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THE EFFECT OF HONEY, WHITE AND BROWN TABLE SUGAR ON LIPID PROFILE AND GLUCOSE LEVEL IN RATS

BY

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DECLARATION

I hereby declare that this thesis is my own research (except for references to other people's work which has been duly acknowledged), undertaken under the supervision of the underlisted below. I am wholly responsible for any flaws in the work. This thesis has not been presented in whole or in part for any degree elsewhere.

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DEDICATION

I dedicate this thesis to the Almighty God and my lovely family.

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ABREVIATIONS

CVD	Cardiovascular disease
NAFLD	Non-alcoholic fatty liver disease
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
TC	Total cholesterol
TG	Total triglycerides
SSB	Sugar sweetened beverage
WHO	World Health Organisation
IDF	International Diabetes Federation
CAD	Coronary artery disease
BS LD	Brown sugar low dose
BS HD	Brown sugar high dose
H LD	Honey low dose
H HD	Honey high dose
WS LD	White sugar low dose
WS HD	White sugar high dose
PBS	Phosphate buffer solution

HbA1c	Glycated haemoglobin
FBG	Fasting blood glucose
PAS	Periodic Acid Schiff
S-D	Sprague-Dawley
WS	White sugar
BS	Brown sugar
H	Honey
CAD	Coronary Artery Disease

ABSTRACT

Background: Honey and table sugar are commonly used sweeteners by many consumers. These sweeteners are examples of disaccharide sugar which forms part of the complex macromolecule, carbohydrate. Excessive consumption of dietary sugars including table sugar and honey is associated with several metabolic abnormalities and adverse health conditions such as obesity, diabetes and cardiovascular disease. There is a general debate by many researchers and consumers that honey is better than brown sugar which is also better than white sugar. However, scientific data supporting these claims are inconclusive on the effect of these natural sweeteners on the various biomarkers of cardio vascular health and glycaemia.

Aim: The aim of the study was to determine the effect of honey, white and brown table sugar on lipid profile, glucose level and pancreatic insulin level and histology using animal models.

Methodology: Thirty-five (35) male Sprague Dawley rats aged 12 - 14 weeks, weighing 150g - 250g were obtained. The groups were set up as follows: Group 1 (G1) – control group, group 2 (G2) - white sugar low dose 0.055 g (WS LD), group 3 (G3) - white sugar high dose 0.22 g (WS HD), group 4 (G4) - brown sugar low dose 0.057 (BS LD), group 5 (G5) - brown sugar high dose 0.230 g (BS HD), group 6 (G6) - honey low dose 0.076 g (H LD) and group 7 (G7) - honey high dose 0.304 g (H HD). One (1) ml of the prepared white sugar, brown sugar or honey solution was administered daily to the treatment group of rats orally by gavage for 12 weeks. After a 12 week administration period, the rats were sacrificed for blood and tissue analysis. Four millimetres (4ml) of blood samples was collected for analysis of lipid profile (total cholesterol, total triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol) glycated haemoglobin (HbA1c) and fasting blood glucose (FBG). The harvested organ was weighed and divided into two parts. Half went into buffered formalin and the other half went into

a container and stored at -80°C for insulin analysis. The pancreas was homogenized using dounce tissue grinder in cold PBS, for each 1 g of pancreas. The sample was centrifuged at approximately 10000 X g for 5 min. The supernatant was collected and insulin was measured. Two way ANOVA was used to compare the means within and between the treatment groups. Where ANOVA was significant, the post hoc test was performed using Bonferroni analysis. A p-value ≤ 0.05 was deemed statistically significant.

Results: In this study, the assessed body weights of animals showed clear and continuous weight gain throughout the treatment groups, even though the difference was not significant between the groups. Also, the effect of intake of the three natural sweeteners white sugar, brown sugar and honey on the HbA1c, glucose level and lipid profile of the rats showed a significant increase in the high dose groups compared to the control group. Furthermore the effect of white sugar brown sugar and honey on the insulin levels of rats showed a significant decrease in insulin level of the high dose groups compare to the control group. However, the effect of white sugar, brown sugar and honey on insulin showed no significant difference between low dose groups when compared with the control group.

Conclusion

A twelve week treatment of honey, white and brown table sugar was found to cause an appreciable increase in FBG, HbA1c and lipid profile and release of insulin at the same dose. The increase was found to occur mostly in the high doses than in low doses with a few disparity.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Honey, white and brown table sugar are commonly used natural sweeteners. Sweeteners are chemical compounds, found in nature or chemically synthesized, which have a sweet taste that determines their usage as sweetening agents (Mooradian et al., 2017). The most common natural sweeteners may contain simple sugars such as monosaccharides (glucose, fructose, and galactose) and disaccharides (lactose, maltose, and sucrose) (Carocho et al., 2017). Added sugars include natural sugars such as white sugar, brown sugar and honey (Bowman, 2017).

The history of honey goes as far back as 3000 BC. It was used as medicine by the Egyptians and Chinese, while Greeks regarded it as food for the gods (Meo et al., 2017). Glucose and fructose are the major monosaccharide combination found in honey and sugar (Kolayli et al., 2012). Honey contains trace quantities of a variety of mineral and vitamins, including zinc, magnesium, iron, potassium, niacin, copper, riboflavin and zinc (Al-Waili et al., 2013). This gives it an added value making it a popularly used sweetener (Al-Waili et al., 2013). Because of its antioxidant properties and a range of health benefits associated with long-term use, honey is considered better than brown or white sugar (Chepulis and Starkey, 2008, Kappico et al., 2012, da Silva et al., 2016).

White sugar is one of the most popular sweeteners used globally today. It also known as refined sugar, table sugar, granulated sugar or regular sugar (Baikow, 2013a). It is obtained from sugar cane or sugar beet juice which is refined to remove molasses (Panda, 2011). White sugar has several uses in the food processing industry. It is a versatile ingredient and contributes many

functional properties to food products such as texture, colour and taste (Brisbois et al., 2014). One tablespoon of white granulated sugar contains about 49 calories (Kumar et al., 2010) while a tablespoon of honey has 68 calories, illustrating why honey is greater in density and weight than sugar. (Kumar et al., 2010).

Brown sugar is the moist and dark sugar produced as a result of sugar cane broth evaporation. (Orlandi et al., 2017). Unlike white sugar, brown sugar is not involved in many chemical processes therefore it is considered a more suitable and healthier alternative (Rodríguez-Entrena et al., 2016, Orlandi et al., 2017). Sugars may be used in foods during preparation, processing, or added at the table (Newens and Walton, 2016). This makes it an inseparable component of our diet and many people consume more sugar than they realize due to it sweet taste.

Several metabolic disorders and detrimental health problems have been associated with excessive intake of these added sugars (Bray and Popkin, 2014). Natural sweeteners are frequently found well above the ideal value in concentrations in food (Bernstein et al., 2016). This consequently led to an absolute dependency or obsessive ingestion of excess sugar (Swarna Nantha, 2014). The amount of sweetness depends on the chemical composition of the sweetener (Clemens et al., 2016b).

Globally, due to an increase in calorie consumption, the incidence of obesity is growing rapidly (Swinburn et al., 2011, Malik et al., 2013b, GBD, 2017). Health complications associated with the excessive consumption of natural sweeteners has been an issue of global concern. Natural sweeteners form a core of meals and the effects of frequent or excessive consumption has been underestimated by majority of the populace. A review of sugar consumption by developed countries found that intake levels in adults living in the United States increased from 13.5% to 24.6% (Bleich et al., 2008). In order to minimize the risk of disease, dietary recommendations for

Americans have been changed to limit intake of added sugar to less than 10% of calories per day (Health and Services, 2017). Similarly, the American Heart Association also suggests that the consumption of added sugar in men and women should not exceed 150 kcal or 100 kcal per day (Vos et al., 2017).

In 2010, it was estimated that among Africa adults 20 years and above, 27% were overweight and 8% obese (WHO, 2010). The increase in the worldwide prevalence of obesity was linked to the excess intake of added sugars (Mooradian et al., 2017). Several studies have shown a significant relationship between the excessive consumption of sugar-sweetened beverages and body weight gain in adults (Chen et al., 2009, De Koning et al., 2012, Pan et al., 2013). Obesity is a known major risk factor of diseases such as diabetes and cardiovascular disease (Te Morenga et al., 2013, Malik et al., 2013a, Saravanan et al., 2014).

In 2015, the prevalence of diabetes in adults aged 20-70 years was 8.8 % and is expected to rise to 10.4 % by 2040 (WHO, 2016a). In 2012, 2.6 million (4.5%) deaths worldwide were related to CVDs (Laslett et al., 2012). The effect of added sugar consumption on a person with diabetes can exacerbate symptoms because diabetes makes it harder for the body to regulate blood sugar levels (Bray and Popkin, 2014). It is also reported in a study that the long term consumption of honey improves weight regulation and decreases blood sugar levels as well as increase levels of HDL cholesterol (Chepulis, 2007).

Potential connection regarding added sugar consumption and dyslipidaemia characterized by elevated low-density cholesterol lipoprotein (LDL-C) and triglycerides (TG) (Kell et al., 2014, Rippe, 2014). Some studies have also linked it with low high density lipoprotein cholesterol (HDL-C) concentrations (Kell et al., 2014, Rippe and Angelopoulos, 2016, Vos et al., 2017). A correlation between sugar intake and fasting and post-prandial concentrations of triglycerides,

which are seen as absolute biomarkers of cardiovascular diseases, has also been found (Siri-Tarino, 2011, Kim and Jee, 2015). Excessive intake of sugar is related to increased levels of triglyceride, which is known to facilitate the onset of coronary heart disease (DiNicolantonio and OKeefe, 2017). However, the association between sugar intake and Low Density Lipoprotein Cholesterol (LDL-C) or High Density Lipoprotein Cholesterol (HDL-C) remains unclear (Johnston et al., 2013, Atangwho et al., 2017). A possible association between added sugar consumption and adult mortality from cardiovascular diseases has also been identified (Yang et al., 2014). Findings of the effect of natural sweeteners on health are controversial especially its effect on glycaemia and lipid profile thus further study on the health effects of these natural sweeteners are warranted.

1.2 PROBLEM STATEMENT

There is a global concern about high consumption of natural sweeteners and its association with a number of health conditions (WHO, 2015). Several studies have shown an indirect correlation between excess sugar intake and obesity, a risk factor for type 2 diabetes, dyslipidaemia and CVDs (Fitch and Keim, 2012, WHO, 2015, Rogers et al., 2016). Due to these health implications, consumers have become more conscious of the use of these natural sweeteners (Bruyère et al., 2015). There is a general debate by many researchers and undocumented claims by many consumers that honey is better than brown sugar which is also better than white sugar (Langlois and Garriguet, 2011, Valli et al., 2012, De Maria, 2013, Roman et al., 2013). It is still unclear if some natural sweeteners are beneficial to good health than others. Scientific data supporting these claims are inconclusive on the effect of these natural sweeteners on the various biomarkers of cardio vascular health and glycaemia (White, 2013, Klurfeld et al., 2013, van Buul et al., 2014). Furthermore, much of the information about the role of sugars on nutrition and

health are inconsistent (Arola et al., 2009, Basu et al., 2013). It is therefore necessary to conduct further studies to determine the effect of honey, white and brown table sugar on biomarkers of cardio vascular health and glycaemia.

1.3 JUSTIFICATION

Sugar forms an essential dietary component in processed or unprocessed foods due to it sweet taste. Nutrition and food knowledge deficit among many consumers has negatively affected food choices and dietary pattern. It is believed that less processed natural sweeteners such as brown sugar and honey are all healthier than white sugars coupled with product advertisement which has increased the use and consumption of added sugars (Bailin et al., 2014). In recent years, metabolic syndrome has emerged as a major global health issue because of excessive sugar intake (Kahn and Sievenpiper, 2014). The rise in metabolic syndrome has accompanied the drastic increase in obesity, dyslipidaemia and insulin resistance worldwide (Johnston et al., 2013).The cost of health care of individuals suffering from the complications of excessive consumption of added sugar or nutritive sweeteners has caused socio economic burden on families (WHO, 2016b). Therefore, it has become necessary to explore the effect of these natural sweeteners on biochemical indices of diabetes and cardiovascular health. This study's results will provide information that will help to distinguish if honey is healthier than brown and white table sugar. It will also provide information on the effect of these natural sweeteners on the release of insulin. Additionally, the effect of these natural sweeteners on blood glucose levels and lipid profile will be established. This knowledge may assist dieticians and nutritionists in designing appropriate interventions for individuals with nutrition and diet related diseases. Findings of this study may also enable other health professionals, consumers and policy makers to make informed decisions when dealing with natural sweeteners. Furthermore, findings from this study

can help in the formulation of food based guidelines and nutritional policies with respect to consumption of added sugars.

1.4 HYPOTHESES

Hɵ: Honey will contribute less to glycaemia than brown and white sugar.

Hɵ: Honey produces a better lipid profile than brown and white sugar.

1.5 AIMS AND OBJECTIVES

The main aim of this study was

To determine the effect of honey, white and brown sugar on lipid profile, glycaemia, pancreatic insulin levels and histology using animal models.

1.5.1 SPECIFIC OBJECTIVES

1. To determine the effect of honey, white and brown sugar on glycaemia by measuring glycated haemoglobin and serum glucose level.
2. To determine the effect of honey, white and brown sugar on cardio vascular disease risk indices by measuring total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C).
3. To determine the effect of honey, white and brown table sugar on insulin level using pancreatic tissue homogenate.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 NATURAL SWEETENERS

Sweeteners are chemical compounds, found in nature or chemically synthesized, which have a sweet taste that determines their usage as sweetening agents (Mooradian et al., 2017). Monosaccharides (fructose, galactose and glucose) and disaccharides (maltose, sucrose and lactose) are the most typical sugars in our diet (Gabius, 2011). Table sugar and honey are a major source of dietary sugar (Edwards et al., 2016). Table sugar is primarily composed of sucrose and is obtained through industrial methods, typically from sugar cane (*Saccharum officinarum L.)* or beet (*Beta alba L.)* (Edwards, 2018). They can be used for methods such as food preservation, fermentation in brewing and wine manufacturing (Erickson and Slavin, 2015).

Many years ago, human diet has included a variety of natural sweeteners from cane sugar, maple syrup, honey, stevia, molasses, date sugar, agave nectar (Grembecka, 2015). However, in the beginning of the 20th century sucrose became the main sweetener used by many consumers and food industry. Currently, table sugar is produced in almost 120 countries with its global production exceeding 165 million tons a year (80% from sugar cane, the rest from sugar beets) (Newens and Walton, 2016). The biggest sugar cane producer in 2013 was Brazil followed by India and China (Li and Yang, 2015).

The terminology used to describe natural sweeteners can be sometimes confusing and unspecific (Lineback and Jones, 2003). They may be classified into natural or synthetic agents, powders and syrups, and caloric and non-caloric (Priya et al., 2011). Added sugars are food substances that are incorporated into food during processing or added at the table (Vos et al., 2017). They may

include white sugar, brown sugar, honey, maple syrup, and high fructose corn syrup (Ervin, 2012, Edwards et al., 2016). These added sugars have similar amount of energy approximately 4 calories per gram, and contain negligible quantities of micronutrients (Clifford and Maloney, 2016).

2.2 PROPERTIES OF NATURAL SWEETENERS

Natural sweeteners are commonly recognizable carbohydrates that are also a source of energy that is simple and fast to digest (Louie and Tapsell, 2015). Natural sweeteners are mostly disaccharides (sucrose) of glucose and fructose unit (Pigman, 2012). It's a naturally occurring carbohydrate found in many fruits, vegetables and grains. Sucrose is a carbohydrate with the formula $C_{12}H_{22}O_{11}$ (Ervin and Ogden, 2013). The refined form of sucrose is known as table sugar or simply sugar. It may come as white or brown granulated crystals in table sugar or a viscose fluid in honey.

Sugar applies to a variety of carbohydrates, such as monosaccharides, disaccharides and polysaccharides, in scientific terms (Pigman, 2012). Monosaccharides are often referred to as simple sugars, with glucose being the most common (Edwards, 2018). It is not possible to hydrolyse them further into simpler chemical. They are known to be the building blocks of disaccharides and polysaccharides (BeMiller, 2018). Disaccharides are sugars consisting of two units of monosaccharides joined by a carbon oxygen carbon bond known as a glycosidic bond. (Yalpani, 2013). This linkage can be broken apart into its two simple sugars in a process called hydrolysis (BeMiller, 2018). Sucrose, maltose, and lactose are common types of disaccharides. Sucrose is the disaccharide of glucose and fructose (McGrath and Fugate, 2012). Polysaccharides are long chain polymeric carbohydrates consisting of units of monosaccharide connected together by glycosidic bonds (Yalpani, 2013). A polysaccharide can be a homopolysaccharide, in

which all the monosaccharides are the same, or a heteropolysaccharide in which the monosaccharides vary (Syrovaya et al., 2018).

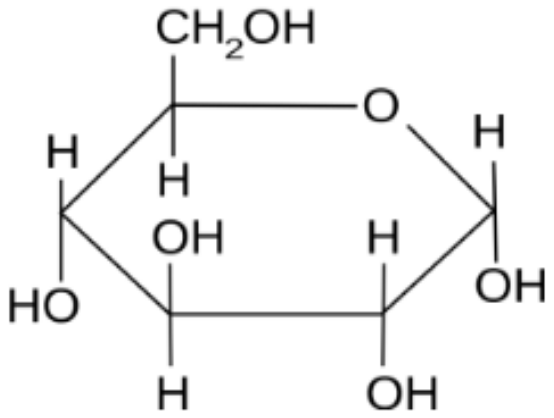


Figure 2. 1: Chemical structure of glucose

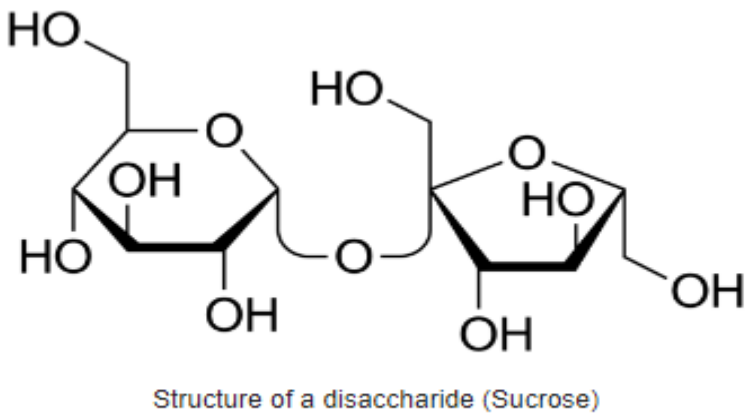


Figure 2. 2: Chemical structure of sucrose

Natural sweeteners have some distinctive properties which enable consumers and food producers to utilize sugar and honey in various ways (Guerra and Mujica, 2010). This includes sweetness, texture, fermentation, preservation and moisture retention, solubility.

2.2.1 Sweetness

Sweetness is marked by the one of the five basic taste sensations that is usually pleasing and commonly perceived when eating foods rich in sugars (Mooradian et al., 2017). It is a sensory experience in the taste receptors on the tongue that is passed on to the brain through the gustatory nerves (Ahmed et al., 2013). Among the factors that influence sweetness are concentration, temperature, pH value and individual sense of taste (Guerra and Mujica, 2010). Honey, white and brown table sugars are naturally sweet therefore it is commonly used in commercial food production and as added sugar. The degree of sweetness is dependent on the chemical structure of the sweetener (Clemens et al., 2016b). Sucrose, either white or brown table sugar is normally used as the standard for sweetness (White, 2014). It has a relative sweetness of 100. However fructose is sweeter with a relative sweetness of 160.

2.2.2 Texture

Texture is an expression of how something feels. It is an important component in the food industry (Forde et al., 2013). By offering volume and consistency in many items such as beverages, jam and bread, sugar influences texture (Lees, 2012). Sugar can add viscosity, consistency and mass to liquid food during commercial production (Mathlouthi and Reiser, 2012). Sugar is used to achieve the right balance between pectin and acid in the processing of jam, marmalade and jelly (Seetal, 2010). When mixed with pectin, the ability of sugar to gel is important to the quality of jam (Seetal, 2010). This may influence the mouth sensation, and thus the sense of taste. (Stieger and van de Velde, 2013).

2.2.3 Fermentation

For years, the fermentation method has been adopted to preserve foods (Lees, 2012). The fermentation process also includes the use of yeast as an energy source and sometimes sugar (Zabed et al., 2014). The form and quantity of added sugar will improve the yield of dough and loaf softness by changing the rate of fermentation (Gisslen, 2012). Sugar activates the yeast in baking, in which the enzymes of the yeast convert the sugar into ethanol and carbon dioxide (Cauvain, 2012). The carbon dioxide raises the dough and renders the baked goods porous (Cauvain, 2012). For this function, sugar in the final product may not always be available. (Lees, 2012). Sugars that remain after fermentation, however, contribute to the final product 's overall taste, colour and texture (Gisslen, 2012). Fermentation of sugar is therefore key in baking and other food processing (Gisslen, 2012).

2.2.4 Preservation

Preservation prevents the growth and activity of microorganism that promote spoilage (Gould, 2012). Foods are preserved to avoid the growth of microorganisms that damage food items (Gould, 2012). This process prevents pathogenic microorganisms that can cause diseases (Ray and Bhunia, 2013). In order to grow, microorganisms need water (Hamad, 2012). They can absorb water using the outer layer of the membrane (Christy et al., 2014). Large quantity of sugar in a food product brings about water loss to the product (Clemens et al., 2016b). This inhibits the growth of microorganisms, as they are deprived a key component for the growth and duplication drops (Christy et al., 2014). The introduction of sugar in food substances can increase the osmotic pressure, thereby minimizing the advantage of microorganism growth. (Goldfein and Slavin, 2015). By creating the most unfavourable conditions for microorganisms, it may increase the shelf life of the food (Hamad, 2012). Sugar can play an important role by

acting as a humectant in maintaining and stabilising the moisture content in foods (Hamad, 2012, Clemens et al., 2016b).

2.2.5 Solubility

Dietary sugars are strongly soluble in water and other polar solvents (Clemens et al., 2016b). They are however insoluble non-polar organic solvents (Clemens et al., 2016b). Due to it solubility, it is a useful ingredient in the food industry (Caraher and Perry, 2017). Dietary sugar is added to various pre-package foods to improve taste and shelf life (Caraher and Perry, 2017). This is made possible because of its ability to dissolve and mix well with other ingredient in food processing (Clemens et al., 2016b). Sugars are more soluble when the temperature of it solvent is increased (Newens and Walton, 2016).

2.3 TYPES OF NATURAL SWEETENERS

2.3.1 White table sugar

Sucrose is found in almost all plants (Li and Yang, 2015). It can be present in sugar cane and sugar beet in concentrations high enough for economic benefit (Bakker, 2012). Sugar cane *Saccharum officinarum* and sugar beets *Beta vulgaris* are popularly cultivated crops (Li and Yang, 2015). Sugarcane is a giant cultivated grass in tropical and subtropical regions (Bakker, 2012). Whereas sugar beet is a root crop that grows in temperate regions (Ma et al., 2014). Processing of sugarcane into table sugar can range from a relatively simple to a multistep process (Baikow, 2013a). The final result varies, depending on the specific steps in the process (Rein, 2016). Mechanically or manually, the sugar cane crop is harvested and cut into lengths (Bakker, 2012). It is either milled and the juice is extracted using a sugar miller or extracted by diffusion (Hugot, 2014). In a series of evaporators, the thin syrup is condensed and water is further extracted (Hugot, 2014). The resulting supersaturated solution is seeded with sugar

crystals, facilitating crystal formation and drying (Baikow, 2013b). Light and dark brown, powdered, and granulated white sugars are all highly refined to give a white granulated sugar (Rein, 2016). White sugar can also be obtained from sugar beet (Cooke and Scott, 2012). Sugar juice is obtained after soaking sliced sugar beet in hot water (Draycott, 2008). The juice is concentrated by boiling under vacuum to remove much water (Cooke and Scott, 2012). Sugar is crystallized from the concentrated solution (Rein, 2016). To isolate the pure sugar crystals from the syrup, it is then spun in centrifuges (Cooke and Scott, 2012). These crystals are then dried and processed in silos until they are packed or sent to consumers in bulk (Baikow, 2013b).

2.3.2 Brown table sugar

Brown table sugar is a moist and dark coloured sugar obtained as a product of the evaporation of sugarcane juice (Orlandi et al., 2017). Evaporated cane juice is essentially a coarse, heavier and coloured version of white sugar (Abdullah et al., 2014b). It is less refined than white table sugar and also contains some trace minerals, vitamins and molasses (Abdullah et al., 2014a). Brown sugar can also be produced by adding sugarcane molasses to white sugar (Yang et al., 2020). The ratio of molasses to sugar crystals is carefully regulated (Yang et al., 2020). This preparation of brown sugar is also much coarser than its unrefined counterpart. (Brekhman and Nesterenko, 2013). Brown sugars can be slightly centrifuged or hold a far greater degree of molasses without centrifugation (Orlandi et al., 2017). Depending on its origin, brown sugar is identified with different names (Valli et al., 2012). Example of such names include panela, rapadura, jaggery, muscovado, turbinado and unrefined sugar (Abdullah et al., 2014a, Jaffé, 2015). Brown sugar is used similarly to granulated white sugar but is known to give a touch of extra flavour (Rein, 2016). The colours vary from light brown to dark brown (Singh et al., 2015). The colour and taste variations between the various brown sugars depend on the amount of molasses present

(Valli et al., 2012). The more molasses, the stickier the crystals, darker the colour and stronger the flavour (Yang et al., 2020).

2.3.3 Honey

The European Communities legislation described honey as the natural sweet substance made from nectar by *Apis mellifera* bees (Machado De-Melo et al., 2018). It can be produced from the secretions of living plant parts. (El Sohaimy et al., 2015). It is a natural material that mainly consists of 80% carbohydrates (Alvarez-Suarez et al., 2014). It includes a variety of other compounds that are expected to confer health benefits when ingested (Machado De-Melo et al., 2018). These compounds may include polyphenols, aromatic compounds, organic acids, amino acids, minerals, vitamins (Omotayo et al., 2010, White, 2013). The type of environment and vegetation affect the constituent of honey (Manyi-Loh et al., 2011a). However, certain external factors such as seasonal, environmental and processing may play a role in its composition (Manyi-Loh et al., 2011a). Since ancient days, honey has not only been seen as a food or a sweetener, but also used as a medicine (Bahrami et al., 2009). The composition of honey is uniquely dependent on the floral source (Schneider et al., 2013). Notably, there are different varieties of honey based on its composition and origin (El Sohaimy et al., 2015). Depending on the variety of plants from which nectar is obtained, the composition of this substance can differ significantly (El Sohaimy et al., 2015). Honey has been used as a regular sugar substitute over the years (da Silva et al., 2016). Consumers decision to buy honey are influenced by economic factors indication the financial situation of many household who can buy them (Roman et al., 2013).

2.4 SUGAR PRODUCTION

The world sugar production from 2015/2016 with a forecast to 2020/2021 shows that, approximately 188 million metric tons of sugars were produced globally (USDA, 2020). Brazil and India are the largest sugar-producing countries in the world, yielding approximately 588 million metric tons of sugar (USDA, 2020). Production in Brazil is expected to recover from 9.6 million tons to 39.5 million metric tons (USDA, 2020). Initial worries about the 2019 dry spell in August-October were compensated by steady rainfall in January-March (USDA, 2020). This was welcomed by an improved sugarcane harvest (USDA, 2020).

2.5 SUGAR CONSUMPTION

The global consumption of sugar amounted to 171.58 million metric tons in 2018/2019, which is expected to grow by 2019/2020 to around 176.45 million metric tons (USDA, 2020). Consumption of sugar is expected to rise to about 177.79 million metric tons in 2020/2021 with the rise in world trade and improved agricultural engineering, among other factors. (USDA, 2020). India is the leading consumer of sugar globally with an estimated consumption of 27 million metric tons in 2019/2020 (USDA, 2020). Overall sugar consumption has increased globally within the last five years by 2.33 million metric tons.

2.6 SUGAR EXPORT AND IMPORT

The global export of sugar is 54.12 million metric tons in 2019/2020 forecast to 65.22 million in 2020/2021. Brazil is the world leading exporter of sugar with an estimated export of 19.3 million metric tons in 2019/2020 forecast to 28.85 million metric tons in 2020/2021 (USDA, 2020).

While there has been an appreciable decrease in global sugar import from 54.63 million metric tons in 2015/2014 to 50.71 million metric tons in 2019/2020. It is forecast to 54.8 million in

2020/2021 (USDA, 2020). Indonesia and China are the world leading importers of sugar with an estimated import of 4 million and 4.1 million metric tons in 2019/2020 forecast to 4.65 and 4.2 million metric tons respectively (USDA, 2020).

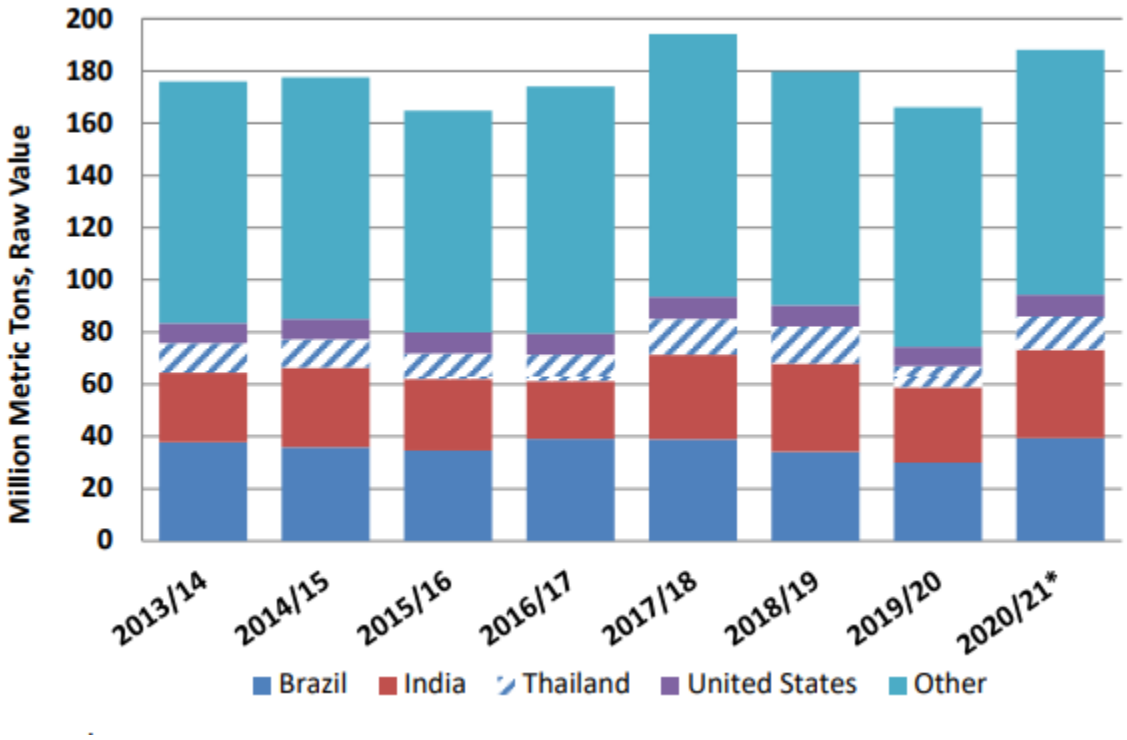


Figure 2. 3: Global sugar consumption from 2013/2014 forecast to 2020/2021

2.6.1 DIGESTION AND METABOLISM OF SUCROSE

2.6.2 Sucrose digestion

The digestion of sucrose does not commence until the sugar reaches the small intestine (Pigman, 2012). Polysaccharides cannot be taken up by the body as it must first isolate sucrose into its component (Fox, 2015). Sucrase, an enzyme in the small intestine, makes it easier for sucrose to break down into glucose and fructose (Fox, 2015). The monosaccharides are transported across the intestinal epithelium into the bloodstream (Pigman, 2012) It is then transferred to the various

body's cells (Pigman, 2012). The developed glucose and fructose monosaccharides are absorbed and can then be used to harness energy in metabolic pathways (BeMiller, 2018).

2.6.3 Sucrose absorption

Glucose and fructose are more recognizable by the body for absorption (BeMiller, 2018). Once it is available in this simple form, the intestinal lining can now absorb both sugars (Pigman, 2012). This happens by various dynamic transporters into the hepatic portal system's bloodstream (Johnson et al., 2010). This system is one of a few that does not return blood directly to the heart (Fox, 2015). Instead, the blood containing all the obtained nutrients is transferred to the liver for further processing (Fox, 2015).

2.6.4 Sucrose Metabolism

The primary energy source, glucose, is transferred from the liver to cells in the body (Johnson et al., 2010). Uptake into cells is facilitated by insulin (Rippe and Angelopoulos, 2013). In a mechanism called glycolysis, glucose converted to pyruvate. (Dashty, 2013). Pyruvate, the end product of glycolysis is an acidic compound (Gray et al., 2014). In order to generate energy, it can then enter either aerobic or anaerobic state (Ruan, 2014). The metabolism of fructose occurs through fructolysis (Harvey and Ferreir, 2011). This process is similar to, but more complex than glycolysis (Harvey and Ferreir, 2011). The breakdown of fructose can make end product which can yield energy (Sun and Empie, 2012). However, fructolysis occurs mainly in the liver, unlike glycolysis, which can take place in almost all tissues (Hannou et al., 2018).

2.6.5 Sugar Storage

Excess glucose which was not utilized in energy production is stored as glycogen (Dashty, 2013). This process binds single subunits of glucose via a chemical bond into long chains, known as glycogen (Adeva-Andany et al., 2016a). Glycogen is hydrolysed back into glucose

during fasted state (Moore et al., 2012). As the body completes its glycogen storage ability, all remaining glucose is converted into fat (Adeva-Andany et al., 2016b). Fructose is not retained in the body for a long time, since the liver converts all fructose into glucose-like molecules. (Hannou et al., 2018).

2.7 NATURAL SWEETENERS AND OBESITY

2.7.1 Obesity

Obesity is defined by the World Health Organization as an excessive fat build-up which affects health (WHO, 2020). The Body Mass Index (BMI) is commonly used to calculate a person's weight; calculated as a person's weight in kilograms divided by meters by the height square (kg / m^2). Overweight and obesity for adult is described by the World Health Organisation as BMI $\geq$ 25 kg/m^2 and BMI $\geq$ 30 kg/m^2 respectively (WHO, 2020). Obesity is a phenomenon of processes, induced by different systems (Rutter, 2018)

2.7.2 Prevalence of Obesity

Obesity is known as a public health epidemic and is a global health concern (Nguyen and Lau, 2012, Piché et al., 2018). Obesity is expected to occur in around 500 million adults worldwide in 2011 (Seidell and Halberstadt, 2015). The prevalence of obesity reported by the WHO in 2014 was 13% globally. In 2016, the WHO reported that 11% of men and 15% of women aged 18 and over were obese (WHO, 2020). In the United Kingdom (UK), the prevalence of obesity almost doubled from 13% to 24% between 1993 and 2011 (Hruby and Hu, 2015) . Also, in 2013 to 2014, approximately one third of adults in the United States of America (USA) were both overweight and obese (Flegal et al., 2016). In a study conducted among Portuguese speaking people, prevalence of obesity was recorded at 28.6% and obesity was higher in females than in males similar to other countries (Gaio et al., 2018, Oliveira et al., 2018).

The prevalence of overweight and obesity and obesity in Africa varies from country to country. A meta-analysis review of the prevalence of adult obesity in Africa reported a prevalence rate of 21.8% among people with less schooling, female gender and disadvantaged status with rising rates over time (Tulp et al., 2018). A 2014 study conducted in Algeria revealed a 32.5% and 30.9% prevalence of overweight and obesity among adults, respectively (Dalichaouch-Benchaoui and Abadi, 2014). The prevalence of overweight and obesity was found to be 9.4% among high school adolescents in Ethiopia. (Alemu et al., 2014). The crude prevalence of overweight and obesity among civil servants in Lagos, Nigeria, was 70.7% (Ajani et al., 2015). In Issele-uku, a community in Nigeria, the prevalence of obesity among adults was 5.5% (Agofure, 2018). Among Sudanese individuals, the prevalence rate was reported at 21.2% (Ahmed et al., 2017). In Zambia it was 24.7% (21.0% among males and 27.3% among females) (Zyaambo et al., 2012).

2.7.3 Effect of natural sweeteners on obesity

Table sugar and honey are common sweeteners that have been part of human diets since ancient times (Priya et al., 2011). These sweeteners enhance the taste and palatability of foods (Amarra et al., 2016). Compared to non-sweetened foods, this triggers a significant rise in the quantity of such foods eaten at a time (Amarra et al., 2016). It is also recognized that its impact on neurotransmitters and pleasant brain sites after prior exposure induces sugar addiction (Berridge and Kringelbach, 2015). This can result in an total dependency on or compulsive intake of excess sugar (Avena et al., 2008). Furthermore, sugar containing foods have been advertised in ways such as associating the consumption of sugar sweetened beverage with happiness (Nestle, 2013). This may have led to the increase patronage and consumption of such products thereby increasing calorie intake. An approximate 15 % of the overall energy consumption in the United

States is generated from added sugars (Marriott et al., 2010). Excess glucose obtained from the consumption of added sugar is stored as glycogen in the liver and muscles or as fat in adipose tissue (Adeva-Andany et al., 2016b). This can increase adiposity. A systematic review was conducted by the WHO in 2010 to address a range of questions regarding the impact of sugar on excess adiposity (Te Morenga et al., 2013). The review was to determine whether the decrease or increase of dietary sugar consumption influenced the assessment of body fat (Te Morenga et al., 2013). The study also analysed whether the existing evidence supported the recommendations to limit the consumption of added sugars to less than 10 % of total energy daily (USDA, 2017). The results showed that the advice to reduce added sugars among free living persons was associated with an average weight reduction of 0.80 kg (Te Morenga et al., 2013). While the suggestion to raise consumption was linked to increase weight of 0.75 kg (Te Morenga et al., 2013). Increase in the consumption of sugar-sweetened drinks does not reduce the calorie intake from other foods, leading to an increase in children's total energy intake and weight gain (de Ruyter et al., 2012). In an animal study, a 20% diet based on honey caused a substantial increase in total body weight (Chepulis, 2007). This was relative to the normal control group of Sprague Dawley rats for 13 weeks (Chepulis, 2007). However, when 10% of the honey-sweetened diet was fed to Sprague Dawley rats for six weeks, there was a significant decrease in body weight. (Chepulis and Starkey, 2008). With respect to the influence of honey on weight, the available evidence is unclear, notably in experimental studies (van Buul et al., 2014, Atangwho et al., 2020). Globally, the prevalence of obesity is increasing at a rapid pace due to an increase in energy intake (Swinburn et al., 2011, Malik and Hu, 2012). Other possible reasons could include increased purchasing power and energy dense food availability, as well as reduced energy

expenditure resulting from urbanization and mechanization. Obesity is a risk factor for diabetes and cardiovascular disease (Hu, 2011).

2.8 NATURAL SWEETENERS AND GLUCOSE LEVELS

2.8.1 Glucose Level

Blood glucose level is the concentration of glucose found in blood at a given period of time (Beck et al., 2018). As part of metabolic homeostasis, the body actively controls blood glucose (Nadkarni et al., 2014). Glucose is stored in glycogen form in the skeletal muscle and liver cells (Adeva-Andany et al., 2016a). In fasting individuals, blood glucose is kept at a stable level at the expense of glycogen stores in the liver and skeletal muscle (Adeva-Andany et al., 2016a). Insulin regulates the glucose homeostasis in the body (Röder et al., 2016). Glucose is the end product of digestion of table sugar and honey (Abdulrhman et al., 2013). Natural sweeteners have a high glycaemic index therefore the tendency to rapidly increase blood glucose. Hyperglycaemia is a situation with increased blood glucose levels above normal range and hypoglycaemia is characterized by persistently low blood glucose (Luitse et al., 2012). Diabetes mellitus is characterized by chronic hyperglycaemia which is the most common condition associated with poorly regulated blood glucose level (Luitse et al., 2012).

2.8.2 Categorization of blood glucose test

Tests to quantify glucose in the blood were established long ago, and hyperglycaemia became the only criteria recommended for diagnosing diabetes (Sacks, 2011). The response to oral glucose tolerance was the subject of the initial diagnostic criteria while subsequent measurement of blood glucose in a fasting person was also appropriate (Sacks, 2011). Fasting blood glucose and oral glucose tolerance test are the most commonly known glucose-based criteria for diagnosis (ADA, 2014). Diagnostic values of (FBG) ≥126 mg/dL or a 2-h plasma glucose ≥200

mg/dL during an oral glucose tolerance test (OGTT) on more than one occasion are used (ADA, 2018). A single spontaneous plasma glucose ≥200 mg/dL viewd diagnostic in a patient with typical symptoms of diabetes (ADA, 2014).

Recently, the HbA1c test has become accepted as a tool to diagnose diabetes (Sherwani et al., 2016). In addition, the American Diabetes Association (ADA) recently revised its diagnostic screening requirements for prediabetes to include HbA1C within a 5.7%-6.4% (ADA, 2019). People with this range of HbA1c are at high risk of developing overt diabetes. HbA1C could be more optimal as it is less tedious than the glucose tolerance test or measurement of postprandial glucose levels (Vijayakumar et al., 2017). Other benefits of HbA1c include the fact that it does not require fasting in patients, represents more long-term glycaemia than plasma glucose levels, and is a standardized and accurate laboratory test (Sherwani et al., 2016). Random blood glucose test can be done at any random time irrespective of the last time food was consumed (Bowen et al., 2015). Fasting blood glucose test is done with blood sample taken after an overnight fast (ADA, 2014). Fasting blood glucose concentration above the reference range may suggest diabetes. Oral glucose tolerance test can be done when the fasting blood sugar level is measured after an overnight fast (Bartoli et al., 2011). This is followed by the consumption of a sugary liquid after which blood glucose levels are tested periodically for the next two hours (Bartoli et al., 2011). These test or a combination can be used to diagnose the onset or development of diabetes.

2.8.3 Effect of natural sweeteners on glucose level

Foods consisting of simple carbohydrates break down rapidly during the digestive process (such as natural sweeteners) and are easily absorbed into the bloodstream. The level of glucose and fructose increase significantly in the blood after the consumption of honey or table sugar

(Abdulrhman et al., 2013). Several animal studies have been done to investigate the effect of natural sweeteners consumption on biochemical parameters including blood glucose (Adesoji and Oluwakemi, 2008, Atangwho et al., 2017). In a study, Sprague-Dawley rats showed a substantial increase in the total weight gain and body fat levels in sucrose-fed rats (Chepulis, 2007). This was comparable for those fed honey or sugar-free diets (Chepulis, 2007). HbA1c levels were significantly reduced whiles HDL-cholesterol increased significantly in honey-fed rats (Chepulis, 2007). This was compared to rats fed sucrose or a sugar-free diet, although no other variations were observed in lipid profiles (Chepulis, 2007). However, a study where healthy rats were fed with 20% honey for 33 days showed that, epididdymal fat weight was 20.1% lower ($P \leq .05$) rats fed honey (Nemoseck et al., 2011). Triglyceride and leptin concentrations were decreased ($P \leq .05$) by 29.6 % and 21.6 % (Nemoseck et al., 2011). In honey-fed rats, non-HDL- cholesterol ($P \leq .05$) was increased by 16.8%. There were no major variations in glucose, TC, insulin and HDL-cholesterol (Nemoseck et al., 2011). Similarly, HbA1c was elevated in the SSB group compared to the control group after six months of treatment in male Wistar rats (Driescher et al., 2019). This means that longer feeding times must be used to produce significant results. In a similar study, there was no significant differences in fasting blood glucose (Erejuwa et al., 2010, Omotayo et al., 2010). Similarly, there was no increase in FBG levels in rats administered with thoracica stingless bee honey compared with the control group (Aziz et al., 2017). Honey was given to the rat as a supplement in this study to ensure to bring about effect.

2.8.4 Diabetes

Diabetes is a chronic condition that develops when the pancreas can no longer produce insulin (IDF, 2015). It can also happen when the body is unable to make effective use of the insulin it

creates (IDF, 2015). The hormone insulin lowers the level of glucose in the blood (Komatsu et al., 2013). It is produced by the pancreas beta cells and released into the circulation when the level of glucose rises (Komatsu et al., 2013). Insulin allows glucose to reach the cells of the body where it can be used for energy (Kubota et al., 2013). It can be also be stored for later usage as glycogen (Kubota et al., 2013). The inability to produce or use insulin efficiently contributes to hyperglycaemia (Bornfeldt and Tabas, 2011). Blood glucose levels remain relatively constant in people without diabetes, as the body retains a normal range.

2.8.5 Prevalence of diabetes

In low and middle-income countries, the prevalence of diabetes has risen sharply than those in high-income countries over the last decade (WHO, 2016b). It is estimated that the global prevalence of diabetes is 9.3 % in 2019 (463 million people) (Saeedi et al., 2019). This is expected to go up to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (Saeedi et al., 2019). In urban locations, the prevalence is greater (10.8%) than in rural locations (7.2%) (Saeedi et al., 2019). High-income countries (10.4%) are also higher than low-income countries (4.0%) (Saeedi et al., 2019). Over the last century, improvements in human behaviour and lifestyle have contributed to a drastic rise in the worldwide incidence of diabetes (Aguiree et al., 2013).

In 2017, about 15.5 million individuals between the ages of 20-79 years lived with diabetes in the African region (IDF, 2018). This reflects a 3.3 % regional prevalence (IDF, 2018). The region 's highest prevalence of diabetes is observed in adults aged 55 to 64 years old (IDF, 2018). The area has the highest prevalence of undiagnosed diabetes, with 69.2 % of adults actually living with diabetes unaware of their condition (IDF, 2018). The prevalence of diabetes varied from 2.6% in rural Sudan to 20.0% in urban Egypt (Bos and Agyemang, 2013). The prevalence

of diabetes among adults aged 50 years and above in Ghana was 3.95% with the prevalence being insignificantly higher in females than males 2.16%, vs. 1.73% (Gatimu et al., 2016).This reflects the gender differences in diabetes prevalence in many African countries (Hall et al., 2011). Ghana has reported a high risk of diabetes with greater general and central obesity (Frank et al., 2013).

2.8.6 Effect of natural sweeteners on diabetes

A review showed that over consumption of sweetened drinks contribute greatly to weight gain and may contribute to an increased risk of type 2 diabetes (Malik et al., 2010). The direct mechanisms of sugar that lead to diabetes involves fructose metabolism (Stanhope, 2012). Without regulating intake, the liver absorbs sucrose, possibly leading to a build-up of liver fats and a decline in insulin sensitivity (Tappy and Lê, 2010). Sensitivity to insulin determines how the cells utilize glucose efficiently, reducing it quantities in the bloodstream (Lecoultre et al., 2013). Blood glucose can become persistently elevated when insulin decreases, eventually resulting in type 2 diabetes (MacDonald, 2016). A review suggest high sugar intake can possibly increase the risk the risk of diabetes (Sonestedt et al., 2012). However, the role of sugar in diabetes is still inconsistent (Stanhope, 2016). In more than 175 countries, a study investigated individuals and found that more sugar in the food resulted in higher rates of diabetes (Basu et al., 2013). In particular, diabetes levels increased by 1 % for every additional 150 calories of sugar consumed per day per person (Basu et al., 2013). There was no change to this trend even when calorie, exercise and obesity was controlled (Basu et al., 2013).

2.9 NATURAL SWEETENERS AND LIPID PROFILE

Lipids are a category of fats and fat-like substances that are essential cell constituents and energy sources (Walther and Farese Jr, 2012). They may be synthesised by body or obtained from

dietary source (Akoh, 2017). Excess glucose is stored as glycogen or fat in the muscles, liver and adipose tissue (Adeva-Andany et al., 2016a). The amount of particular lipids in the blood is determined by a lipid profile test. Lipoproteins are transported in the blood by two essential lipids, cholesterol and triglycerides (Rodwell et al., 2015). A mixture of cholesterol, triglycerides, proteins, and phospholipid molecules is found in each form of lipoprotein (Rodwell et al., 2015). This test is used for the detection of dyslipidaemia (different cholesterol and triglyceride disorders), many of which are known risk factors for cardiovascular disease (Teramoto et al., 2013).

2.9.1 Categorization of lipid profile

Particles of the lipid profile are classified by their density as low-density lipoproteins (LDL), high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL) (Nordestgaard, 2017). Total cholesterol, which tests all cholesterol in all lipoprotein particles, is a traditional lipid profile examination. (Nordestgaard et al., 2016). Cholesterol in HDL particles is measured by high-density lipoprotein cholesterol (HDL-C) (Nordestgaard et al., 2016). The cholesterol in LDL particles is calculated by Low Density Lipoprotein Cholesterol (LDL-C) (Martin et al., 2013). In addition, triglycerides that measure all triglycerides in lipoprotein particles (Arsenault et al., 2011). The level of LDL-C is typically estimated using total cholesterol, HDL-C, and triglyceride data (Martin et al., 2013).

2.9.2 Dyslipidaemia

Dyslipidaemia refers to abnormal blood lipid levels (Klop et al., 2013). The word describes a broad spectrum of conditions, but the most common type of dyslipidaemia is hyperlipidaemia (Klop et al., 2013). Which refers to high levels low-density lipoproteins cholesterol (LDL-C), high-density lipoproteins cholesterol (HDL) and triglycerides (Schofield et al., 2016).

Hyperlipidaemia is usually asymptomatic, so a lipid profile is the best way to diagnose it (Schofield et al., 2016). Dyslipidaemia is clinically expressed as high or low levels of triglycerides and/or high or low cholesterol level (Björnson et al., 2017).

2.9.3 Prevalence

More than 12% of adults age 20 and older had higher total cholesterol and more than 18% had lower high-density lipoprotein cholesterol levels less than 40 mg/dL in America (Carroll et al., 2017). In men aged between 30 and 39 years, the peak prevalence of dyslipidaemia is (48.2 %) in China (Qi et al., 2015). Similarly, the prevalence of dyslipidaemia increased with age in women, with the highest prevalence occurring after 60 years of age (46.3%) in China (Qi et al., 2015). In Ireland, a 30% decrease in the death rate of heart disease was due to a 4.6% decrease in the national level for total cholesterol (Nichols et al., 2013). Similarly, in Finland, the drop in population blood cholesterol levels has explained % of the decrease in ischaemic heart disease mortality (O'Flaherty et al., 2013). In South Africa, dyslipidaemia prevalence rates of between 14% and 69% have been found using community level assessments (Reiger et al., 2017). The prevalence of dyslipidaemia in Nigeria varied from 60% among seemingly healthy Nigerians to 89% among diabetic Nigerians (Oguejiofor et al., 2012). The prevalence of hypercholesterolemia, hypertriglyceridemia and hyperlipidaemia were 56%, 7.11% and 1.9% respectively in a study in Senegal (Doupa et al., 2014). A study undertaken in the Ga-East municipality showed a moderate level of hypercholesterolemia (2.8%) among school children who are overweight and obese (Steiner-Asiedu et al., 2012).

2.9.4 Effect of natural sweeteners on lipid profile

Triglycerides are enormously influenced by increased consumption of processed carbohydrates and added sugars (Kell et al., 2014). Low HDL levels and high triglyceride levels are indicators

of poor cholesterol levels (Klop et al., 2013). A research has also shown that women who consume more added sugar appear to have higher LDL-C levels (Yu et al., 2018). Sugar sweetened beverage intake has a strong association with human weight gain (Hu and Malik, 2010). An animal study found leptin and triglycerides levels were 21.6% and 29.6% lower ($p \leq .05$) respectively (Nemoseck et al., 2011). Non-HDL-cholesterol was also found to be 16.8% higher ($p \leq .05$) in honey-fed rats in comparison with sucrose-fed rats (Nemoseck et al., 2011). Another systematic study and meta-analysis showed that higher sugar consumption was correlated with a higher blood lipid profile. This was comparable to low intake of sugar which showed a reduce level of lipid profile (Te Morenga et al., 2014).

Honey is known to contain some trace elements phenolic acids and flavonoids therefore it provides more in terms of health and wellness (Manyi-Loh et al., 2011b). A research on male students aged 18-30 years on the consumption of natural sweeteners was conducted at Isfahan University of Medical Sciences in Iran (Rasad et al., 2018). Honey intake was found to be able to minimize total cholesterol, TG and LDL-C (Rasad et al., 2018). In addition, there was an increase HDL-C in young healthy subjects (Rasad et al., 2018). Intake of sucrose, however, increased TC, LDL, TG and decreased HDL-C (Rasad et al., 2018). Similarly, honey administration raised HDL cholesterol significantly ($p < 0.05$) (Erejuwa et al., 2016). Although glucose, TG and very-LDL-C ($p<0.05$) decreased significantly (Erejuwa et al., 2016, Mohammadimanesh et al., 2019). However it was concluded that further clinical trials should be conducted to confirm these findings (Rasad et al., 2018). In a study, there was a significant increase in HDL-C and LDL-C after subjects consumed 3 different isocaloric solution (Jameel et al., 2014). The solutions contained 50 g of either sucrose, fructose or glucose (Jameel et al., 2014). In another study, neither solution significantly influenced cholesterol or triglyceride

values in the treatment groups (Münstedt et al., 2009). Subjects obtained honey solution or sugar solution equal to honey once daily for a duration of 14 days (Münstedt et al., 2009). In comparison, substantial reductions in plasma LDL and total cholesterol levels were found in the test as opposed to the control group ($P<0.01$) (Alagwu et al., 2014).

Some studies however, showed no effects of sucrose or fructose on LDL- cholesterol or HDL-cholesterol levels (Kelishadi et al., 2014, Lowndes et al., 2014) . The aforementioned studies placed more emphasis on fructose which is only one component of table sugar and honey. These irregularities in lipid profile are significantly associated with an increased consumption of dietary sugar (Xi et al., 2015). Dyslipidaemia has been clearly established as a significant cardiovascular disease risk factor in developed and developing countries. (Hendrani et al., 2016).

2.9.5 Cardiovascular disease

Cardiovascular diseases are a category of cardiac and blood vessel disorders (Nichols et al., 2014). This include, cerebrovascular disease, ischemic heart disease, coronary heart disease and other conditions (Wilkins et al., 2017). Cardiovascular disease is considered to be the number one cause of death worldwide (WHO, 2013). Heart attacks and strokes account for four out of five CVD deaths (WHO, 2017c). One third of these premature deaths occur in persons under 70 years of age (WHO, 2017c). Lifestyle factors such as heavy use of alcohol, tobacco use, unhealthy diet, and inadequate physical activity are risk factors for CVD (Dahlöf, 2010). High blood pressure, high cholesterol in the blood and high blood sugar are other factors. (Dahlöf, 2010).

2.9.6 Prevalence

An estimated 17.5 million individuals died from CVD in 2012, accounting for 31% of all deaths worldwide (WHO, 2017c). An approximate 7.4 million of these deaths were caused by coronary

artery disease (CAD) and 6.7 million by stroke (WHO, 2017a). The World Health Organization estimates that approximately 20 million CVD-related deaths would occur globally (WHO, 2017b). It is estimated that about 80% of global CVD-related deaths happen in low- and middle-income countries (WHF, 2018). This is concurrent with 87% of CVD-related disabilities which happen mostly in this region (WHF, 2018).

Young people are known to be the most populated age group of people in Sub Saharan Africa (Ofori-Asenso and Garcia, 2016). Instead, the trend of morbidity and mortality associated with CVD has increased (WHF, 2018). Sub-Saharan Africa remained the only region of the world within 1990 and 2013 where CVD-related deaths increased (Roth et al., 2015a). CVD-related fatalities account for almost 9.2% of all deaths (Meier et al., 2019). It is also the leading cause of death in the African region among individuals aged over 45 years (Roth et al., 2015b). Approximately 7-10% of all adult patient admissions to hospitals in Africa were also for cardiovascular diseases (Mocumbi, 2012). In these admissions, heart failure alone accounts for about 3-7% (Mocumbi, 2012).

Ghana is estimated to have a 20 % chance of people dying from diabetes, cancer, CVD or chronic respiratory disease between the ages of 30 and 70 years (WHO, 2016c). In Ghana's capital, Accra, CVD developed from being the 10th to 7th cause of death in 1953 and 1966, respectively (Agyei-Mensah and Aikins, 2010). In 2014, even in a peri-urban area in the eastern region of Ghana, CVD was ranked as the leading cause of death (Ofori-Asenso and Ofei, 2015). A one-year study of in-patient records described stroke as comprising 9.1% of overall adult admissions (Agyemang et al., 2012). It was responsible for 13.2% of all adult death in the review (Agyemang et al., 2012).

2.9.7 The effect of natural sweetener on cardiovascular disease

An inconclusive body of evidence indicates that increased consumption of added sugars can increase the risk of CVD (Chiavaroli et al., 2012, Wang et al., 2014, Zhang et al., 2013). In a study, rats were fed high doses of sucrose and honey has been used to establish a correlation between added sugar ingestion and risk of CVD (Nemoseck et al., 2011, Atangwho et al., 2020). Similarly, human subjects fed a variety of sugars have been used to establish risk of CVD (Madero et al., 2011). In the Framingham Heart study a significant increase in risk of CVD and sugar intake was observed (Ma et al., 2015).

Cross-sectional studies sugar sweetened beverage intake with higher calorie intake, increased weight and poor nutrition (Narain et al., 2016). It has also been suggested that excessive intake of fructose plays a role in hypertension, dyslipidaemia and obesity (Malik et al., 2010, Tappy et al., 2010). Likewise, a study found a link between a high intake of sugar and a higher risk of mortality from heart disease (Yang et al., 2014). In the aforementioned study, subjects that consumed les calories from sugar were at a lower risk getting CVD (Yang et al., 2014).

Similarly, a study among overweight and obese adults showed that consumption of fructose containing sugar leads to dyslipidaemia (Stanhope, 2016). In addition, it can lead to increased visceral adiposity and decreased insulin sensitivity (Stanhope, 2016). The consistent intake of foods high in sugar can increase fasting plasma triglycerides and low-density lipoproteins cholesterol (Miller et al., 2011, Welsh et al., 2011). In short, while the mechanisms remain uncertain compared to other sources of carbohydrates, the consumption of sugar seems to be related to dyslipidemia, a recognised risk factor for CVD. (Johnson et al., 2009).

2.10 THE EFFECT OF NATURAL SWEETENERS ON INSULIN

2.10.1 Gross Anatomy of the Pancreas

The pancreas is situated behind the peritoneum. The head of the pancreas is located between the C shaped structure of the second and third part of the duodenum. The spleen is next to the pancreatic tail. The areas of the pancreas are the head, body, tail and uncinate process (Pandol, 2011).The classification of the rat pancreas as an intermediate pancreas is due to the fact that the splenic portion of the pancreas is quite compact and the duodenal aspect being dispersed within the mesentery (Pandiri, 2014). Currently different authors describe the lobes of rat pancreas the duodenal, gastric and splenic lobes (Pandiri, 2014), the right lobe, body and left lobe (Chandra et al., 2013), gastric lobe, duodenal head and tail (Pandiri, 2014) and head, body and tail (Brenneman et al., 2014). The rat pancreas is likened to the human pancreas based on its anatomical descriptions (Suttie et al., 2017).

Anteriorly, the pancreas is bounded by the stomach, transverse colon, greater omentum, and loops of small intestines (Pandol, 2011). Posteriorly, it is bounded by the portal vein, inferior vena cava, aorta, superior mesenteric artery and vein, kidneys and Lumbar vertebra (Pandol, 2011). The inferior surface is enclosed by the peritoneum (Gray, 2000). The pancreas lies on the duodenojejunal flexure and some loops of the jejunum. Its left boundary lies on the left colic flexure (Gray, 2000). The superior border starts from the omental tuberosity and it is related to the celiac artery (Gray, 2000). The hepatic artery runs to the right just above the gland whilst the splenic artery runs towards the left in a groove along this border (Gray, 2000).

The main arterial blood supply is from branches of the splenic artery. The pancreaticoduodenal and pancreatic arteries also supply the pancreas with blood (Moore, Dalley, & Agur, 2014). Nerve supply is by the parasympathetic and sympathetic nervous systems. Within the divisions

of the vagus nerve, is the efferent parasympathetic system that originates in the dorsal vagal complex (tenth cranial nerve nucleus) of the brain.

2.10.2 Histology of the Pancreas

The exocrine pancreas is a compound tubulo-acinar gland and consists of serous acini of atypical form and appearance (Craigmyle, 1986), ductal and the stellate cells (Clemens et al., 2016a).

The cells are truncated pyramids whose infra-nuclear cytoplasm is intensely basophilic and whose apical cytoplasm contains large eosinophilic zymogen granules (Craigmyle, 1986). The center of the acinus contains centro-acinar cells (Craigmyle, 1986). The exocrine acinar produces inactive zymogens which are released through the pancreatic ducts to the duodenum (Clemens et al., 2016a). The inactive zymogens now become activated in the duodenum (Clemens et al., 2016a). The intercalated ducts lined with cuboidal cells invaginate into the acinus of the pancreas (Craigmyle, 1986). The intralobular ducts are very rare in the pancreas (Craigmyle, 1986). Interlobular ducts are lined with tall columnar epithelium which runs in the connective tissue septa of the gland and joins up to form the main and accessory ducts which open into the duodenum (Craigmyle, 1986). Bicarbonate produced from the cells of the ducts facilitates the passage and release of digestive enzymes to the duodenum (Clemens et al., 2016a). The pancreatic stellate cells are also involved in the production and breakdown of extracellular matrix proteins (Clemens et al., 2016a).

The endocrine pancreas consists of about one million islets of Langerhans. Three cell types known as alpha, beta and delta cells are found in the islets. The alpha, beta, and delta cells produce glucagon, insulin and somatostatin respectively (Craigmyle, 1986).

2.10.3 Effect of natural sweeteners on insulin

When food containing natural sweetener is ingested, the digestive system breaks it down into glucose and fructose, which enters the blood. Dietary sugar may have a direct or indirect impact on the pancreas (Michaud et al., 2002). Due to their basic chemical structure, dietary sugar can be easily and rapidly used by the body for energy, sometimes contributing to a faster increase in blood sugar and pancreatic insulin secretion, which can have negative health effects. The pancreas produces specialized hormones that help regulate a variety of body functions. These hormones are insulin and glucagon. Insulin controls how much glucose, is taken up by the body's cells while glucagon stimulate the synthesis of glucose (Röder et al., 2016). Impairment to the normal function of the pancreas may affect it release of insulin (Czech, 2017). Furthermore, excess fat in storage and blood circulation may contribute to insulin insensitivity (Hardy et al., 2012). In this case blood sugar levels may be high even though pancreas produces adequate insulin. The risk factors associated with an inflamed pancreas include age (with mortality increased in patients 60 years and above) (Gardner et al., 2008, Wu et al., 2008), and overweight (body mass index >30 kg/m^2). The relationship between body weight and diabetes may suggest that insulin resistance plays a role in the function of the pancreas (Gapstur et al., 2000). Too much intake of foods sweetened with sugar is associated with overweight or obesity (Te Morenga et al., 2013). Epidemiological studies have shown that as the body mass index (BMI) increases, the risk of diabetes and insulin resistance increases (Tsugane and Inoue, 2010). An overworked pancreas may cause damage to the islet and other components and hence loss of function (Gerber and Rutter, 2017).

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY DESIGN

An experimental study design was used for this study.

3.2 STUDY SITE

The experiment was carried out at the Animal Experimentation Unit of the Department of Microbiology, University of Ghana, Korle-Bu.

3.3 STUDY POPULATION

Thirty-five (35) male Sprague Dawley rats aged 12 - 14 weeks, weighing 150g - 200g were acquired from the Noguchi Memorial Institute for Medical Research, University of Ghana. Rats were kept in the Animal experimentation unit of the Department of Microbiology, (University of Ghana). The rats were weighed and placed into seven (7) groups of five (5) animals in each cage using a random sampling technique. The animals were kept in a rat cage of dimensions of 20.3 cm (W) × 28.7 cm (L) × 17.3 cm (H) with soft wood shavings as bedding. The animals were acclimatized to their environment for two weeks. The groups set up were as follows: Group 1 (G1) – control group, group 2 (G2) - white sugar low dose (WS LD), group 3 (G3) - white sugar high dose (WS HD), group 4 (G4) - brown sugar low dose (BS LD), group 5 (G5) - brown sugar high dose (BS HD), group 6 (G6) - honey low dose (H LD) and group 7 (G7) - honey high dose (H HD).

3.4 ACCLIMATIZATION OF ANIMALS AND FEEDING

Rats were kept in the unit throughout the study under the following conditions; a temperature of $28^{O}C \pm 4\ ^{O}C$, relative humidity of 70 ±4% and under alternating 12-hour period of light and 12-

hour period of darkness (12-hour light/dark cycle). Furthermore, the animals were fed on standard rat chow and water *ad libitum* for a period of two (2) weeks before the commencement of the experiment. The animals were given distilled water. Rat chow diet was formulated and supplied by AGRIMAT, Accra, Ghana.

3.5 ETHICS

The study was approved by the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences (Ethics Identification Number: SBAHS-DT.//SA/2018-2019). All animal procedures and techniques used in this study were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (N.R.C., 2010).

3.6 Diet Regimen of Sweeteners

The Microdiet Nutritional Analysis Software (Version 3 Downlee Systems, UK) was used to estimate the same amount of carbohydrate for the different treatment groups in both the high and the low dose groups. This was to ensure that animals in the treatment group received the same amount of carbohydrate for the different diet treatments. For the purpose of this study, the rats were given the same amount of carbohydrate which translates into weight as follows:

Table 3. 1: Recommended sugar intake/day of white sugar, brown sugar and honey

RECOMMENDED SUGAR INTAKE/ DAY			
Natural Sweetener	Weight (g)	Carbohydrate (g)	Calorie (kcal)
Honey	52.10	39.80	150
White sugar	38.10	40.06	150
Brown sugar	41.50	42.04	150

The average weight of carbohydrate given per natural sweetener: 39.80+ 40.06+42.04/3 = 40.63 g. The weight of each natural sweetener was converted to the same amount of carbohydrate per natural sweetener. Half of the recommended sugar intake per day was calculated as low dose and twice of the recommended sugar intake per day as calculated as high dose.

Table 3. 2: recommended sugar intake/day per average weight of carbohydrate.

RECOMMENDED SUGAR INTAKE/DAY			
Natural Sweetener	Weight (g)	Carbohydrate (g)	Calorie (kcal)
Honey	53.19	40.63	153.13
White sugar	38.64	40.63	152.13
Brown sugar	40.11	40.63	144.90

Table 3. 3: High dose estimation per average weight of carbohydrate.

HIGH DOSE			
Natural Sweetener	Weight (g)	Carbohydrate (g)	Calorie (kcal)
Honey	106.39	81.27	306.29
White sugar	77.29	81.27	304.31
Brown sugar	80.23	81.27	289.97

Table 3. 4: Low dose estimation per average weight of carbohydrate.

LOW DOSE			
Natural Sweetener	Weight (g)	Carbohydrate (g)	Calorie (kcal)
Honey	26.60	20.32	76.58
White sugar	19.33	20.32	76.09
Brown sugar	20.06	20.32	72.50

3.6.1 TRANSLATING INTO ANIMAL DOSE

For a 70kg adult = 106.39 g honey

106.39 g/70 kg

1.52g/kg

For a 200 g rat = 0.2 kg

Animal dose = 0.2 kg x 1.52g/kg

= 0.304 g honey

Table 3. 5: Estimated animal high dose.

RAT HIGH DOSE		
Natural Sweetener	Rat weight (g)	Amount (g)
Honey	200	0.304
White sugar	200	0.220
Brown sugar	200	0.230

Table 3. 6: Estimated animal low dose.

RAT LOW DOSE		
Natural Sweetener	Rat weight (g)	Amount (g)
Honey	200	0.076
White sugar	200	0.055
Brown sugar	200	0.057

3.6.2 Preparation of diet

The high dose diet treatment was prepared by dissolving 0.304 g, 0.220 g and 0.230 g of honey, white table sugar and brown sugar respectively in 1ml of distilled water. Similarly, the low dose diet treatment was prepared by dissolving 0.076 g, 0.055 g, and 0.057 g of honey, white sugar and brown sugar respectively in 1 ml of distilled water. The solute was allowed to completely dissolve before administration.

3.7 ADMINISTRATION OF TREATMENT

One (1) ml of the prepared white sugar, brown sugar or honey solution was administered daily to the treatment group of rats orally by gavage for 12 weeks. Rats in the control group received one (1) ml of distilled water orally by gavage for 12 weeks.

3.8 BLOOD SAMPLE AND TISSUE COLLECTION

After 12 weeks of oral administration of sweetener daily, rats in each group were anesthetized with ethyl ether and blood was drawn by cardiac puncture. Four millimetres (4 ml) of blood samples was collected and discharged as follows: 2 ml into gel-separator tubes and 1 ml into fluoride tubes and kept on ice (One millilitre (1 ml) of the blood sample was discharged into an

EDTA tube for analysis outside the scope of this work). The samples were centrifuged at 3000 rpm for 10 minutes to separate serum. Sera were transferred into Eppendorf tubes, labelled and stored in a laboratory freezer at -80°C until analysis. The stored samples were allowed to thaw at room temperature of 20˚C before assaying. Rats were later euthanized and the pancreas harvested. The harvested organ was weighed and divided into two parts. Half went into buffered formalin and the other half went into a container and stored at -80°C for later analysis.

3.9 PREPARATION OF RAT PANCREAS HOMOGENATE

The frozen pancreas was placed on a glass plate and minced into small pieces by use of razor blade until a paste like consistency was formed. The minced pancreas was collected into a beaker containing 1 ml of cold PBS (phosphate buffer solution). The pieces were swirled gently and allowed to settle at the bottom of the beaker before being decanted to remove blood. The pancreas was homogenized using dounce tissue grinder in cold PBS, for each 1 g of pancreas. The sample was centrifuged at approximately 10000 X g for 5 min. The supernatant was collected and insulin was measured using ELISA Kit.

3.10 BIOCHEMICAL ANALYSIS

Lipid profile comprising total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) were analysed with an auto-analyser machine and test kits according to the manufacturer's instructions (Mindray Bio-Medical Electronics Bs-200e, Shenzhen, China). Glycated haemoglobin (HbA1c) and fasting blood glucose (FBG) were also analysed following the manufacturer's manual (ELITech, SEES, France). Insulin level was determined using an insulin ELISA kit from (Crystal Chem High Performance Assay, France) according to the manufacturer's instructions. In brief, 5 µL sera was introduced into each well alongside standards. Wells were coated with rat insulin antibodies. The

reaction was allowed to incubate for two (2) hours at 4^{o}C. After "washing" 100 µL conjugate was added to each well and further incubated at room temperature for 30 min. After the final wash, 100 µL substrate was added and incubated at room temperature for 40 min. After adding 100 µL of stop solution, the final chromogen was read at 450 nm with a reference wavelength of 630 nm.

3.11 HISTOLOGICAL ANALYSIS

Pancreatic tissue samples were processed through the Leica TP 1020 tissue processor with various solvents. Samples were then embedded in paraffin wax and sectioned at 5µm. Sectioned samples were placed on slides to dry. Samples were later stained with Periodic Acid Schiff (PAS) and after mounting, samples were examined using an Olympus microscope at times forty magnification (x40).

3.12 STATISTICAL ANALYSIS

The GraphPad Prism software (version 8) was used for the analysis. All results were expressed as means and standard error of the mean, at 95% confidence interval (CI). Two way ANOVA was used to compare the means within and between the treatment groups. Where ANOVA was significant, post hoc test using Bonferroni analysis was done. Corrections were determined using Pearson correlation analysis. A p-value ≤ 0.05 was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 LOW DOSE WEIGHT CHANGES

The mean weights recorded at week 1 for control, white sugar low dose, brown sugar low dose and honey low dose group were 270.40±23.31 g, 266.40±4.93 g, 207.60±4.01 g and 218.20±12.02 g respectively but there was no significant difference between them. There was no significant difference in the mean weight of rats from week 1 to week 6 between the control group and the low dose treatment groups. However, there was an increase in weight of rats from week 7 to week 12 in the brown sugar low dose and honey low dose groups 230.25±2.66 and 279.67±17.42 respectively as compared to the control group. The mean weight of low dose groups of brown sugar and honey were found to be significantly higher ($p < 0.001$ and $p < 0.001$, respectively) compared to the control group.

The mean weights recorded at week 1 for control, white sugar high dose, brown sugar high dose and honey high dose groups were 270.40±23.31 g, 230.00±10.48 g, 171.00±5.68 g, and 221.20±7.72 g respectively were not significantly different. There was no significant difference in the mean weight of rats from week 1 to week 6 between the control and high dose treatment groups despite an increase in weight. However, there was an increase in weight of rats from week 7 to week 12 from the white sugar high dose, brown sugar high dose and honey high dose groups 272.00±13.05 g, 226.75±2.02 g and 259.67±10.68 g respectively as compared to the control group. High dose groups of white sugar, brown sugar and honey were found to be significantly higher ($p < 0.001$) compared to the control group (Figure 4.1).

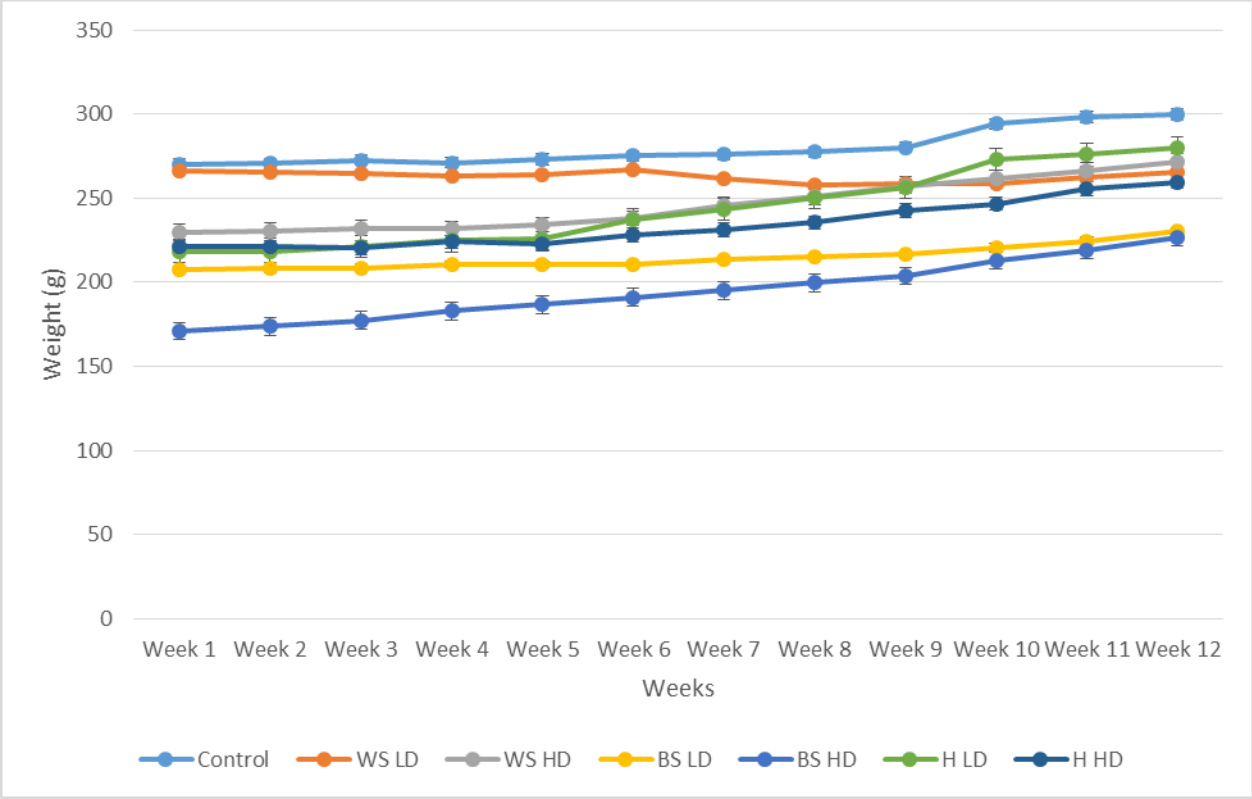


Figure 4. 1: Mean weights of experimental rats measured from week 1 to week 12 administration of white sugar, brown sugar and honey. Data represents mean ± standard error of mean (SEM) for values statistically different as indicated with $p < 0.001$.

4.2 EFFECT OF NATURAL SWEETENERS ON HBA1C

Figure 4.2 shows the effect of intake of the three natural sweeteners (white sugar, brown sugar and honey) on the HbA1c level of the rats.

Both the WS HD and WS LD were significantly higher ($p<0.001$) and ($p=0.003$) respectively in comparison with control group. Similarly, BS HD was significantly higher ($p<0.001$) when compared with the control group. Differences between BS LD, H LD and the control group were not significantly different ($p>0.05$). Honey high dose group (H HD) was significantly higher ($p<0.001$) when compared with the control group.

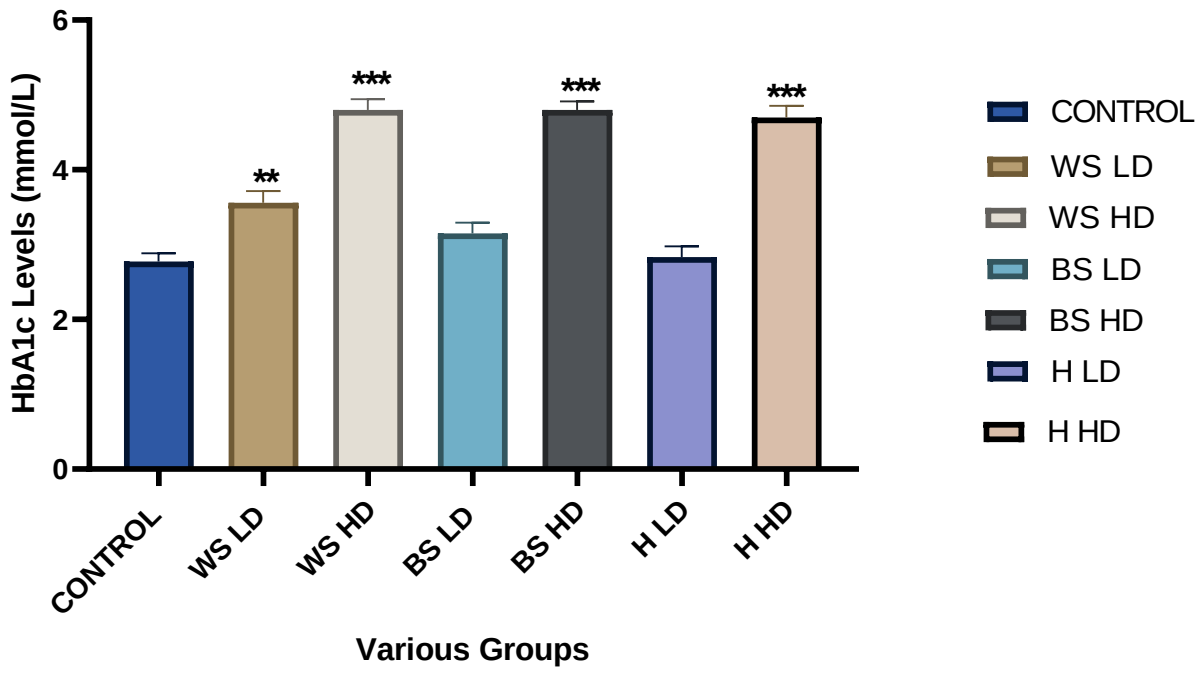


Figure 4. 2: The effect of white sugar, brown sugar and honey on HbA1c of rats after 12 weeks administration. Each column represents mean with SEM as error bars for values statistically different as indicated with $p < 0.001$.

4.3 EFFECT OF NATURAL SWEETENERS ON FASTING BLOOD GLUCOSE (FBG)

The following results show the effect of intake of the three natural sweeteners white sugar, brown sugar and honey on the fasting blood glucose levels of the rats measured. There was no significant difference between WS HD and WS LD groups when compared with the control group. Also, BS HD group was significantly higher (p= 0.010) when compared with the control group. However, there was no significant difference between BS LD group and the control group. Honey high dose H HD were a significantly higher difference (p= 0.04) when compared with the control group. However, there was no significant difference between the H LD group and the control group (Figure 4.3).

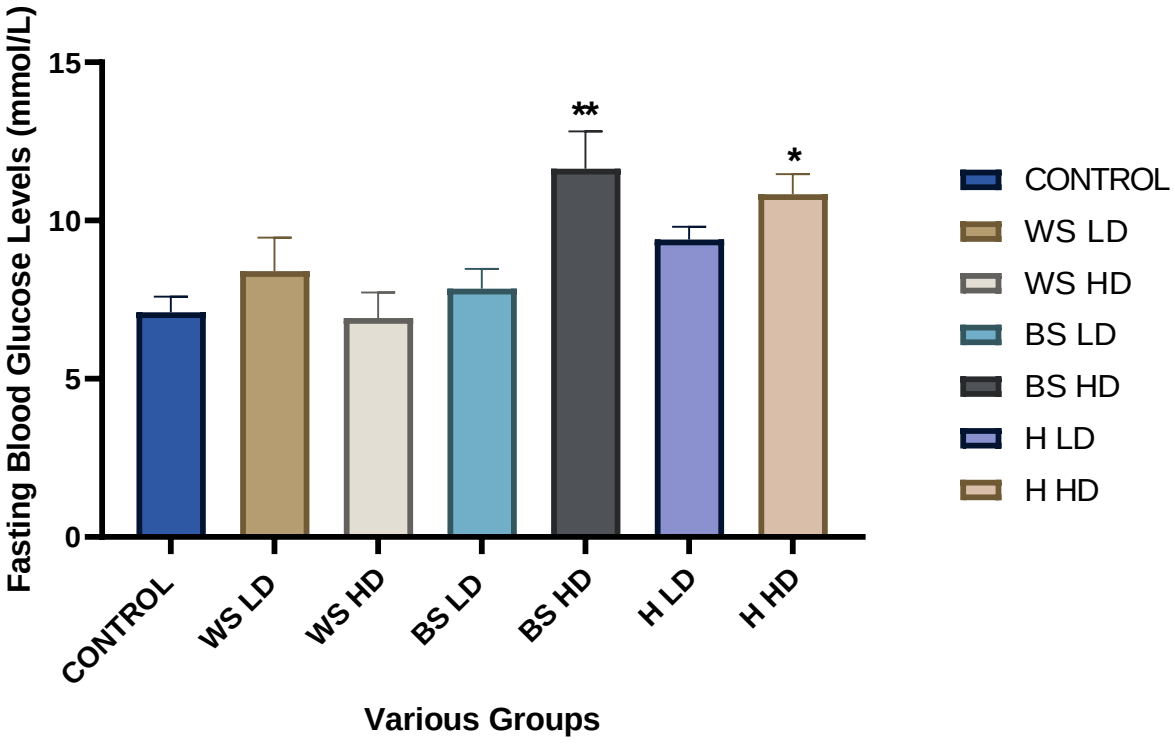


Figure 4. 3: The effect of white sugar, brown sugar and honey on fasting blood glucose of rats after 12 weeks administration. Each column represents mean with SEM as error bars for values statistically different as indicated with $p < 0.001$.

4.4 EFFECT OF NATURAL SWEETENERS ON LIPID PROFILE

The following results show the effect of intake of three natural sweeteners white sugar, brown sugar and honey on the lipid profile of the rats by the levels of biochemical parameters measured. These included Total Cholesterol (TC), Low Density Lipoprotein cholesterol (LDL-C), High Density Lipoprotein cholesterol (HDL-C), Triglyceride (TG).

4.4.1 Effect of natural sweeteners on Total Cholesterol (TC)

The following results show the effect of intake of three natural sweeteners white sugar, brown sugar and honey on the total cholesterol levels of the rats measured. Control 3.92±0.25 mmol/L,

WS LD 4.43±0.31 mmol/L, WS HD 3.94±0.24 mmol/L, BS LD 3.95±0.21 mmol/L, BS HD 5.06±0.94 mmol/L, H LD 3.60±0.28 mmol/L, H HD 4.69±0.72 mmol/L. The Total Cholesterol (TC) level in all the treatment groups was not statistically significant compared to the control group (Figure 4.4).

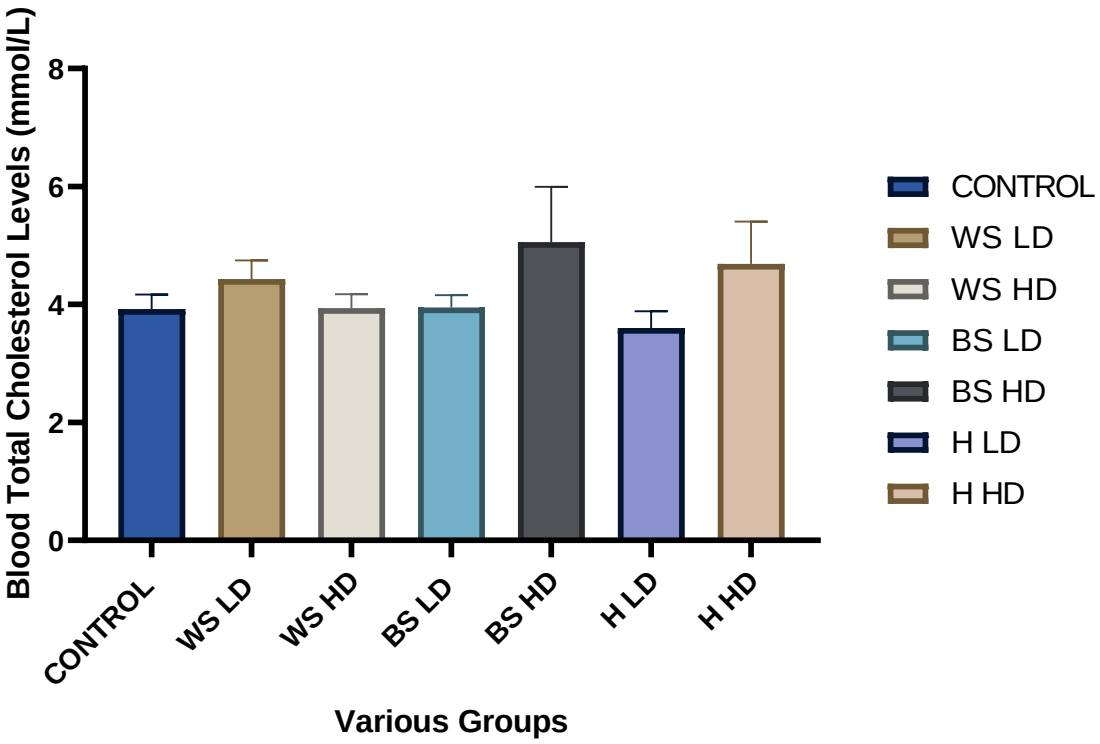


Figure 4. 4: The effect of white sugar, brown sugar and honey on total cholesterol of rats after 12 weeks administration. Each column represents mean with SEM as error bars for values statistically different as indicated with $p < 0.001$.

4.4.2 Effect of Natural Sweeteners on Triglyceride (TG)

The following results show the effect of intake of three natural sweeteners white sugar, brown sugar and honey on the triglyceride levels of the rats measured. Control 1.245±0.35 mmol/L, WS LD 1.17±0.14 mmol/L, WS HD 0.79±0.09 mmol/L, BS LD 0.83±0.08 mmol/L, BS HD 0.78±0.13 mmol/L, H LD 1.02±0.21 mmol/L, H HD 0.91±0.14 mmol/L. The Triglyceride (TG)

level in all the treatment groups was not statistically significant compared to the control group (Figure 4.5).

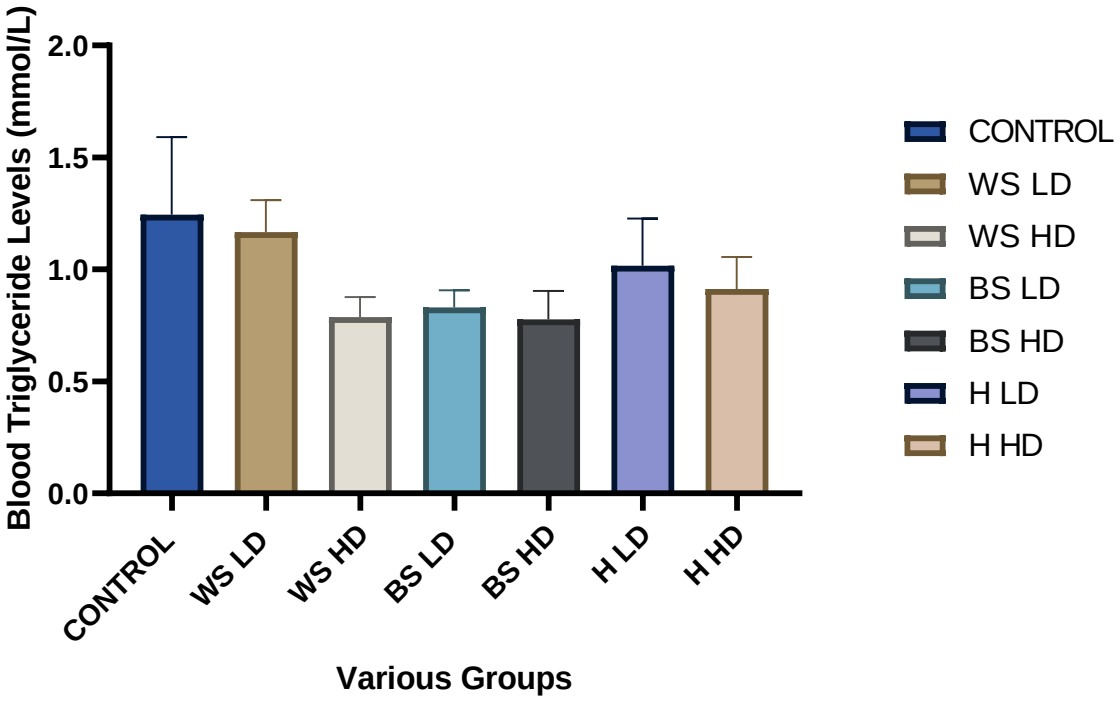


Figure 4. 5: The effect of white sugar, brown sugar and honey on triglycerides of rats after 12 weeks administration. Each column represents mean with SEM as error bars for values statistically different as indicated with $p < 0.001$.

4.4.3 Effect of Natural Sweeteners on High Density Lipoprotein Cholesterol (HDL-C)

The following results show the effect of intake of three natural sweeteners white sugar, brown sugar and honey on HDL-C levels of the rats measured. High Density Lipoprotein Cholesterol (HDL-C) in all the treatment groups was not statistically significant compared to the control group (Figure 4.6).

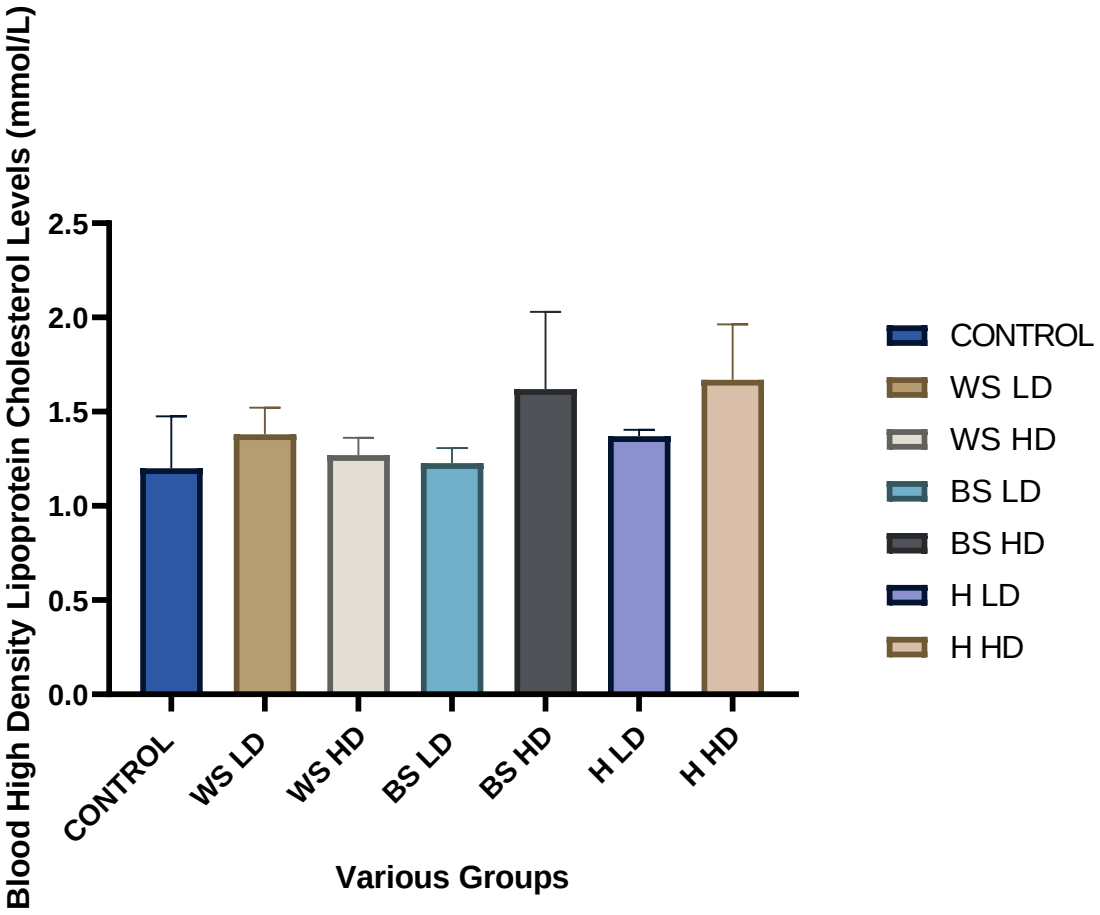


Figure 4. 6: The effect of white sugar, brown sugar and honey on high density lipoproteins cholesterol of rats after 12 weeks administration. Each column represents mean with SEM as error bars for values statistically different as indicated with $p < 0.001$.

4.4.4 Effect of Natural Sweeteners on Low Density Lipoprotein Cholesterol (LDL-C)

The following results show the effect of intake of the three natural sweeteners (white sugar, brown sugar and honey) on the LDL-C levels of the rats measured. There was no significant difference between WS LD and WS HD group when compared with the control group. The following; BS LD, BS HD, H LD and H HD groups were significantly lower ($p<0.001$), ($p<0.001$), ($p<0.001$) and ($p<0.001$) in comparison with control group (Figure 4.7).

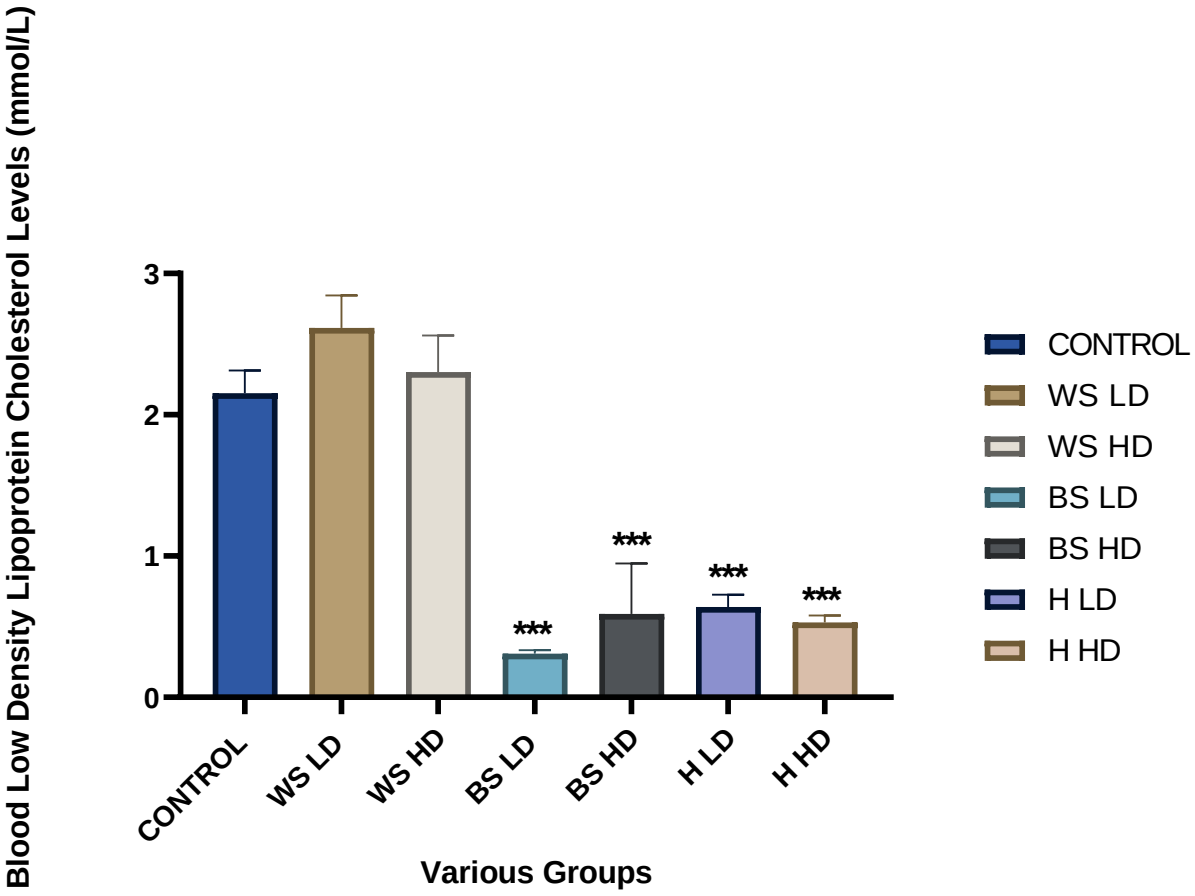


Figure 4. 7: The effect of white sugar, brown sugar and honey on low density lipoproteins cholesterol of rats after 12 weeks administration. Each column represents mean with SEM as error bars for values statistically different as indicated with $p < 0.001$.

4.5 EFFECT OF NATURAL SWEETENERS ON INSULIN

The effect of intake of the three natural sweeteners (white sugar, brown sugar and honey) on the insulin levels of the rats measured.. There was no significant difference between BS LD group and H LD group when compared with the control group. However, WS LD , WS HD , BS HD and H HD were significantly lower compared with control group ($p=0.03$, $p<0.001$, $p<0.001$, $p<0.001$, respectively) (Figure 4.8).

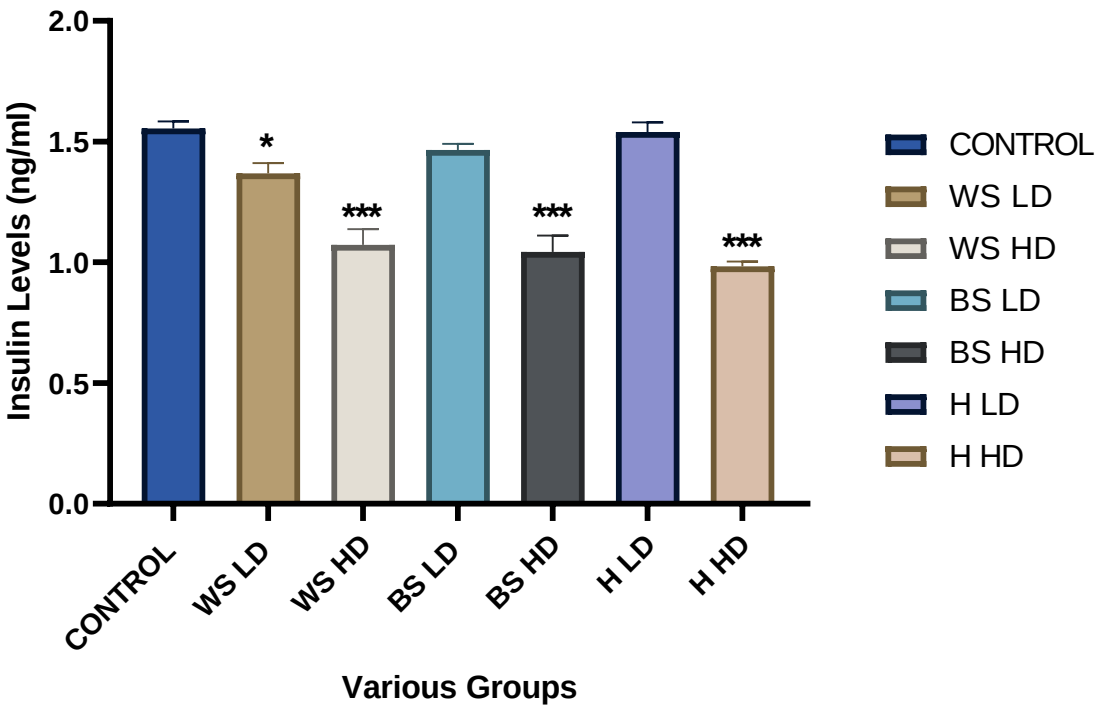
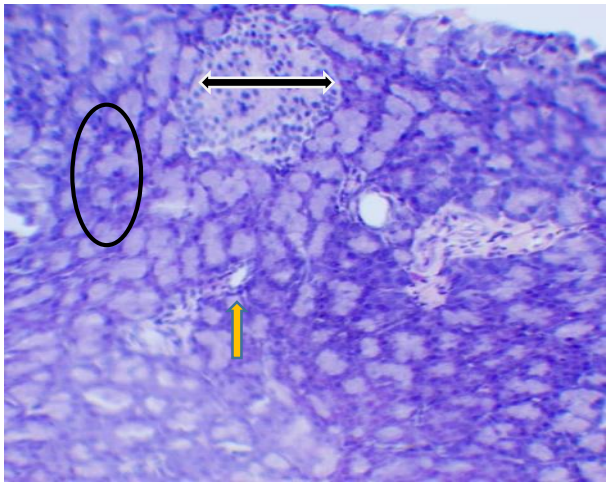


Figure 4. 8: Bar chart showing the effect of white sugar, brown sugar and honey on low insulin level of rats after 12 weeks administration. Each column represents mean with SEM as error bars for values statistically different as indicated with $p < 0.001$.

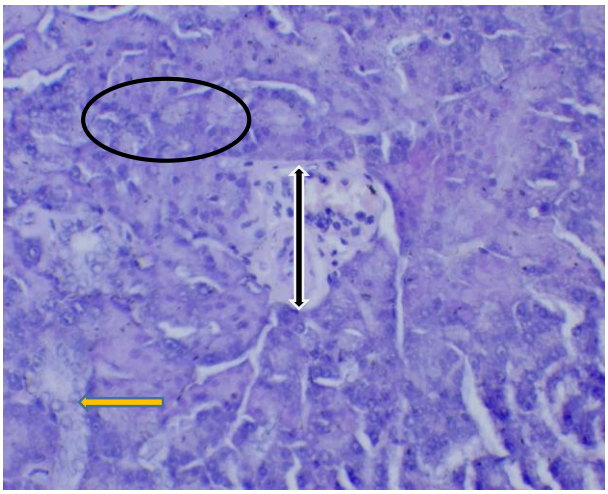
4.6 EFFECT OF WHITE SUGAR, BROWN SUGAR AND HONEY ON HISTOLOGY OF THE PANCREAS.

Histological assessment of the effect of honey, white and brown table sugar showed that control, WS LD, BS LD and H LD groups had compact islets with well-defined borders. The islet cells in the control, WS LD, BS LD and H LD groups were evenly distributed than those in the WS HD group. The acini were found to be more compact and congested with little degenerations in the control group, WS LD, BS LD and H LD. However, the islet were distorted with less defined borders while the acini were degenerated, less compact and congested WS HD, BS HD and H LD compared to the control group (Figure 4.9).

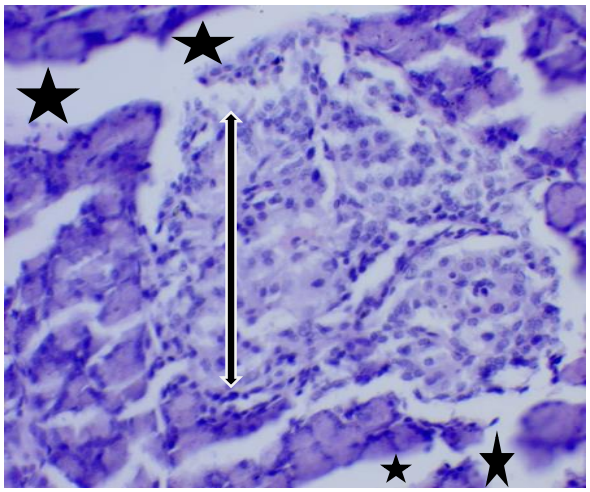
CONTROL



WS LD



WS HD



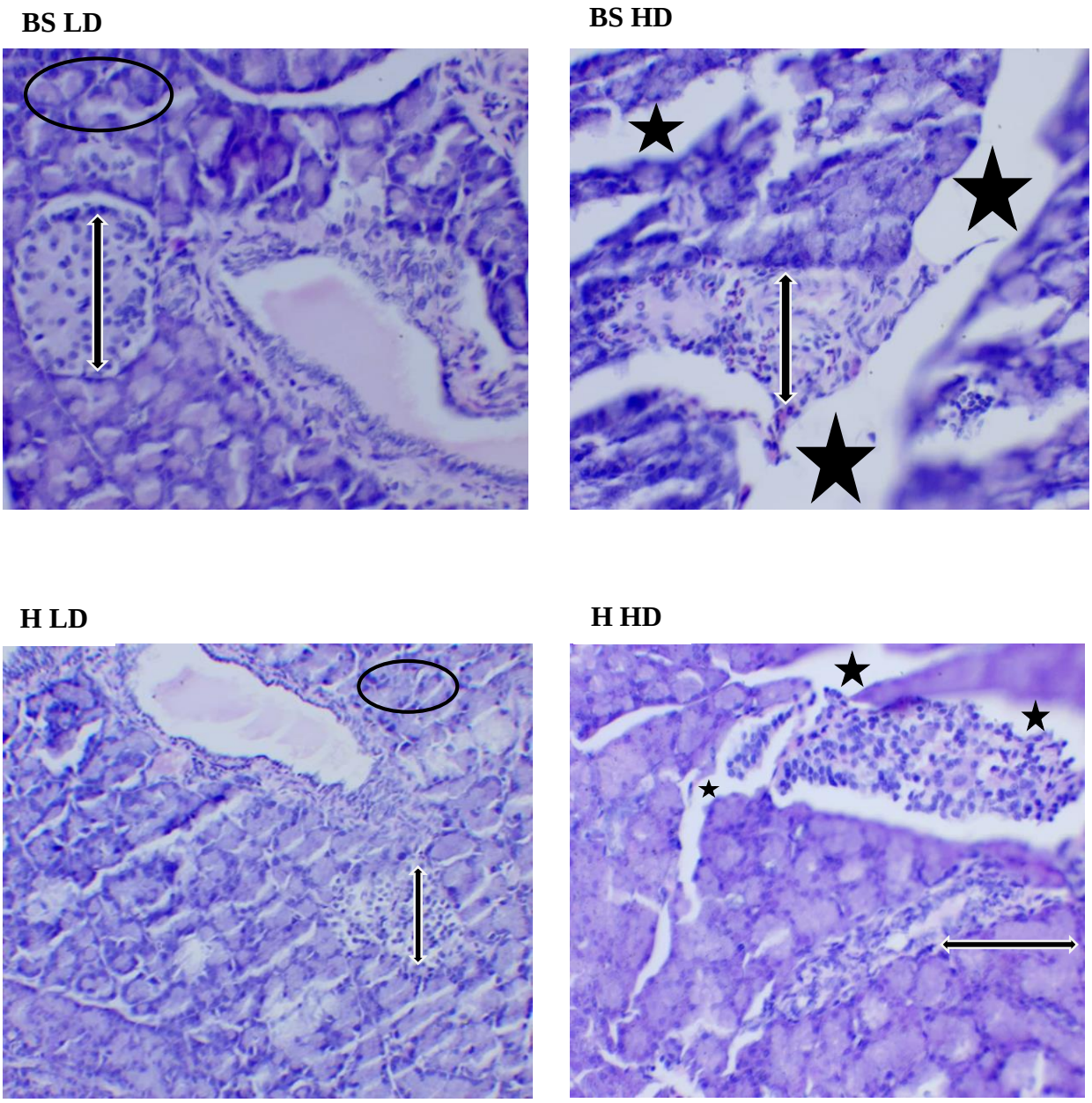


Figure 4. 9: Photomicrographs of PAS staining showing representative sections of pancreatic tissue of rats studied after 12 weeks administration. Black double headed arrows indicate islet. Yellow double head arrow indicate duct system. Solid yellow arrows indicate epithelial lining of the duct. Circle indicate acini. Five-point stars indicate degenerated acini.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Added sugar continues to be a major problem worldwide. It is termed as the hidden ingredient with many names and has a wide range of uses. Sugars are added to foods during preparation, processing, or added at the table and this makes it an inseparable component of our diet. Many people consume excess sugar due to its sweet taste. Over consumption of sugar has led to an increased prevalence of obesity globally (Malik and Hu, 2012). The increase in chronic conditions as a result of overweight or obesity has overburdened the livelihood of many individuals and families. Due to it health implications, consumers have become more conscious of the use of these sweeteners and prefer some natural sweeteners over another.

Much of the information about the role of sugars on nutrition and health are inconsistent. It is unclear if some natural sweeteners are beneficial to health than others. However, there is a gradual but substantive preference on the use of honey as a better choice of sweetener than brown and white table sugars. This preference is justified by the fact that honey contains some trace elements and is not subjected to any form of processing therefore it is more natural and healthier (Roman et al., 2013).

Animal studies using normal Sprague-Dawley rats have demonstrated varying effect of honey and sucrose on weight gain, HbA1c, FBG and lipid profile (Chepulis, 2007, Chepulis and Starkey, 2008, Nemoseck et al., 2011, Atangwho et al., 2020). Having obtained inconclusive results in the aforementioned studies, it was incumbent to further investigate anecdotal claims. In this study, rats were fed WS LD (5.5%), WS HD (22%), BS LD (5.7%), BS HD (23%), H LD

(7.6%) and H HD (30.4%) for 12 weeks. In an earlier study, Wistar rats were fed a similar dose including 8% and 16% table sugar, 10% and 20% honey diets, for 29 weeks (Atangwho et al., 2020). Similarly, a diet which contained 8% sucrose, 8% mixed sugars as in honey, or 10% honey freely were fed to Sprague-Dawley (S-D) rats for 6 weeks (Chepulis, 2007). In the aforementioned studies different animal models were used to study the effect of these natural sweeteners on parameters such as weight, glycaemia, lipid profile and insulin.

In this study, measured animals body weights showed straight and continuous gain across the treatment groups, with the continuous weight gain not significantly different among the groups. This indicates that the three sweeteners, honey, white and brown table sugar did not have any distinguishable result on body weight gain. This finding is consistent with a report by (Atangwho et al., 2020) which showed weight gain across the treatment groups of female Wistar rats fed 20% honey and 16% sugar sweetened diet, were comparable to the control fed rat chow only. Similarly, a study by (Adesoji and Oluwakemi, 2008) reported that the control non diabetic rats fed with honey and both honey and fructose led to progressive weight gain 50.27% and 19.32% respectively.

An earlier study by (Atangwho et al., 2017) showed weight gain across the treatment groups where rats were fed with 15% and 30% sugar, 12% and 24% honey compared to the control group for 13 weeks. On average, the recommended intake of dietary sugar in the form of added sugar should not exceed 150 and 100 calories for men and women respectively (Johnson et al., 2009). This is equivalent to 38 g WS, 41 g BS and 52 g H per day. Weight increase is expected if no effort is made to reduce the intake of added sugar (Hu, 2013). This was demonstrated in a trial of sugar free or sugar sweetened beverage and body weight in children (de Ruyter et al., 2012). However, (Chepulis and Starkey, 2008) reported a significant reduction in body weight gain of

10% honey-sweetened diet fed to Sprague-Dawley rats compared to rats given 7.9% sucrose-based diet for six weeks. Likewise (Nemoseck et al., 2011) reported a lower weight gain for honey-fed rats than rats fed sucrose after 33 days of treatment. This may be a result of the effect of fructose which is a major component in honey in modulation of appetite regulating hormones such as leptin, ghrelin and peptide (Klok et al., 2007). Further to this, the rats in the treatment groups were fed natural sweetener treatment diet *ad libitum* which may explain reduced diet intake and subsequently weight gain. Honey, white and brown sugar contains glucose and fructose, which when consumed in the same quantity will affect energy balance similarly. It is therefore comprehensible that honey, white and brown table sugar fed in equivalent quantities of carbohydrate may have contributed equally to weight gain.

In this study, the effect of intake of the three natural sweeteners white sugar, brown sugar and honey on the HbA1c level of the rats showed a significant increase in the high dose groups compared to the control group. This is consistent with an earlier study by (Chepulis, 2007) where the authors reported that HbA1c levels were significantly higher in the sucrose-fed group (4.14 ± 0.07 mmol/L), mixed sugars-fed group (4.09 ± 0.11 mmol/L) and the honey-fed group (4.11 ± 0.19 mmol/L) compared with rats fed a sugar free diet (4.02 ± 0.12 mmol/L). Similar to a recent study by (Driescher et al., 2019), sugar sweetened beverage consumption increased HbA1c levels after three and six months of continous sugar intake. (Bahrami et al., 2009) also reported a significant increase in HbA1c levels in human subject fed with natural honey for eight weeks compared with the control group.

There was no significant increase in HbA1c levels among the low dose groups except for WS LD. This may be due to the high glyceamic nature of white sugar. This is at variance with an earlier study by (Chepulis and Starkey, 2008) which recorded a significant decrease of HbA1c

levels in a long-term honey (3.97 ± 0.12 mmol/L), sucrose (4.19 ± 0.14 mmol/L) and sugar free (4.07 ± 0.17) feeding in Sprague-Dawley rats for 52 weeks. It could be that the natural sweetener administered in low dose groups were relatively small to cause an increase in HbA1c levels.

In this study, the effect of intake of the three natural sweeteners white sugar, brown sugar and honey on the fasting blood glucose (FBG) level of the rats showed there was no significant difference between WS LD, BS LD, H LD and control group. This is similar to a recent study by (Atangwho et al., 2017) which reported that FBG concentrations did not significantly differ between the test groups and/or the normal control. Likewise, a previous study (Erejuwa et al., 2016) showed that there was no significant difference in the glucose lowering effect on honey-fed rats. Honey and table sugar contain glucose and fructose in a relatively close amount plus other components depending on it source. This implies that its' consumption over a period will affect FBG in a similar way especially where the same amount of carbohydrate was given to the rats in this study.

In this study, the effect of the intake of the three natural sweeteners white sugar, brown sugar and honey on the TC, TG, HDL-C and LDL-C level of rats showed there was no significant difference between TC, TG, HDL-C levels in all the treatment groups and that the LDL-C levels of WS LD and WS HD groups were comparable to the control groups. This is similar to a study that found no significant difference between the HDL-C, TC and TG levels in the treatment group compared with control group in honey fed rats. (Mohammadimanesh et al., 2019). Furthermore, found no significant differences in triglyceride levels when rats were fed 10% honey or sucrose-based diet after 6 or 52 weeks (Chepulis, 2007, Chepulis and Starkey, 2008). Further, a similar study observed no significant difference in TC and HDL-C between the rats fed the honey and sucrose-based diet (Nemoseck et al., 2011). It is imperative to point out that

the afore-mentioned study was designed to examine the potential differences of a honey based diet and a sucrose based diet. However, these diets were not compared with a more neutral control diet such as a sugar free diet and this may imply that honey may be a healthier replacement for sucrose.

Further, in a study on diabetic rats it was observed that TC levels of normal non-diabetic rats, slightly increased following honey treatment which is similar to the observation made in this present study (Aziz et al., 2017). Furthermore, not all studies ascertained the modulation of lipid profiles with the consumption of honey (Chepulis, 2007, Münstedt et al., 2009). In this present study, LDL-C levels of rat were significantly lower BS LD (0.31+0.2 mmol/L), BS HD (0.59+0.36 mmol/L), H LD (0.64+0.09 mmol/L) and H HD (0.53+0.05 mmol/L) groups when compared with the control group (2.15+0.16 mmol/L). White sugar, brown sugar and honey treatment groups had the same effect on lipid profile except the effect of brown sugar and honey on LDL-C. This may be as a result of antioxidant, polyphenols found in honey as well as trace elements in brown sugar which may be cardioprotective compared to white sugar which has none of these components. This is in line with a study by (Alagwu et al., 2014) which reported lowered LDL-C in honey fed-rat when compared with the control group ($p<0.01$). This is an indication of the positive impact of honey on the level of LDL-C in rats. However, the same can be said for brown sugar which equally showed a lower LDL-C of rats compared to the control group in this study. This similiarity does not put honey above brown sugar in terms of cardioprotective property.

The effect of intake of the three natural sweeteners white sugar, brown sugar and honey on the insulin levels of rats showed no significant differences between BS LD group and H LD group when compared with the control group. This explains the lower levels of FBG and HbA1c

measured in rat in this groups. This is concurrent with a study (Aziz et al., 2017) where serum insulin levels of normal non-diabetic rats, were almost twice that in non-treated diabetic rats, and were not affected by honey treatment. However, there were significant differences between WS LD, WS HD, BS HD and H HD groups compared with control group. This was shown by high FBG and HbA1c levels in this groups (WS LD, WS HD, BS HD and H HD) due to lower insulin secretion as revealed by distorted islet with less defined borders. The function of insulin is to help transport glucose into the cells to be used in energy production. Inadequate amount of insulin will make cause make the glucose level rise in the blood.

This implies the ability to produce near normal insulin by the pancreas in the rat low dose groups was supported by near normal histological features of the pancreatic islets that appears compact and evenly distributed with well-defined borders and than in the rat high dose groups.

5.2 CONCLUSION

In the current study, honey, white and brown table sugar were found to cause an appreciable increase in FBG, HbA1c and lipid profile and release of insulin at the same dose adminitration after the twelve week period. The increase was found to occur mostly in the high doses than in low doses with a few disparity. Additionally, high doses of honey, white and brown sugar were found to cause weight gain after the twelve week period. Although this study was conducted in an animal model, this finding is noteworthy, as this may serve as a basis for future studies.

5.3 LIMITATIONS

To allow the testing of HbA1c the study had to change to a chronic study.

5.4 RECOMMENDATION

There is the need for further research to understand the mechanism on the effect of brown table sugar on lipid profile especially on LDL-C to ascertain if there is threshold of added sugars below which there are no negative effects on cardiovascular health.

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APPENDICES

APPENDIX A

Calculation of Animal Equivalent Dose of Honey

Recommended human dose = 53.19 g

Average human weight = 70 kg

Dose = 53.19 g/70 kg = 0.76 g/kg

Animal weight = 200 g = 0.2 kg

Animal equivalent dose = 0.2 kg x 0.76 g/kg = 0.152 g/kg

High animal dose = 0.152 g/kg x 2 = 0.304 g/kg

Low animal dose = 0.152 g/kg /2 = 0.076 g/kg

Calculation of Animal Equivalent Dose of Brown Sugar

Recommended human dose = 40.11 g

Average human weight = 70 kg

Dose = 40.11 g/70 kg = 0.573 g/kg

Animal weight = 200 g = 0.2 kg

Animal equivalent dose = 0.2 kg x 0.573 g/kg = 0.115 g/kg

High animal dose = 0.115 g/kg x 2 = 0.229 g/kg

Low animal dose = 0.115 g/kg /2 = 0.057 g/kg

Calculation of Animal Equivalent Dose of White Sugar

Recommended human dose = 38.64 g

Average human weight = 70 kg

Dose = 38.64 g/70 kg = 0.552 g/kg

Animal weight = 200 g = 0.2 kg

Animal equivalent dose = 0.2 kg x 0.552 g/kg = 0.110 g/kg

High animal dose = 0.110 g/kg x 2 = 0.220 g/kg

Low animal dose = 0.110 g/kg /2 = 0.055 g/kg

APPENDIX B

COLLECTION OF BLOOD FOR BIOCHEMICAL ANALYSIS (CARDIAC PUNCTURE)

Rats were anaesthetized by ether inhalation until consciousness was lost.

A 2 ml syringe was fixed to a 23G × 1" hypodermic needle was inserted into the left ventricle by moving about 1 cm superiorly above the xiphisternum and 1 cm laterally to the left and into the 5^{th} intercostal space.

The plunger of the syringe was drawn back to collect about 4 ml of blood into various sample bottles.

APPENDIX C

PREPARATION OF 10% BUFFERED FORMALDEHYDE PH 7.26

10% buffered formaldehyde, pH 7.26

Formalin (37 - 40% w/v - BDH, England) …….……100 mL

Distilled water……. 900 mL

Sodium hydrogen orthophosphate (NaH_2PO_4) ….…….4g

Disodium hydrogen orthophosphate (Na_2HPO_4) …….6.5g

Apparatus and equipment

Electronic balance (Mettler CH – 8606)

1000 mL flask

Magnetic stirrer

pH meter (Philips, PW9418)

Conical flasks and beakers

Plastic weighing container

Measuring cylinder

PROTOCOL FOR PERIODIC ACID SCHIFF STAINING

With the aid of Leica Auto Sectioner XL with the following programmed methods, the paraffin embedded pancreatic blocks were sections at 5 micrometre prior to PAS staining for histomorphometry.

Staining Technique

De-wax sections in xylene for 1 minute.

Bring tissue sections down to water, rinse in distilled water

Apply periodic acid for 5 minutes and rinse with distilled water

Apply Schiff reagent for 20-30 minutes

Wash under running water for 10 minutes

Counter stain in haematoxylin for 15 minutes.

Wash in water for 2-3minutes

Blue under running water

Dehydrate in graded series of alcohol, clear in xylene for subsequent mounting with DPX

APPENDIX D

PROTOCOL FOR TISSUE PROCESSING

Rinse tissue in ice-cold PBS and weigh before homogenization

Mince the tissues to small pieces and homogenize

Sonicate the resulting suspension till the solution is clarified

Centrifuged the homogenate for 5 minutes at 10,000×g.

Collect the supernates and assay immediately

ASSAY PROCEDURE

Add 50µL standard or sample to each well.

Add 50µL prepared Detection Reagent A immediately.

Shake and mix. Incubate 1 hour at 37°C;

Aspirate and wash 3 times

Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37°C

Aspirate and wash 5 times

Add 90µL Substrate Solution. Incubate 10-20 minutes at 37°C

Add 50µL Stop Solution. Read at 450 nm immediately