable antioxidants, all of which may mean that honey could be less harmful to health than refined granulated sugar in the human body (Atangwho *et al*., 2020). The aim of this study was to determine the effect of the golden liquid from honeybees and refined sugar on the blood glucose and serum iron levels of albino rats.

MATERIALS AND METHODS

Study Area

The study was carried out in the Animal House of the Department of Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria. The experimental site lies between latitude 6° 15′ 18.06″ N and longitude 7° 06′ 41.37″ E. Awka is town is located on latitudes 6°9'19"N 7°07'12"E and stretches 8 km in an east-west direction along the Enugu-Onitsha expressway and about 5 km in a North-south orientation. The dimension of Awka is 1,207,800m2 or 12,007 ha. Ecologically, Awka lies in the Guinea Savanna and has experienced 1798.52mm of rain annually between 1977 and 2019. It experiences two seasons: the dry and the wet seasons with a spell of harmattan between December and January (Abajue and Ewuim, 2020).

Source of Experimental Materials

Twenty-five (25) healthy albino rats with mean weights ranging from 41.21±1.247g to 47.27±1.769g were used in the study which was purchased from Mr. Onyewuchi farms in Awka, Anambra State. The albino rats were carefully transferred to the Animal House of the Department of Biochemistry, Nnamdi Azikiwe University, Awka, where the experimental animal was housed for the whole period of the experiments. The refined sugar (Dangote granulated sugar) used for this study was used for the experiment was purchased from Eke-Awka market, Awka Anambra State while the freshly processed natural honey (Benbeela natural honey) was purchased from Uzoben Integrated Services in Awka Anambra State.

Management of experimental Animals

The albino rats were intensively managed in an improvised metallic cage. Each cage consists of length 1m, width 0.5m and height 1m was used to house four albino rats. The top of the cages was covered with wire to allow proper ventilation and the floor was made of tin metal covered with saw dust. During the period of the experiment, the albino rats were subjected to similar sanitary condition so that the only source of variation is the dietary inclusions. Also, clean water was provided for the albino rats daily from 9am to 12pm for the period of 1 month.

Experimental Design

For easy identification, the experimental rats were marked at the head, abdomen, centre, neck and tail then labelled H, Ab, C, N and T respectively. The albino rats were grouped into five treatment groups based on the dose of natural honey and granulated sugar namely: T1(1.02g of honey /kg BW), T2(1.40g of honey /kg BW), T3(1.02g of granulated sugar /kg BW), T4(1.40g of granulated sugar /kg) while rats in T5 was not administered with honey and granulated sugar hence was served as control. The experiment was set up in a completely randomized design. Each treatment comprised of five albino rats.

Glucose Content Determination

The glucose content of the albino rats was measured using the glucose strips with glucometer. A puncture was made on the tale to collect the blood sample. A drop of blood was applied to a chemically active disposable test strip on which the chemical reaction occurred due to the action of glucose deoxyreductase that resulted in color change. Glucometer verified this colour change and showed glucose level. At the same time, a drop of capillary blood, (drawn from the heel) was sampled and applied to the test strip to measure level of glucose. Before sampling, the heel was warmed up by hand massage followed by disinfecting the spot, and then blood sample was taken (Flavigny, 2014).

Serum Iron Analysis:

Method for the analysis of Serum iron

Serum iron analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method of APHA 1995 (American Public Health Association) as reported by Lopes *et al*. (2019).

Sample Dilution

1ml of sample (serum) was measured followed by a 5 dilution with distilled water. This was made up to 10ml for analysis. The absorbance was then measured using FS2400AA Agilent atomic absorption spectroscopy.

Working principle*:* Atomic absorption spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since micro nutrients have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

Preparation of reference solutions

A series of standard micronutrient solutions in the optimum concentration range are prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the micronutrient stock solutions. Calibration curve for each micronutrient was prepared by plotting the absorbance of standards versus their concentrations.

Statistical Analysis

The data obtained from the study was recorded and captured Microsoft excels 2019. The data on the concentrations of blood glucose and serum iron levels in the five treatments was subjected to analysis of variance (ANOVA. Turkey honest significant difference (HSD) was used to separate means using IBM SPSS statistics version 23 at 5% significance ($P<0.05$).

RESULTS

The result from Table 1 showed the weekly blood glucose level of albino rat orally administered natural honey and refined granulated sugar. The result showed that the mean weekly blood glucose level in week 1 was highest in T3 (112.95mg/dl), followed by T5 (92.200mg/dl), followed by T4 (93.700mg/dl) while the least value was recorded in T2 (74.866mg/dl). Statistical analysis showed that there were no significant differences in the albino rats orally administered natural honey and refined granulated sugar at varying levels ($P>0.05$).

Table 1: Blood glucose concentration in albino rats orally administered natural honey and refined granulated sugar for a period of three weeks.

	Weekly mean concentration of blood glucose level ±SD			
Treatment	**Week 1**	**Week 2**	**Week 3**	**Mean blood glucose level ± SD**
T1	78.2^{a}±8.871	84.7^{a}±19.709	104.7^{b}±23.905	89.20^{a}±20.808
T2	70.95^{a}±36.502	85.2^{a}±22.51	68.45^{a}±7.098	74.86^{a} ±24.457
T3	97.7^{a}±22.726	144.95^{b}±34.956	96.2^{a}±21.993	112.95^{a} ±34.407
T4	93.7^{a}±21.058	94.45^{a}±18.341	87.45^{a}±16.908	91.86^{a} ±17.749
T5	92.45^{a}±22.19	94.95^{a}±21.045	89.2^{a}±3.962	92.20^{a} ±16.663

Columns with different superscript are significantly different (P<0.05)

Effect of Natural Honey and Refined granulated sugar on the serum iron levels of albino rats at different dosages.

The result from Table 2 showed that the highest serum iron level was recorded in T2 (1.22±0.115ppm) followed by T3, while the least serum iron level was recorded in T1 (0.88±1.319ppm). It was observed that there were no significant differences (p>0.05) between the treatment groups orally administered natural honey and refined granulated sugar.

Table 2 serum iron concentration of albino rats fed with natural honey and refined granulated sugar

Treatment	**Mean Serum iron level ± SD**
T1	0.88^{a}±1.319
T2	1.22^{a}±0.115
T3	1.04^{a}±0.277
T4	0.99^{a}±0.299
T5	1.31^{a}±0.395

Columns with different superscript are significantly different (P<0.05)

Discussion

Findings of the study indicates that natural honey does not significantly increase the blood glucose level of albino rat as granulated sugar does. This finding is similar to that reported by Sohaimy *et al*. (2015) who noted that Dextrose (glucose) and laevulose (fructose) are the main sugars in honey. Similarly, Kek *et al*. (2017). These are the building blocks for the more complex honey sugars and account for about 85 percent of the solids present in honey. Furthermore, Adekanmbi *et al*., (2019) reported that honey is rich in fructose, glucose, and minerals such as magnesium, potassium, calcium, phosphate, sulphur, ferrous, and sodium chloride as well as vitamins such as B1, B2, B5, B6, and C.

The result from the weekly glucose level in albino rats orally administered natural honey and refined sugar showed that highest value was found in T3. The findings of this study were in line with previous studies (Tappy and Le, 2010; Bezborodkina *et al*., 2014; Sun *et al*., 2010) on the effect of natural honey on regulation of blood glucose level and it benefits in the treatment of type 2 diabetes. Thus, bee honey is useful for diabetic patients as a tasty substance and as a nutritious substance. Honey may have a role in diabetic complications, so more effort is needed to be exerted in this field (Pasupulet *et al*., 2020).

The effect of natural honey and refined sugar on the serum iron levels of albino rats at varying doses showed that the highest serum iron level was recorded in T2 (1.40g of honey /kg BW) (1.22±0.115) while the least serum iron level was recorded in T1 (0.88±1.319 kg BW). the result of this study showed that honey inclusion at 1.40g of honey /kg BW has high serum. This has been reported by Erejuwa *et al*., (2012) who reported that honey administration was found to increase serum iron levels of while it reduced serum concentrations of glucose and fructosamine in diabetic rats. It was further concluded that natural honey could affect the levels of many blood (haematological) elements in the body.

Conclusion

This study showed that honey possess many advantages over commercial sugar, since the natural honey in this study honey possess high fructose content it means that honey can be used as a good source of carbohydrate. Also, natural honey in this study was found to contain substantial amount of copper and manganese which is good for human body compared to refined sugar. It was further observed that natural honey can be used to manage blood glucose level in the body and also that the consumption of natural honey increases the serum iron level in the blood. It was therefore recommended that natural honey should be used over refined sugar especially to people suffering from diabetes.

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