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THE EFFECT OF NATURAL SWEETENERS ON ATHEROGENIC RISK INDEX IN SPRAGUE-DAWLEY RATS.

BY

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DECLARATION

I hereby declare that this research, under the supervision of the individuals named below, was conducted and written by me (with the exception of references to other people's work that have been properly acknowledged). No portion or the entirety of this thesis has ever been submitted for a degree elsewhere.

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DEDICATION

This thesis is dedicated to the Almighty God and my beloved family.

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ABBREVIATIONS

AIP	Atherogenic index of plasma
BLNA	Body Length Nose to the Anus
BS	Brown sugar
BS LD	Brown sugar low dose
BS MD	Brown sugar medium dose
BS HD	Brown sugar high dose
CVD	Cardiovascular disease
CAD	Coronary artery disease
FBG	Fasting blood glucose
FPG	Fasting plasma glucose
H	Honey
HbA1c	Glycated hemoglobin
HDL-C	High density lipoprotein cholesterol
H LD	Honey low dose
H MD	Honey medium dose
H HD	Honey high dose
IDF	International Diabetes Federation
LDL-C	Low density lipoprotein

	cholesterol
NS	Nutritive Sweetener
NMIMR	Noguchi Memorial Institute for Medical Research
NSS	Non-nutritive Sweetener
S	Stevia
S LD	Stevia low dose
S MD	Stevia medium dose
S HD	Stevia high dose
S-D	Sprague-Dawley
SSB	Sugar sweetened beverage
TC	Total cholesterol
TG	Total triglycerides
UG-IACUC	University of Ghana Institutional Animal Care and Use Committee
US	United States
WHO	World Health Organization
WS LD	White sugar low dose
WS MD	White medium dose
WS HD	White sugar high dose
WS	White sugar

ABSTRACT

Background: Human preferences for sweet taste in what they eat is responsible for the increased production and consumption of sweeteners. Excessive consumption of natural sweeteners including honey, white granulated sugar, brown sugar and stevia have been linked to a number of atherogenic risk factors, including obesity, diabetes, and abnormal cholesterol and triglyceride levels, although the evidence remains unclear. Subjective evidence suggests some sweeteners are healthier than others, whereas other policies imply all sweeteners have similar impact on the body. Without comparing how different sweeteners affect various health outcomes, previous studies grouped all sweeteners together. Many studies frequently use the traditional lipid measurements in clinical investigations without considering lipid ratio formulas in forecasting atherogenic risks. Therefore, scientific evidence supporting claims regarding the impact of these natural sweeteners on several indices of atherosclerosis are inconclusive.

Aim: To determine the effect of sweeteners on atherogenic risk index in Sprague-Dawley rats.

Method: This research employed an experimental design and included seventy-eight (78) Sprague Dawley rats aged 10-13 weeks weighing 150-200 g. Rats were randomized into 13 groups: 6 rats per group. Three nutritive sweeteners (white sugar, brown sugar, honey) and one non-nutritive sweetener (stevia) were used. Doses for each sweetener were categorized into low, medium and high dose. Sweeteners were administered by oral gavage for 112 days. Water was provided *ad libitum*. Rat chow diet for each experimental group was weighed before and after feeding. The following were determined; fasting plasma glucose (FPG), serum lipids (total cholesterol, HDL, LDL and triglyceride), body weight, length and BMI. Descriptive statistics were run for continuous variables and a 2-way ANOVA was conducted to compare means across groups. A Linear mixed model was used to evaluate the effect of the sweeteners over time on body weight and fasting plasma glucose (FPG).

Results: Honey, brown sugar and stevia were found to cause an increase in triglycerides after the seventeen weeks period with a corresponding reduction in LDL-C and VLDL-C concentrations.

The increase was observed in high doses than in low doses of sweeteners. Also, medium doses of stevia elevated glucose levels. Additionally, high doses of white, honey, and stevia increased weight after the seventeen weeks period. Further, doses of honey and stevia increased the atherogenic index of plasma after the seventeen weeks period, indicating an increased risk of atherosclerosis.

Conclusion: This study highlighted that, stevia irrespective of the dosage has tendencies of inducing a higher atherogenic risk than the other natural sweeteners by increasing triglyceride levels, bodyweight and fasting plasma glucose, thus, may not be a better sugar alternative. However, high intake of all the natural sweeteners may exert different effects on metabolism, which may ultimately determine their suitability for different people due to differences in their constituent sugars and nutrients. Therefore, natural sweeteners should be taken with caution by avoiding excessive consumption.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Human preferences for sweet taste in what they eat is responsible for an increased production and consumption of sweeteners. This intrinsic human characteristic has led to the discovery of several natural and artificial compounds with strong sweet flavors and taste-altering qualities (González- Montemayor et al., 2019).

Natural Sweeteners (NS) have been grouped into two main categories; nutritive/caloric and non-nutritive/ low caloric sweeteners (Tou et al., 2011). Honey, white granulated sugar (invert sugar) and brown sugar (malt syrup), are the commonly used nutritive natural sweeteners (Das & Chakraborty, 2016). These NS are also known as caloric sweeteners because, they contain simple carbohydrates such as fructose, glucose, and sucrose, which are quickly metabolized to produce energy, as well as complex carbohydrates such as starch, which are stored for long periods of time and provide long-lasting energy (Saraiva et al., 2020).

These simple carbohydrates can either be found naturally in food or added during food processing or right before individuals consume their food (Carocho et al., 2017). However, due to the high caloric content of these nutritive sweeteners, which can contribute to obesity, diabetes, and cardiovascular diseases, there is a significant market for sugar alternatives that have fewer or no calories but greater sweetening power (Ashwell, 2015).

Despite the fact that there are numerous artificial zero-calorie sweeteners available on the market, such as potassium, cyclamates, aspartame, saccharin, and others, their use have been curtailed due to varied health concerns (Saraiva et al., 2020). As a result, there is a constant search for naturally derived, high intensity, low calorie or non-caloric sweeteners that are also safe for use.

Stevia, which has a significant role as a natural sweetener without any nutritional value, has emerged as a safe sugar substitute with no risk to human health (Ashwell, 2015).

According to Ashwell, (2015), stevia can be considered to have no caloric value because, stevia (or any of its components or byproducts) moves through the body as part of metabolism rather than accumulating there and the energy produced by the fermentation of glucose units is typically only 2 kcal/g. Thus, Stevia has the potential to help people consume less calories, which could reduce and prevent obesity (Ashwell, 2015). Over the years, people have utilized stevia as a flavoring agent and a natural sugar alternative (Prakash et al., 2014). Food researchers continue to investigate new ways stevia extracts are being used in food and beverages (Ashwell, 2015).

Honey is one of the oldest natural sweeteners produced by bees from the nectar of plants or the secretions of living parts of plants (Erejuwa et al., 2012). The chemical composition of honey is dependent on a variety of factors; geographical location, plant from which the nectar was obtained, environmental and climatic conditions and the processing techniques (González-Montemayor et al., 2019; Erejuwa et al., 2012). Honey is made up of monosaccharaides, disaccharides, oligosaccharides and other constituents, as well as other bioactive substances; phenolic compounds, flavonoids, carotenoids, minerals, vitamins, enzymes and aromatic compounds which gives it an additional value and makes it an ultimate preference for people (da Silva et al., 2016; Al-Waili et al., 2013; Kolayli et al., 2012). Honey is thought to be healthier than brown or white sugar due to its antioxidant properties and several long-term health advantages (Kolayli et al., 2012).

In recent times, white sugar, also referred to as table sugar, has been the commonly used nutritive sweetener worldwide (Baikow, 2013). White sugar is made from juice that has been refined to eliminate molasses from sugar cane or sugar beet juice (Panda, 2011). There are several applications for white sugar in the food manufacturing sector. It is a versatile component that gives food products several useful qualities, including texture, color, and flavor (Brisbois et al., 2014).

According to Kumar et al., (2010), a scoop of white sugar using a tablespoon provides roughly 49 calories, whereas that of honey gives about 68 calories, showing that honey is denser than sugar.

Brown sugar is now a commonly used table sugar also derived from sugar cane broth (Orlandi et al., 2017). The processes involved in the production of brown sugar is slightly different from that of white sugar. Brown sugar is usually limed, clarified and repeatedly evaporated by open pan-drying without removing the molasses component. Its dark color is attributed to products from the Maillard reaction, caramelization and the presence of phenolic compounds (Shyu et al., 2019). This makes it an indispensable part of our diet, and because of its sweet taste, many individuals eat more sugar than they realize.

Excessive consumption of these added sugars has been linked to metabolic abnormalities and harmful health issues, including elevated blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol and triglyceride levels raising the risk of atherosclerosis (Bray & Popkin, 2014). Depending on the source, an excess of the undesired nutrients will affect body homeostasis and induce pathophysiological conditions since these nutrients are metabolized by bodily cells and tissues. (Ahmed et al., 2015).

Atherosclerosis is a vascular disease or disorder characterized by the buildup of cholesterol and lipid particles in the walls of the arteries resulting in less room for blood to flow and increases the risk of blockage (Libby et al., 2019). This can lead to ruptures in the vessels and hemorrhages, progressing to various complications including bleeding, thrombosis or stroke, and severe heart problems (Libby et al., 2019). Along with age, other variables that may raise the risk of atherosclerosis include dyslipidemia, diabetes, obesity, smoking and other tobacco use, and hypertension. Lack of exercise, a poor diet, and a history of early heart disease in the family are included (Monta Alvidrez et al., 2017).

Among individuals with atherosclerosis, dyslipidemia has been shown to be one of the primary significant risk factors which may play many roles before other risk factors appear. However, most people with dyslipidemia also have concurrent hypertension which often occurs alongside diabetes and obesity, due to their synergistic effect on each other (Kolderup & Svihus, 2015).

Cholesterol is essential for several bodily processes, encompassing the production and regulation of a variety of hormones and the construction of cell membranes. However, altered blood lipid levels has been identified as a major risk factor for cardiovascular diseases (Osuji et al., 2012).

Major indicators that are frequently used to predict atherosclerosis are abnormal lipid profile values (Nikkos & Rehman, 2021). However, several studies have shown that lipid ratios like the atherogenic index of plasma (AIP), Castelli Risk Index I and II (CRI) and atherogenic coefficient (AC) are the diagnostic alternatives that have been found to predict the risk of developing atherosclerosis when the lipid profile parameters (TG, HDL C, LDL C, and TC) remain ostensibly normal (Olamoyegun et al., 2016). Therefore, understanding these cholesterol sub fraction levels is more important than simply knowing the levels of plasma cholesterol (Ahmed et al., 2015).

1.2 PROBLEM STATEMENT

Atherosclerosis is a chronic condition that progresses during a person's lifetime; clinical manifestations start to show up after years of undetected progression. The leading factor in fatalities in developed countries is still atherosclerotic disease, notwithstanding recent advancements in the treatment of cardiovascular disease that were linked to a considerable drop in death rates from 1990 to 2000 (Libby et al., 2019).

Obesity, diabetes, dyslipidemia, and hypertension are new developments in human evolution that raise the risk of atherosclerosis. These conditions are caused by lifestyle alterations such as poor dietary habits, particularly excessive sugar consumption (Rogers et al., 2016; WHO, 2015; Fitch & Keim, 2012).

In reducing overall caloric intake and combat the growing incidence of obesity associated with the intake of natural sweetness and its effect in developing atherosclerosis, zero-caloric natural sweeteners such as stevia have gained an increasing attention with a gradual shift in their use (Swithers, 2016; Romo-Romo et al., 2016; Pereira, 2014).

Unfortunately, several studies have proposed that, increased usage of sugar substitutes with no calories, like stevia, may alter gut bacteria resulting in reduced levels of protective cells in the gut, responsible for preventing metabolic diseases and promoting optimum health. (Pepino, 2015). Therefore, variations in gut bacteria may have a negative impact on an individual's body weight as well as lipid levels which are strong indicators for atherosclerosis, although the evidence remains inconclusive (Fu et al., 2015)

Many researchers and consumers seem to suggest, that some natural sweeteners are healthier than others (Valli et al., 2012, Roman et al., 2013). Policies that classify natural sweeteners together also imply that; they all have a comparable impact on the body. However, as sweeteners differ in their constituent sugars and have diverse chemical structures, they may exert different effects on metabolism, which may ultimately determine their suitability for different people. There is still a debate on whether the various sweeteners have superseding benefits over each other.

Without comparing how different sweeteners affect various health outcomes, previous studies grouped all sweeteners together (Nwachuku et al., 2017). There is no study that has examined in one study, the different types and doses of natural sweeteners on various markers of atherosclerosis since most of the studies conducted use a maximum of three sweeteners.

Also, the correlation between excessive intake of sugar and elevated triglycerides levels, a major biomarker of atherogenic risk, is uncertain (DiNicolantonio & OKeefe, 2017). In addition, the traditional lipid measures are frequently used in clinical investigations to forecast health consequences regarding the impact of natural sweeteners on lipid profiles. There is, however, limited information on how well lipid ratio formulas may be used to predict atherogenic risks.

Therefore, there is a need for further research on the effects of natural sweeteners on the atherogenic risk index using a murine model such as Sprague-Dawley rat, given the controversy surrounding the findings regarding their effects on health, particularly their impact on lipid profiles.

1.3 SIGNIFICANCE OF STUDY

This research will provide information on how different concentrations and types of natural sweeteners acts on the lipid profile, thereby ascertaining which sweetener is better in relation to atherogenic risk. This study will complement efforts in providing strategies, including lifestyle modification in using sweeteners. Additionally, it will provide evidenced-based information which will address the general debate and undocumented claims on natural sweetness, to assist individuals in their choices. Furthermore, Dieticians and nutritionists may use this information to help them create the best interventions for people with conditions that are related to diet and nutrition. The study's findings may also help consumers, policymakers, and other healthcare professionals make knowledgeable choices when using natural sweeteners and can assist in the development of dietary recommendations and nutritional policies on the use of added sugars.

1.4 HYPOTHESES

H0 -There is no statistical difference in body weight and BMI among rats on the various types of natural sweeteners.

H0– There is no statistical difference in the FPG, lipid profile and lipid ratios among rats on the various types of natural sweeteners.

1.5 AIM AND OBJECTIVES

This research aimed at determining the effect of natural sweeteners on atherogenic risk index using Sprague-Dawley rat models.

1.5.1 SPECIFIC OBJECTIVES

1. To measure the effect of natural sweeteners on anthropometric measurement (Body weight and Body Mass Index) of the experimental animals.
2. To access the effect of natural sweeteners on atherogenic risk index by measuring concentrations of fasting plasma glucose (FPG) and lipid profile of experimental animals.
3. To compare the effect of natural sweeteners on atherogenic risk by using the lipid ratios formulas.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 NATURAL SWEETENERS

For a very long time, individuals all over the world have utilized natural sweeteners in varied ways due to a natural human preference for sweetness. This intrinsic human trait has spurred the discovery of a number of natural and synthetic compounds with strong sweet tastes or taste-altering abilities (González- Montemayor et al., 2019). To preserve and advance health, the public is growing more informed of how important maintaining a balanced diet is, thus, they are going in for meals with less or no sugar since it is difficult to break the habit of ingesting too much sugar (Saraiva et al., 2020).

Today, the food business now uses strong sweeteners in large quantities to replace sugar (sucrose) in food products, providing consumers with variety of options to satisfy their health needs (Saraiva et al., 2020). As a result, customers are even more ready to purchase and consume foods that are made with natural components, have clear labelling, and ideally, also have additional beneficial qualities (Asioli et al., 2017). However, a product being termed as natural does not assure their commercial success thus, it cannot negate the requirement for conducting in-depth scientific research to reveal how safe the natural components are as well as the quantities suitable for human consumption (Saraiva et al., 2020).

2.2 CLASSIFICATION OF NATURAL SWEETENERS

Although the nomenclature used to define natural sweeteners is frequently unclear and general, they can be divided into natural and synthetic agents, powders and syrups, nutritive/caloric and non-nutritive/non- caloric' substances (Kalakoti et al., 2011).

2.2.1 Nutritive/Caloric sweeteners

Nutritive sweeteners are referred to as caloric because they provide energy (~4kcal/g) in the form of carbohydrate after ingestion or as sugar alcohols (~ 2kcal/g) (Fitch & Keim, 2012; Tou et al., 2011). Caloric sweeteners are often present as simple sugars; monosaccharaides ($C_6H_{12}O_6$) and disaccharides ($C_{12}H_{22}O_{11}$) (Ervin & Ogden, 2013). Nutritionally relevant monosaccharaides are glucose, fructose and galactose. Disaccharides consist of two monosaccharaides units covalently bonded. Commonly consumed disaccharides are lactose (glucose and galactose), sucrose (glucose and fructose) and maltose which is made up of two glucose units (Tou et al., 2011). Nutritive sweeteners are naturally present in foods or added during food processing and manufacturing. Naturally occurring sweeteners include lactose found in milk and its products and fructose in fruits. Other commonly consumed nutritive sweeteners are refined sugars popularly known as white sugar, brown sugar, honey, fructose corn syrup, maple syrup, corn-based sweetener and agave nectar (Fitch & Keim, 2012).

2.2.1 Honey

Honey, a delectable food produced by Apis mellifera bees from blossom nectar, has been shown to have therapeutic value on both a curative and preventive levels (De-Melo et al., 2018). Monosaccharaides, disaccharides, oligosaccharides, and polysaccharides make up the majority of the sugars in honey (Alvarez-Suarez et al., 2014). It includes enzymes that, when consumed, are anticipated to have positive health effects, including glucose oxidase, diastase, invertase, catalase, and peroxidase (Nwachuku et al., 2017). Additional bioactive components found in honey include flavonoids, antioxidants, organic acids, ascorbic acid, trace elements, vitamins, amino acids, proteins, and products of the Maillard reaction (White, 2013). When consumed, foods rich in glucose and fructose can be digested to produce energy and aid in tissue regeneration. (Nwachuku et al., 2017).

Honey has been used as a medication since the beginning of time in addition to being a food and a sweetener (Erejuwa et al., 2014). The components of honey are influenced by the type of flora and environment (Manyi-Loh et al., 2011a). However, extrinsic elements like season, environment, and processing could have an impact on the composition (Manyi-Loh et al., 2011a). Consumer purchasing decisions are impacted by economic considerations that show the financial status of many households that can afford those products (Roman et al., 2013).

2.2.3 White table sugar

Sucrose is present in nearly all plants with high levels found particularly in sugar beet and cane at levels high enough to have a positive economic impact (Li & Yang, 2015; Bakker, 2012). Two commonly grown crops are sugar beets and sugar cane (Saccharum officinarum and Beta vulgaris, respectively) (Li and Yang, 2015). In tropical and subtropical areas, sugarcane is a massively farmed grass (Bakker, 2012). The root crop sugar beet, on the other hand, is only grown in temperate climates (Ma et al., 2014).

The method used to make sugar from sugarcane may be either simple or complex depending on the procedures employed and these procedures can also affect the outcome (Rein, 2016; Baikow, 2013a). The sugar cane crop is harvested and chopped into pieces either mechanically or manually (Hugot, 2014). Alternatively, it is processed and the juice is extracted by diffusion or by using a sugar miller followed by water extraction through a series of evaporators (Hugot, 2014). As a result, sugar crystals are dispersed throughout the supersaturated solution, promoting crystal growth and drying (Baikow, 2013).

Sugar beets can also be used to produce white sugar (Cooke & Scott, 2012). Sliced sugar beet is soaked in hot water to produce sugar juice and then heated under a vacuum to eliminate a lot of water to get a concentrated juice (Cooke & Scott, 2012). Crystallization of sugar occurs from the concentrated solution (Rein, 2016). The syrup is then spun in centrifuges to separate the pure sugar crystals from the syrup, packaged or shipped to consumers in bulk after being dried and processed in silos (Baikow, 2013b; Cooke & Scott, 2012).

2.2.4 Brown sugar

Brown sugar is produced through sugarcane juice evaporation (Abdullah et al., 2014a). Compared to white sugar, it appears rougher, darker, and pigmented after evaporation due to the presence of molasses and also contains certain trace minerals as well as vitamins (Orlandi et al., 2017). White sugar can also be converted to brown sugar by mixing it with sugarcane molasses (Yang et al., 2020). Carefully measured proportion of molasses to sugar crystals is used (Yang et al., 2020). Additionally, this brown sugar product is substantially coarser than its raw equivalent and can store significantly more molasses without centrifugation or moderate centrifugation (Brekhman & Nesterenko, 2013). Granulated white sugar and brown sugar are used similarly, however brown sugar is thought to add a little extra flavor (Rein, 2016). The amount of molasses present determines how different the various brown sugars taste and look (Yang et al., 2020). More molasses results in gummier crystals, a deeper color, as well as a moe intense flavor (Yang et al., 2020).

2.2.5 Non-nutritive/low Caloric sweeteners

Non-nutritive sweeteners (NNS) are compounds that are used in place of sugar to sweeten foods, beverages, and other items like oral care products and some medications (Tou et al., 2011). When ingested, non-nutritive sweeteners provide very little or no energy (Jain et al., 2017). They might be made from plants, herbs, or even sugar itself. Because their sweetening agent is substantially sweeter than sucrose, they are also known as high intensity sweeteners (Kalakote et al., 2011). They are either generally accepted as safe or are approved by Food and Drug Administration (FDA) as food additives. NNS are choices for those who want to restrict their calorie consumption and avoid gaining weight (Jain et al., 2017). Aspartame, saccharin, neotame, sucralose, and stevia are among the non-nutritive sweeteners that are permitted and approved for use (Fitch & Keim, 2012).

2.2.6 Stevia

For many years, people have utilized the naturally occurring, calorie-free sweetener, stevia, as a natural sugar substitute and flavoring agent (Ashwell, 2015). It has been widely utilized for more than 400 years throughout the world and for centuries by the people of Paraguay (Jain et al., 2017). The Latin word stevia is derived from a Spanish botanist and physician, Petrus Jacobus Stevus, who was the first to examine this NNS (Abdullateef & Osman, 2012). Stevia is a general word for several types of sweeteners, including the entire Stevia plant (Srebaudiana Bertoni) and the leaves, which contain the sweet compound (Ashwell, 2015).

The term stevia extract refers to a preparation that is created by steeping Stevia plant leaves to draw out the plant's sweet-tasting components (Chowdhury et al., 2015). Comparable to how other plant-based components, like cane sugar or natural vanilla extract, are created through a series of procedures starting with the harvested, raw plant material to the finished product, the process of purifying stevia into high- purity stevia leaf extract is similar (Ashwell, 2015). The procedure starts with the leaves being dried before they are steeped in hot water, followed by liquid extract, purification and filtering using water, or in some circumstances, water and food-grade alcohol (Abdullateef & Osman, 2012). If food-grade alcohol is used, it is subsequently eliminated, leaving virtually no alcohol in the finished product (Prakash et al., 2014). In some circumstances, different procedures may be employed. The original leaf compounds, known as steviol glycosides, are present in the purified form (Ashwell, 2015). The method for extracting steviol glycosides from the stevia leaf is depicted in Figure 2.1, along with the filtration and purification steps.

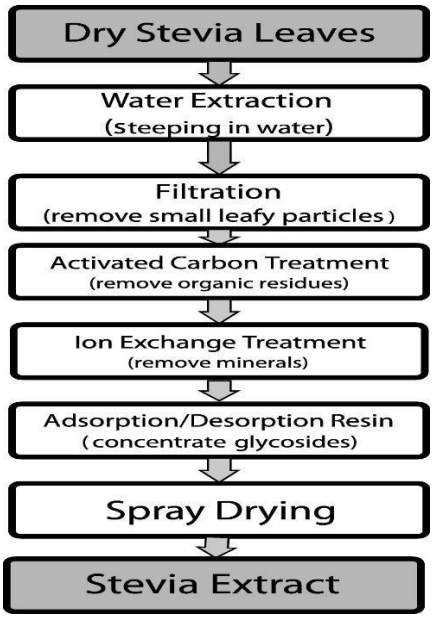

Figure 2.1: Processes involved in stevia extraction (Ashwell, 2015).

Rebaudioside and stevioside, two important chemicals, are primarily responsible for its sweetness (Chowdhury et al., 2022). Stevia is heat resistant, pH stable, not fermentable, and minimally affect metabolic functions (Jain et al., 2017). Numerous antioxidant substances, such as flavonoids, triterpenes, tannins, caffeic acid, kaempferol, and quercetin, can be found in stevia (Clemente et al., 2021).

It is used by many food and beverage producers in yogurt, coffee, tea, smoothies, and other goods to provide items with less calories (Prakash et al., 2014). Stevia has made headlines as a sugar alternative that poses no threat to human health and offers a number of health advantages, including the ability to manage diabetes, aid in weight loss, and lower blood pressure and cholesterol levels (Ashwell, 2015). Stevia has other medicinal properties that is useful for skin diseases like eczema and dermatitis since it functions as a steroid and aids in reducing the growth of bacteria (Clemente et al., 2021).

2.3 FUNCTIONAL PROPERTIES OF SWEETENERS

Natural sweeteners have some unique qualities that allow consumers and food manufacturers to use them in a variety of ways (Guerra & Mujica, 2010). This comprises solubility, fermentation, preservation, moisture retention, texture and sweetness.

2.3.1 Sweetness

Sweetness is a distinct sensory experience usually felt after eating sugary foods which can be altered by certain factors, including concentration, temperature, pH level, and individual preferences of a particular product (Mooradian et al., 2017; Guerra & Mujica, 2010). The major functional role of caloric sweeteners is to provide sweetness whereas alternative sweeteners is to provide sweet taste without contributing kcals (Tou et al., 2011). Since honey, white, brown, and stevia are all naturally sweet, they are frequently employed as additional sugar in the creation of commercial foods. The chemical makeup of the sweetener determines the sweetness level (Clemens et al., 2016b). Fructose is 160 times sweeter than sucrose whereas stevia has 200 to 300 more sweetening power than both fructose and sucrose (Ashwell, 2015; White, 2014).

2.3.2 Texture

Sweeteners affect texture by providing volume and consistency in numerous products, including beverages, jam, and bread (Lees, 2012). When used in commercial food manufacturing, sweeteners can give liquid foods more viscosity, consistency, and bulk (Mathlouthi & Reiser, 2012). When making jam, marmalade, and jelly, the proper balance of pectin and acid is achieved with the aid of sweeteners (Seetal, 2010). The jam's quality depends on the sweetener's ability to gel when combined with pectin which could affect mouth feel (Seetal, 2010). Sweeteners can alter the texture of food by acting as a tenderizer to give cakes a soft texture and full volume or by providing foods like cookies with crispness and brittleness (Tou et al., 2011). They can also reduce water activity, allowing them to be used as humectants to keep moisture in a variety of products like baked goods (Tou et al., 2011).

2.3.3 Fermentation

For many years, the fermentation method has been used to preserve foods ((Gisslen, 2012). Sugar is sometimes used together with yeast in fermentation processes (Zabed et al., 2014). The kind and amount of sugar used will determine the rate of fermentation in product (Gisslen, 2012).

In baking, sugar activates the yeast, causing the yeast's enzymes to break down the sugar into ethanol and carbon dioxide (Cauvain, 2012). The carbon dioxide makes the baked items porous and raises the dough, increasing the production of dough and loaf softness (Cauvain, 2012). Sugar might not always be present in the finished product for this function. (Lees, 2012). However, sugars that are left over after fermentation contribute to the overall flavor, color, and texture of the finished product.

2.3.4 Preservation

Preservation stops the growth and activity of bacteria that cause spoiling (Gould, 2012). Foods are preserved to prevent the development of bacteria that harm food products (Gould, 2012). This procedure eliminates potentially harmful pathogenic bacteria (Ray & Bhunia, 2013). Microorganisms require water to grow (Hamad, 2012). They can use the membrane's outer layer to absorb water (Christy et al., 2014). A food product loses water because of the amount of sugar it contains (Clemens et al., 2016b).

Microorganisms are deprived of a necessary component for growth, and as a result, their ability to multiply and thrive is inhibited (Christy et al., 2014). By increasing osmotic pressure, the addition of sweeteners to food items can reduce the benefit of microbial growth (Goldfein & Slavin, 2015). It could lengthen the food's shelf life by making conditions that are unfriendly to microbes (Hamad, 2012).

2.3.5 Solubility

Natural sweeteners are highly soluble in polar solvents such as water and they work well as ingredients in the food business due to their soluble nature (Caraher & Perry, 2017). To enhance flavor and shelf life, sweeteners are added to a variety of pre-packaged goods since they have the capacity to dissolve and combine well with other ingredients during food processing (Caraher & Perry, 2017; Clemens et al., 2016b). They become more soluble when the temperature of their solvent is raised (Newens & Walton, 2016).

2.4 PRODUCTION AND CONSUMPTION OF NATURAL SWEETENERS

China had the highest per capita consumption of sugar and sweeteners in 2018, followed by India and the United States (USDA, 2020). The global consumption of sugar amounted to 171.58 million metric tons in 2018/2019, which was expected to grow by 2019/2020 to around 176.45 million metric tons (USDA, 2020). In 2013, the global average for sweetener intake per person was 24.0 kg causing an increase up to about 0.95% from previous years (USDA, 2020). USA and Canada are among the top consumers of natural sweeteners (Rez et al., 2019). Overall sugar consumption has increased globally within the last five years by 2.33 million metric tons (USDA, 2020). Because of its largest market, the market for diet beverages, having reached maturity, high- intensity sweetener consumption is anticipated to stay stable in the near future (Rez et al., 2019). However, due to the saturated market, it is anticipated that western countries' demand for sweeteners will rise more slowly than that of emerging nations (Rez et al., 2019).

Over the coming years, the demand for non-sugar sweeteners is anticipated to rise due to a growth in the demand for low-calorie products from end-use sectors (Rez et al., 2019). The demand for sweeteners is anticipated to increase during the next ten years due to the increasing use of natural and sugar alcohol sweeteners, including stevia, in the diet beverage market (USDA, 2020). Up until 2027, the demand for sugar and caloric sweeteners is anticipated to increase 1.5%, which is about 213 Mt. (OECD-FAO, 2018).

Few chemical substances that have sweet or sweetness-enhancing qualities have high commercial potential to support the development of a procedure for their manufacture, according to Philippe et al. (2014). According to these writers, the availability of sweeteners on a large scale, the quality of their flavor, their stability, their solubility, their cost, and their patentability all affect how successful they will be commercially (Rez et al., 2019). Since customers typically prefer the taste of NS to that of NNS, the quality of the flavor is one of the most crucial factors (DuBois & Prakash, 2012). Additionally, sweeteners must be safe for consumers, not poisonous and stable under a variety of pH, temperature, and light settings without experiencing unfavorable reactions or producing dangerous derived chemicals (Rez et al., 2019).

2.5 METABOLISM OF NATURAL SWEETENERS

2.5.1 Sucrose Digestion and Absorption

Sucrose digestion begins in t intestine where it is broken down into its component (Fox, 2015). The small intestine's sucrase enzyme facilitates the breakdown of sucrose into glucose and fructose (Fox, 2015). Once they are broken down into its simple form, it is absorbed via the gut lining, through the intestinal epithelium, into the bloodstream and then distributed to various bodily cells to bas energy (Pigman, 2012). This occurs when different dynamic transporters enter the circulation of the hepatic portal system (Johnson et al., 2010). This is one of the few systems that does not send blood back to the heart immediately, Instead, the blood is sent to the liver for additional processing while still carrying all the acquired nutrients (Johnson et al., 2010). Cells in the body receive glucose, which is the body's main energy source, from the liver and insulin facilitates uptake into cells (Rippe & Angelopoulos, 2013). Glucose can also be turned into pyruvate (an acidic substance) during glycolysis to produce energy (Gray et al., 2014; Ruan, 2014; Dashty, 2013).

Fructose can also be broken down to provide energy through a fructose metabolism pathway called Fructolysis (Sun & Empie, 2012; Harvey & Ferreir, 2011). However, unlike glycolysis, which can occur in practically all tissues, fructolysis mostly takes place in the liver (Hannou et al., 2018).

2.5.2 Sugar Storage

Glycogen is formed when excess glucose that was not used for energy production is stored (Dashty, 2013). Through this process, short chains of glucose that are made up of just one subunit are joined together to form glycogen (Adeva-Andany et al., 2016a). When fasting, glycogen hydrolyzes back into glucose (Moore et al., 2012). The body uses up all its glycogen storage capacity and turns the remaining glucose into fat (Adeva-Andany et al., 2016b). Due to the liver's conversion of all fructose into molecules similar to glucose, fructose is not stored in the body for an extended period of time (Hannou et al., 2018).

2.5.3 Stevia metabolism

Steviol glycosides are completely unharmed as they transit through the upper gastrointestinal tract. By cutting off their glucose units, gut bacteria in the colon hydrolyze steviol glycosides into steviol (Guo et al., 2022). Following its ingestion through the portal vein, steviol is predominantly metabolized by the liver to produce steviol glucuronide, which is primarily eliminated in the urine (Ashwell, 2015). According to research, stevia (or any of its components or byproducts) travels through the body during metabolism and does not accumulate there (Ashwell, 2015). Since the energy produced by the fermentation of glucose units is typically only 2 kcal/g, stevia can be considered to have no caloric value (Guo et al., 2022).

2.6 ATHEROSCLEROSIS

The primary cause of cardiovascular disorders is atherosclerosis, an inflammatory disease of the arteries accompanied by cholesterol and other metabolic changes (Barquera et al., 2015). Ischemic heart disease (IHD) and cerebrovascular disease (mostly ischemic stroke) are the two main disorders that make up atherosclerotic cardiovascular disease (ACD) (Barquera et al., 2015). The abnormal accumulation or buildup of cholesterol and lipid particles in the walls of medium- and large-calibre arteries is the hallmark of the vascular disease or ailment known as atherosclerosis (Monta Alvidrez et al., 2017). The second decade of life is when this buildup is most frequently found, and it worsens with age (Monta Alvidrez et al., 2017). Vascular lesions grow and progress, leaving less space for blood to pass through the vessel to the dependent tissues and raising the possibility of obstruction (Kolderup et al., 2015). This can result in blood vessel ruptures and hemorrhages, which can develop into several complications include bleeding, thrombosis or stroke, and serious cardiac issues (Libby et al., 2019).

Atherosclerotic lesions can develop everywhere in the arteries, but they are more likely to do so around vessel curves and bifurcations disrupting the normal flow of blood and other vital bodily processes (Winkel et al., 2015). This may result in corresponding clinical symptoms of myocardial ischemia and infarction, stroke, and peripheral vascular disease (Monta Alvidrez et al., 2017).

2.7 PATHOPHYSIOLOGY OF ATHEROSCLEROSIS

Atherosclerosis develops by a complicated pathologic process frequently accompanied by high levels of LDL-c (Martin et al., 2014). The main players in the emergence of this illness are endothelial cells, leukocytes, and intimal smooth muscle cells (Jufri et al., 2015). The primary purpose of these structures is to act as a semi-permeable barrier to control the flow of fluid, nutrients, gases, and waste materials between the blood and tissues. Endothelial cells also control a variety of additional, less visible functions (Spacek et al., 2018). They offer a special surface that, in most cases, permits blood's cellular components to flow without sticking to the artery lining until some sort of damage disturbs the cells (Monta Alvidrez et al., 2017).

Endothelial cells release cytokines in response to disturbances, which start and sustain an inflammatory response (Libby, 2012). Monocytes and T-lymphocytes stick to the endothelium and then migrate beneath it by squeezing between the endothelial cells as the endothelial cells start to manufacture cell surface adhesion molecules (Seneviratne et al., 2013). Chemoattractant cytokines bind to the sites of damage and draw circulating monocytes and T-lymphocytes there (chemokines). Increased permeability to fluid, lipids, and leukocytes results from the endothelial cells changing shape and the tight connections between them becoming looser (Monta Alvidrez et al., 2017). The artery wall is where lipoprotein particles, particularly low-density lipoprotein (LDL), enter and proceed through the oxidation process. Nitric oxide, macrophages, and certain enzymes like lipoxygenase all contribute to the oxidation of LDL in the artery wall (Spacek et al., 2018). Once they have gone into the intima, monocytes undergo differentiation into macrophages, they start to ingest and accumulate more oxidized LDL and then die (Seneviratne et al., 2013). Macrophages are known as "foam cells" in this process (Monta Alvidrez et al., 2017). Damaged endothelium may develop dysfunctional characteristics that are proatherogenic and can accelerate atherosclerosis regardless of the etiology (Chen et al., 2015).

2.7.1 Special features of Atherosclerotic Plaques

Early in the process of atherogenesis, the scavenging macrophages remove the atherogenic lipoproteins from the intima, causing intracellular lipid buildup (formation of foam cells) (Spacek et al., 2018). When the foam cells decompose and leave the lipid behind as a soft, unstable, and mostly inactive necrotic (athermanous) core within the plaque, this generally helpful role may be outweighed and transformed into a harmful disease-causing route (Spacek et al., 2018). Without first passing through foam cells, atherogenic lipoproteins can potentially be maintained and accumulate within the intima (Jufri et al., 2015). The lipid-rich athermanous core lacks all supporting collagen and becomes avascular, hypocellular, and mushy like gruel (Spacek et al., 2018).

Foam cells break down and release lipid into the extracellular space in response to an intensified atherogenic stimulus, creating a lipid pool that is primarily acellular (Spacek et al., 2018). Activated smooth muscle cells move into the intimal layer and multiply at the same time, dramatically improving its capacity to make collagen and maintain the fibrous cap (Libby, 2012). The adventitial vasa vasorum may produce angiogenesis and micro vessel proliferation in response to the plaque's deeper layers, becoming hypoxic as it thickens (Spacek et al., 2018). However, due to their immaturity, fragility, and susceptibility to bleeding, these neovessels may facilitate the conversion from a stable to an unstable lesion by producing fast changes in plaque size and composition (Spacek et al., 2018). This harmful plaque destabilization process is also aided by matrix metalloproteinase activation, inflammation- induced necrosis of the surrounding cells, and bleeding of erythrocytes with cholesterol-rich membranes, which is made worse in hypercholesterolemia patients (Khoury et al., 2021; Spacek et al., 2018).

The development of plaques is now known to be a sequence of instances of clinical injury and healing rather than a gradual process (Khoury et al., 2021). In the case of an acute incident, exposure of highly thrombogenic fatty core components triggers a thrombogenic cascade, which results in the development of an acute occlusive thrombus.

The process of occlusion may be countered by the fibrinolytic system if erosion occurs in place of plaque rupture (Chen et al., 2015). The outcome might either be thrombus advancement, which carries the danger of occlusion and distal embolization, or thrombus retreat and healing, which may once more be demonstrated by plaque lamination (Spacek et al., 2018).

2.8 PREVALENCE OF ATHEROSCLEROSIS

People in industrialized countries are more likely to have atherosclerosis than those in developing countries, and it is becoming a bigger problem there (Herrington et al., 2016). According to data released by the American Heart Association, the leading cause of death worldwide in 2013 was cardiovascular disease, which claimed more than 17 million lives (Mozaffarian et al., 2016). A total of 800,937 fatalities from cardiovascular disease, or around 31% of all deaths in 2013 occurred in the United States (Mozaffarian et al., 2016).

Atherosclerosis is the primary factor in the majority of these fatalities. However, age related ones have decreased for a number of reasons, including better medical care and risk factor reduction (Kolderup et al., 2015). Unfortunately, during that time, there has been an increase in the long-term consequences of atherosclerotic illnesses. In actuality, heart failure-related fatalities rose by almost 100%. (Herrington et al., 2016). This suggests that many people who might have otherwise died from atherosclerosis now live but are disabled by the disease's long-term effects (Mozaffarian et al., 2016). Unfortunately, the bad news does not stop there. Mortalities associated with atherogenic risk factors are increasing in emerging nations because of the rise in disease risk factors linked to a Western lifestyle (Herrington et al., 2016).

2.9 ATHEROGENIC RISK FACTORS

Atherosclerosis is a complicated, multifaceted disease caused by both genetic and environmental causes (Barquera et al., 2015). Ageing, hypertension, obesity, high cholesterol, diabetes mellitus, and smoking are all generally acknowledged risk factors significant for the development of atherosclerosis (Huang et al., 2014; Yang et al, 2014). Atherosclerosis is also more likely to affect men than women, however this disparity may be decreasing with the increase some modifiable risk factors in women (Morales-Villegas, 2014). Strong risk factors for atherosclerosis include abnormal lipid levels (Barquera et al., 2015).

Exercise and dietary factors are linked to the appearance of atherosclerotic lesions either directly through altering established risk factors or indirectly through yet-unidentified mechanisms (Torres et al., 2015; Babu et al., 2014). The risk of atherosclerotic problems can be effectively reduced by therapeutic measures to treat various risk factors, including hypertension, diabetes, smoking, and abnormal lipid levels although several of them have genetic components (Fitch and Keim, 2012). Other risk variables have been suggested, although their magnitude or importance is unclear (Rogers et al., 2016). Another important risk factor for atherosclerotic disease is a history of early atherosclerosis in the family (Torres et al., 2015). Dyslipidemia has been identified as one of the key risk factors for atherosclerosis patients, and it may play a variety of functions before other risk factors manifest (Niroumand et al., 2015). Due to their mutually reinforcing effects, most persons with dyslipidemia also have concomitant hypertension, which frequently coexists with diabetes and obesity (Kolderup & Svihus, 2015).

2.9.1 Overview of Obesity

Obesity can be defined as an accumulation of fat that negatively affects one's health (WHO, 2020). An individual's weight can be measured using the Body Mass Index (BMI), which is done by dividing weight in kilograms by the square of height in meters (kg/m 2). Being overweight and obese is classified as having a BMI of ≥ 25 kg/m2 and BMI ≥ 30 kg/m2 respectively (WHO, 2020).

2.9.2 Incidence of Obesity

According to research, obesity is a global health issue and is known as a public health epidemic (Piché et al. 2018). Around 500 million persons globally were anticipated to be obese in 2011 (Seidell & Halberstadt, 2015). 13% of people worldwide were obese in 2014, according to the WHO. According to data from the WHO, 11% of men and 15% of women over the age of 18 were obese in 2016 (WHO, 2020). United States of America (USA) recorded about one-third of their population falling within the overweight and obese categories in 2013 and 2014 (Flegal et al., 2016). Obesity has been the cause of death compared to underweight globally due to the increased number of obese persons to those who are underweight worldwide, excluding some areas of sub-Saharan Africa and Asia (WHO, 2021).

2.9.3 Overview of blood glucose levels

The amount of glucose present in the blood at any particular time is known as the blood glucose level (Beck et al., 2018). Blood sugar is actively regulated by the body as part of metabolic homeostasis (Nadkarni et al., 2014). In the cells of the skeletal muscle and liver, glucose is stored as glycogen and insulin carries glucose in the blood to body cells to be used up as energy, thus, individuals who have low insulin levels are likely to have high blood glucose levels (Röder et al., 2016). Blood glucose is stabilized in fasting people at the expense glycogen reserves (Adeva-Andany et al., 2016a). Because natural sweeteners are high in glucose, they tend to quickly raise blood sugar levels because of their high glycemic index (Abdulrhman et al., 2013). Blood glucose levels that are elevated above normal ranges are known as hyperglycemia, and chronically low blood glucose levels are known as hypoglycemia (Luitse et al., 2012). The most prevalent disorder linked to improperly controlled blood glucose levels, persistent hyperglycemia, is a feature of diabetes mellitus (Luitse et al., 2012).

2.9.4 Categorization of blood glucose test

Long ago, blood tests to measure blood glucose were developed, and hyperglycemia was subsequently recommended as the only criterion for a diabetes diagnosis (Sacks, 2011). Initial diagnostic criteria focused on the response to oral glucose tolerance, while subsequent measurements of blood glucose in a fasted person were also adequate (Sacks, 2011). The most well-known glucose-based diagnostic criteria are the oral glucose tolerance test (200 mg/dL) and fasting blood glucose levels (126 mg) (ADA, 2014) A patient with typical diabetes symptoms is considered to have diabetes if their single spontaneous plasma glucose reading is less than 200 mg/dL. (ADA, 2014).

The glycated hemoglobin test (HbA1c) has recently gained acceptance and has been identified as the most suitable diagnostic tool for diabetes (Sherwani et al., 2016). Individuals with HbA1c levels within a range of 5.7% to 6.4% are at a high risk of developing overt diabetes (ADA, 2019). HbA1c also has the advantages of not requiring patients to fast (Sherwani et al., 2016).

Fasting blood glucose test is done by drawing blood from a subject following an overnight fast to perform a fasting blood glucose test (ADA, 2014). It may be a sign of diabetes if your fasting blood sugar level is higher than the upper reference range (6.0 mmol/l). A fasting blood sugar test can be performed when the level falls between 6.0 and 7.0 mmol/l after an overnight fast. Although diabetes is present when readings are higher than 7.0 mmol/l (Bartoli et al., 2011). A sugary beverage is then consumed, and for the next two hours, blood glucose levels are checked occasionally (Bartoli et al., 2011). To identify the beginning or progression of diabetes, these tests may be used singly or in combination.

2.9.4 Overview of lipid profile

An important component of cells and a source of energy are lipid (Walther & Farese Jr, 2012). They are fatlike substances that could come from dietary sources or bodily synthesis (Akoh, 2017). Adipose, liver, muscles, and the liver all store extra glucose as glycogen or fat (Adeva-Andany et al., 2016a).

A lipid profile test can quantify specific lipid concentrations in the blood (Rodwell et al., 2015). Two important lipids, triglycerides and cholesterol, carry lipoproteins in the blood with different molecules made up of proteins, phospholipids, triglycerides, and cholesterol (Adeva-Andany et al., 2016a). This test is used to identify dyslipidemia, a term that refers to a variety of problems involving cholesterol and triglycerides, which predisposes individuals to having atherosclerotic disease (Teramoto et al., 2013).

2.9.5 Categorization of lipid profile

Low-density lipoproteins cholesterol (LDL-C), high-density lipoproteins cholesterol (HDL-C), and very low-density lipoproteins cholesterol (VLDL-C) are the three categories used to classify the lipid profile's particle types (Nordestgaard, 2017). Traditional lipid profile testing includes total cholesterol measurements as well as assays for cholesterol in all lipoprotein particles (Nordestgaard et al., 2016). HDL-C is the term used to describe the cholesterol found in HDL particles (Nordestgaard et al., 2016). LDL-C, is the name given to the cholesterol found in LDL particles (Martin et al., 2013). Triglycerides also count every triglyceride included in lipoprotein particles (Arsenault et al., 2011). Data on triglycerides, HDL cholesterol, and total cholesterol are commonly used to estimate the level of LDL-C (Martin et al., 2013).

2.9.6 Categories of lipid ratio formulas

Major indicators that are frequently used to predict atherosclerosis include a lipid profile that evaluates total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides (Nikkos & Rehman, 2021). However, several studies have shown that lipid ratios like the atherogenic index of plasma (AIP), Castelli Risk Index I and II (CRI) and atherogenic coefficient (AC) are the diagnostic alternatives that have been found to forecast atherogenic risks when the conventional lipid profile measures remain ostensibly normal (Olamoyegun et al., 2016). Various studies have identified AIP formular as the most accurate in forecasting atherosclerosis compared to the others (Cure et al, 2018). It makes use of serum triglyceride, and serum HDLc in calculation for atherogenic risk (Sujatha & Kavitha, 2017).

Research has shown that the AIP is able to reflect the exact association between protective and atherogenic lipoprotein according to the size of atherogenic lipoprotein particle (Niroumand et al., 2015). AIP is calculated as, log (TG/HDL-C) (Mudhaffar, 2013; Kanthe et al., 2012). An AIP value < 0.11 is related with a low risk of cardiovascular disease, while levels between 0.11 and 0.21 and more than 0.21 are, respectively, linked to intermediate and elevated risks (Niroumand et al., 2015).

CRI-I and CRI-II are also useful in predicting atherogenic risk. They are calculated as: CRI-I = Serum total cholesterol/Serum HDLc, CR-I II = Serum low-density lipoprotein (LDL)-cholesterol/Serum HDLc (Sujatha & Kavitha, 2017). AC is also calculated as: Serum total cholesterol−Serum HDLc/HDLc (Sujatha & Kavitha, 2017).

Understanding these cholesterol sub fraction levels that may offer new insights to data, is more important than simply knowing the level of plasma cholesterol, which suggests that the higher the LDL cholesterol level, the greater the risk of atherosclerotic heart disease, while the higher the HDL cholesterol level, the lower the risk of coronary heart disease (Ahmed et al., 2015).

2.9.7 Overview of dyslipidemia

The term dyslipidemia describes variety of problems involving cholesterol and triglycerides (Klop et al., 2013). Although a wide range of disorders are covered by the term, hyperlipidemia is the most prevalent form (Klop et al., 2013). Hyperlipidemia refers to excessive triglyceride, LDL, and HDL values (Schofield et al., 2016). The easiest technique to diagnose hyperlipidemia is with a lipid profile because it usually has no symptoms (Schofield et al., 2016). According to Björnson et al., (2017), abnormal lipid levels are the clinical manifestations of dyslipidemia and have been linked to a number of atherogenic risk factors and as a result, the lipid ratios are used in evaluating how high or low an individual is at risk (Niroumand et al., 2015; Mudhaffar, 2013).

2.9.8 Prevalence of dyslipidemia

In America, more than 12% of persons aged 20 and older had greater total cholesterol and more than 18% had HDL-C values below 40 mg/dL (Carroll et al., 2017). Chinese men between the ages of 30 and 39 had the highest frequency of dyslipidemia (48.2%) and that of women rose with age, reaching its maximum level in China after 60 years of age (46.3%) (Qi et al., 2015). A 4.6% drop in the nation's total cholesterol level contributed to a 30% drop in the death rate from heart disease in Ireland (Nichols et al., 2013).

According to O'Flaherty et al. (2013), Finland's population blood cholesterol levels have decreased, which has led to a fall in the mortality rate from ischemic heart disease. Utilizing community-level assessments, it has been discovered that dyslipidemia is prevalent at rates ranging from 14% to 69% in South Africa (Reiger et al., 2017). Among Nigerians who appeared to be in good health, the frequency of dyslipidemia ranged from 60% to 89% (Oguejiofor et al., 2012). In research conducted in Senegal by Doupa et al., (2014), hypertriglyceridemia, hypercholesterolemia, and hyperlipidemia were all prevalent to varying degrees (56 percent, 7.1 percent, and 1.9%, respectively). According to a study done in the Ga-East municipality in Ghana, schoolchildren who are overweight and obese had a moderate level of hypercholesterolemia (2.8%) (Steiner-Asiedu et al., 2012).

2.10 EFFECTS OF NATURAL SWEETENERS ON ATHEROGENIC RISK INDEX

2.10.1 Natural sweeteners and obesity

Since ancient times, people have used the popular sweeteners table sugar and honey in their diets (Priya et al., 2011). These sweeteners improve the palatability and flavor of food (Amarra et al., 2016). This leads to a noticeably higher intake of such products at a time as compared to foods that aren't sweetened (Amarra et al., 2016). It is also known that following previous exposure, its effects on neurotransmitters may result in sugar addiction (Berridge & Kringelbach, 2015). This may lead to a complete reliance on or obsessive use of sugar (Amarra et al., 2016). Additionally, advertisements for items containing sugar have included associations between drinking sugar-sweetened beverages and happiness (Nestle, 2013).

This might have increased consumer interest in and consumption of these goods, increasing calorie intake. The United States' overall energy consumption is generated by added sugars to a degree of about 15%. (Marriott et al., 2010).

Consuming additional sugar results in an increase in adiposity because excess glucose is stored as fat or glycogen in adipose tissue, the liver, and muscles (Adeva-Andany et al., 2016b). WHO undertook a comprehensive review in 2010 to address a number of queries on how sugar affects increased adiposity (Te Morenga et al., 2013). The review sought to ascertain whether dietary sugar consumption had an impact on how much body fat was assessed, either positively or negatively (Te Morenga et al., 2013). The study also looked at whether the evidence was consistent with the recommendations to keep daily intake of added sugars to less than 10% of total energy (USDA, 2017). The findings revealed that among people who were not pregnant or nursing, the recommendation to cut back on added sugars was linked to an average weight loss of 0.80 kg (Te Morenga et al., 2013). While the recommendation to increase consumption was associated with a 0.75 kg rise in weight (Te Morenga et al., 2013). Increased use of sugar-sweetened beverages does not result in a decrease in calories from other foods, which causes children's overall energy intake to rise and their weight to increase (de Ruyter et al., 2012).

In experimental research using Sprague Dawley rat models, a 20% honey diet led to a significant rise in overall body weight (Erejuwa, 2017). 13 weeks were spent comparing this to the Sprague Dawley rat control group (Chepulis, 2007). But after six weeks of feeding animals with 10% of the honey- sweetened diet, their body weight dropped significantly (Chepulis and Starkey, 2008). The information available regarding how honey affects weight, particularly in experimental investigations is still unclear (Atangwho et al., 2020).

In a study by Becker et al. (2020), it was discovered that mice on a high-fat diet had no significant weight loss when stevia was consumed. After ingesting foods and beverages for one week that contained stevia as a sweetener, students in a different study by Duram Aguero et al. (2015) reported normal weight and

nutritional status. There are various clinical trials that have not identified a substantial effect on BMI, despite the fact that few human and animal research have demonstrated that Stevia has positive effects on food consumption and weight loss (Masoumi et al., 2020). No long-term studies examining the efficiency of stevia in managing weight have been published (Ashwell, 2015). The prevalence of obesity is quickly increasing around the world as a result of an increase in calorie intake. The availability of foods that are high in energy and increased purchasing power, as well as decreased energy, are additional potential causes.

2.10.2 Natural sweeteners and glucose levels

Simple carbohydrate foods, including natural sweeteners, breakdown quickly and are readily absorbed into the bloodstream. After consuming honey or table sugar, blood levels of glucose and fructose rise noticeably (Abdulrhman et al., 2013). The impact of natural sweetener ingestion on biochemical variables like blood glucose has been studied in a number of animal research (Atangwho et al., 2017; Adesoji & Oluwakemi, 2008). A study on Sprague-Dawley rats who were fed sucrose revealed a significant increase in their overall weight growth and body fat percentages (Chepulis, 2007). Those who consumed honey or sugar-free diets experienced similar results (Chepulis, 2007). In rats given honey, HbA1c levels were markedly lowered while HDL-C levels increased (Chepulis, 2007). Although there were no additional differences in the lipid profiles between this and rats given sucrose or a sugar- free diet (Chepulis, 2007). However, a 33-day research in healthy rats fed 20% honey revealed that the weight of the epididymal fat was 20.1% smaller (P .05) in the rats fed honey (Nemoseck et al., 2011). Triglyceride and leptin readings fell by 29.6% and 21.6%, respectively (P .05). (Nemoseck et al., 2011). Non-HDL- cholesterol rose by 16.8% in rats fed honey (P .05). No significant changes in glucose, TC, insulin, or HDL cholesterol were observed (Nemoseck et al., 2011). An increased HbA1c level was observed in a similar study using male Wistar rats treated for six months with SSB compared to the control (Driescher et al., 2019). This implies that longer feeding intervals are required to yield meaningful outcomes. There were no significant variations in fasting blood glucose levels in a related trial (Erejuwa et al., 2010, Omotayo et al., 2010).

Similar to this, animals given thoracic stingless bee honey as a supplement did not have higher FBG levels than those in the control group (Aziz et al., 2017).

2.10.3 Natural sweeteners and lipid profile

An increase in refined carbohydrate and added sugar consumption has a significant impact on triglycerides (Kell et al., 2014). Poor cholesterol levels are indicated by high triglyceride levels and low HDL-C values (Klop et al., 2013). Women who consume more added sugar appear to have higher LDL- C levels, according to studies (Yu et al., 2018). Consumption of sugar-sweetened beverages is strongly associated with human weight growth (Hu and Malik, 2010). Leptin and TG levels were 21.6% and 29.6% lower in an animal study (($p \leq .05$), respectively) (Nemoseck et al., 2011). Additionally, it was discovered that rats fed honey had non-HDL cholesterol levels that were 16.8% higher ($p \leq .05$) than rats fed sucrose (Nemoseck et al., 2011). A more comprehensive investigation and meta-analysis revealed a relationship between increased sugar intake and an improved blood lipid profile. This was similar to minimal sugar intake, which demonstrated a reduced level of the lipid profile (Te Morenga et al., 2014).

Honey is more beneficial in terms of overall health because it contains certain trace amounts of phenolic acids and flavonoids (Manyi-Loh et al., 2011b). At Iran's Isfahan University of Medical Sciences, researchers studied the usage of natural sweeteners among male students between the ages of 18 and 30 for six weeks (Rasad et al., 2018). It was discovered that consuming honey could reduce LDL-C, TG, and TC (Rasad et al., 2018). Additionally, young healthy subjects had higher HDL-C levels (Rasad et al., 2018). But sugar consumption increased TC, LDL, TG, and lowered HDL-C (Rasad et al., 2018). In a different trial, administering honey increased HDL-C considerably (p 0.05). (Erejuwa et al., 2016). However, there was a considerable reduction in glucose, TG, and very-LDL-C (p0.05) (Erejuwa et al., 2016; Mohammadimanesh et al., 2019). To corroborate these results, more clinical trials were recommended (Rasad et al., 2018).

In a study, participants who took three different isocaloric solutions (50 g of either sucrose, fructose, or glucose) found a significant rise in HDL-C and LDL-C (Jameel et al., 2014). In a different trial, there was no significantly changed in the TC and TG levels of the treatment groups fed with honey and sugar solution for a period of 14 days (Münstedt et al., 2009). Compared to the control group, significant decrease in LDL and TC levels were reported in the treatment group ($P<0.01$) (Alagwu et al., 2014). However, some research revealed that sucrose or fructose had no impact on LDL or HDL-C levels (Kelishadi et al., 2014; Lowndes et al., 2014). The research cited above focused primarily on fructose, which is just one of the ingredients in honey and table sugar. These lipid profile abnormalities are strongly linked to increasing dietary sugar consumption (Xi et al., 2015). In both industrialized and developing nations, dyslipidemia is a significant risk factor for cardiovascular disease (Hendrani et al., 2016)

Animal studies involving hypoglycemic activity of Stevia rebaudiana extract using rabbits weighing 1400–1500 g indicated a decreasing effect of stevia on triglyceride (Aghajanyan et al, 2017). Similarly, 1g/day stevia consumption for 60 days in diabetic patients led to reduced levels of cholesterol, triglyceride, and LDL cholesterol (Ritu & Nandini, 2016). Likewise, in a study conducted by Ahmed et al., (2018) revealed a significant decrease in TC, TG, LDL, VLDL levels in stevia fed hyperlipidemic rats for 8 weeks. This may imply that, stevia may be beneficial in managing hyperlipidemia and its consequences that may be linked to it (Ahmed et al., 2018). In contrast, a study by Masoumi et al., (2020) found no significant decrease in lipid profile in diabetic and healthy people upon stevia consumption. This shows an inconsistency in the findings regarding the effect of stevia on cholesterol level (Onakpoya et al, 2015).

2.10.4 Natural sweetener and cardiovascular disease (CVD)

Increased consumption of added sugars may raise the risk of CVD, according several research (Wang et al., 2014; Zhang et al., 2013; Chiavaroli et al., 2012). Cross-sectional investigations evaluating the consumption of sugar-sweetened beverages revealed increased caloric intake, weight gain, and poor nutrition (Narain et al., 2016).

Additionally, it has been hypothesized that consuming too much fructose contributes to hypertension, dyslipidemia, and obesity (Malik et al., 2010). Similarly, a study discovered a connection between a high sugar intake and a higher risk of death from heart disease (Yang et al., 2014). Likewise, a study using overweight and obese adults revealed that ingestion of sugars containing fructose causes dyslipidemia and can also result in diminished insulin sensitivity and more visceral obesity (Stanhope, 2016). Consuming sugary foods frequently can raise cholesterol levels in low-density lipoproteins, triglycerides, and fasting plasma glucose (Miller et al., 2011; Welsh et al., 2011). In summary, consumption of sugar appears to be linked to dyslipidemia, a known risk factor for cardiovascular disease, even though the processes are still unclear when compared to other sources of carbohydrates (Chiavaroli et al., 2012, Wang et al., 2014, Zhang et al., 2013).

Recent research suggests that stevia, together with its metabolites stevioside, rebaudioside A, and steviol, helps to maintain cardiovascular health and lowers blood pressure. In a study (Geeraert et al., 2010), participants were double LDL and leptin receptor knock-out (DKO) mice, which display many characteristics of the metabolic syndrome. The study looked at the effects of stevia on cardiovascular measures in these animals (Geeraert et al., 2010). After 12 weeks of daily administration of 10 mg/kg stevia or saline solution, researchers examined a number of markers, including adiponectin, IL-6, TNF and autoantibodies against MDA-modified low-density lipoprotein, a kind of oxidized low-density lipoprotein (ox-LDL) (Geeraert et al., 2010). The researchers observed that the stevia-treated group had significantly lower levels of total cholesterol and ox-LDL than the control group, which had values of 13.51 mmol/L and 9.20, respectively (Geeraert et al., 2010). However, there were no significant variations in weight or triglyceride levels between the two groups (Geeraert et al., 2010). Researchers stained the smooth muscle and plaque elements in the mice's extracted aorta tissue using immunohistochemistry to evaluate atherosclerosis (Geeraert et al., 2010). Stevioside treatment resulted in lessened infiltration of macrophages, lipids, and LDL in the DKO mice, which in turn resulted in lessened atherosclerosis (Geeraert et al., 2010).

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY DESIGN

This study used an experimental design.

3.2 STUDY SITE

The research was carried out at the department of Animal Experimentation (DAE), Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. Noguchi Memorial Institute for Medical Research was set up in 1979 as a semi-autonomous institute of the University. It is the leading biomedical research facility in Ghana. The institute has nine (9) departments; Animal Experimentation, Nutrition, Bacteriology, Parasitology, Virology, Clinical Pathology, Electron Microscopy, Epidemiology and Immunology.

3.3 STUDY POPULATION AND DESIGN

Seventy-eight (78) Sprague Dawley rats aged 10-12 weeks, weighing between 100 g – 200 g were obtained from the DAE at the NMIMR. The rats were weighed and randomized into thirteen (13) groups of six (6) animals in each cage. The animals were acclimatized in rat cages with soft wood shavings as bedding for 7 days. The experimental groups were set up as follows: Group 1- Control (C), Group 2 – White sugar low dose (WSLD), Group 3- white sugar medium dose (WSMD), Group 4 – white sugar high dose (WSHD), Group 5 – Brown sugar low dose (BSLD), Group 6 – Brown sugar medium dose (BSMD), Group 7 – Brown sugar high dose (BSHD), Group 8 – Honey low dose (HLD), Group 9 – Honey medium dose (HMD), Group 10 – Honey high dose (HHD), Group 11 – stevia low dose (SLD), Group 12 – Stevia medium dose (SMD), Group 13 – Stevia high dose (SHD).

NS were prepared weekly and administered once daily, usually in the morning (7-8am) from week 1 to week 17 after the body weight, length and FBS were measured. The body weight of rats was measured with a weighing scale and recorded weekly whereas the length and the FBS were measured bi-weekly with a measuring tape and glucometer respectively. Blood samples were collected at the week of the experiment for the analysis of FPG and lipid biomarkers (Figure 3.1.1).

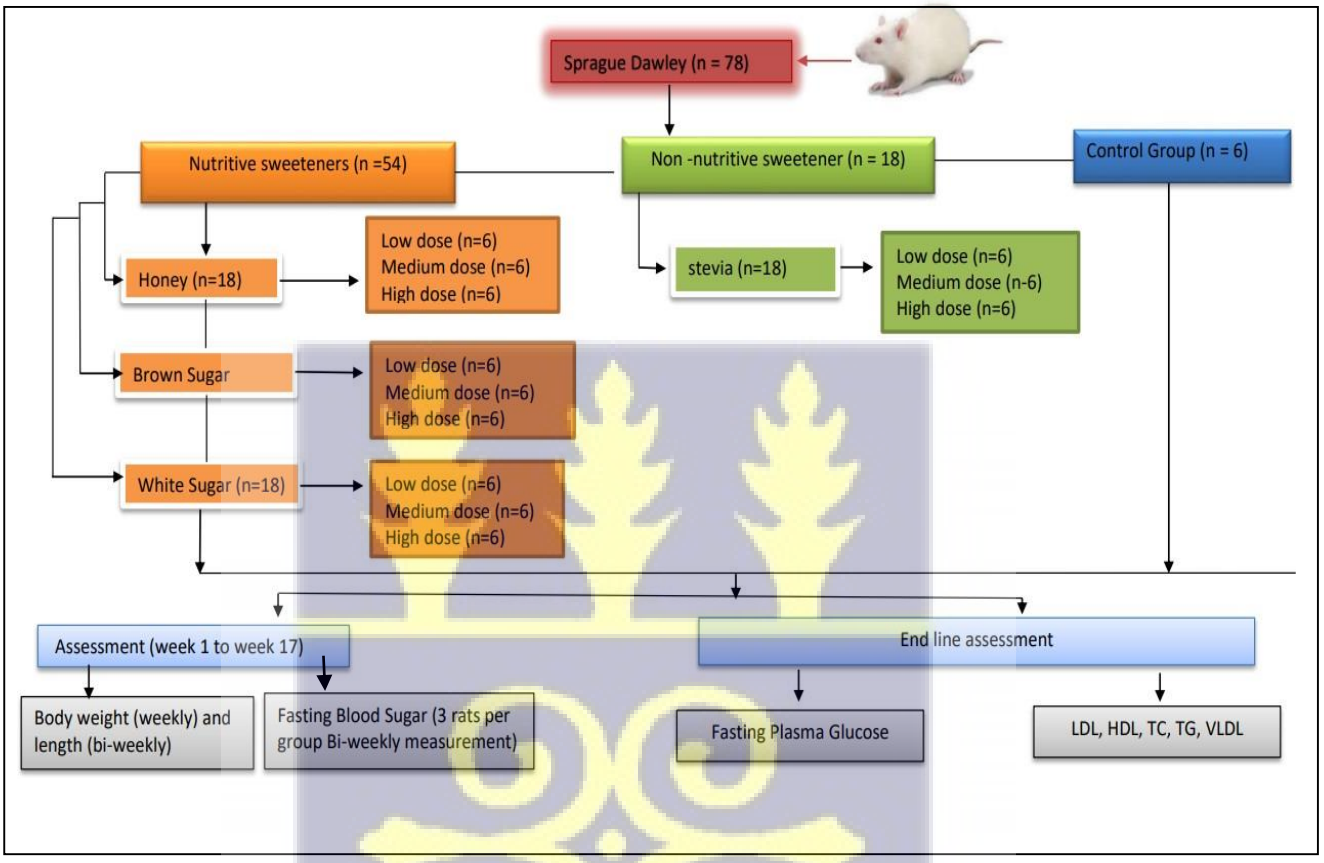

Figure 3.1 1: *Flow chart for study design. A total of 78 female SD rats were divided into 13 groups of 6 animals, including a control group. Bi-weekly fasting plasma glucose was assessed by tail clipping. After 17 weeks of administration, all 78 rats were euthanized and blood was collected by cardiac puncture.*

3.0 Housing and feeding of Experimental Animals

Rats were kept at a in their cages at the unit at a temperature of 24 º C ± 2º C and relative humidity (60-70%) with a 12-hour light/dark cycle ensured. The animals were fed on standard rat chow and distilled water ad libitum for a period of 1 week before the commencement of the experiment. Rat chow was formulated and supplied by Agricare limited. Agricare limited is a company located in Kumasi which supplies nutrient rich feeds for poultry, livestock and aquaculture on a commercial basis.

3.1 Determination of diet regimen of sweeteners

The percentage of sugar solutions for nutritive sweeteners was calculated according to the WHO recommendation of reducing the intake of free sugars to less than 10% of total energy intake and a further reduction of the intake of free sugars to below 5% of total energy intake (World Health Organisation, 2018). Thus, for an average adult (70kg) with a calorie intake of 2000 kcal, 5 per cent of total energy intake is equivalent to 100 kcal. For the purpose of this study, the dosages were categorized into low doses (5% of total energy intake), moderate doses (10 % of total energy intake) and high doses (15 % of total energy intake). Thus, the weight of each nutritive sweetener to provide 100 kcal was estimated from their proximate analysis (Table 3.1).

Table 3.1: Weight of Sugar to provide low, moderate and high sugar intake (human and animal dosage)

	Human dose			**Animal dose**		
Natural Sweeteners	Low dose (g)	Medium dose (g)	High dose (g)	Low dose (g)	Medium dose (g)	High dose (g)
White sugar	12.5	25	37.5	0.035	0.07	0.1
Brown Sugar	12.8	25.6	38.4	0.036	0.075	0.11
Honey	16	32	48	0.047	0.094	0.14
Stevia	3	6	9	0.004	0.008	0.013

Using white sugar as an example,

White sugar: 25 g of white sugar was equivalent to 100 kcal

For a 70 kg adult requiring 2000 kcal, medium dose = 25 g/70 kg = 0.36 g/kg body weight Thus, for a 200 g (0.2 kg) rat, the required dosage = 0.2 kg x 0.36 g/ kg = 0.072

0.07 g of the white sugar was dissolved in 2 ml of distilled water.

A stock solution was prepared at 0.035 g/ml for low-dose white sugar.

Moderate (10 % of total energy intake) = 0.07 g/ml

High dose (15 % of total energy intake) = 0.1 g/ml

3.2 ANTHROPOMETRIC MEASUREMENT

Body weight: Rats were weighed weekly with a rat scale (TGC.INC, Japan) and weekly differences in the body weight were recorded. Height was measured using a Pesfer® measuring tape. Body length was measured as the distance from the nose tip to the anus (BLNA) (Aguh, Yahaya, Saidu, Ayeku, & Agba, 2013). BMI was calculated as body weight (g)/ length^2 (cm)^2 (Samat et al., 2017).

3.0 BLOOD SAMPLE COLLECTION

Animals were anesthetized with ketamine (75 mg/kg) and xylazine (5 mg/kg) intraperitoneally at the conclusion of the research, and blood was collected from the heart (cardiac puncture) (Parasuraman, Raveendran, & Kesavan, 2010). This method made it possible to obtain more blood from each animal. The animals were given general anesthesia before being laid on their backs, facing away, and having the left index finger positioned at the lowest rib level without applying any pressure.

When the heart was located, a 5 ml syringe with G25 needles was inserted at a 45° angle between the two ribs. 4 ml of blood was taken from each rat and aliquots were placed into lithium heparin and fluoride anticoagulant tubes (3ml and 1ml, respectively). Samples were centrifuged at 4000 rpm for 5 minutes and plasma samples were transferred into cryotubes for storage at -20^{o}C until use. Organs were harvested. One portion was placed in buffered formalin and another, snap frozen in liquid nitrogen for storage at -80^{o}C. Isoflurane was used to euthanize the animals, and then they were incinerated. Organ studies are outside the scope of this work.

3.1 BIOCHEMICAL ANALYSIS

3.1.1 Measurement of Fasting Plasma Glucose

The fasting plasma glucose of the rats was measured using Accu-Chek Active Blood Glucose Glucometer bi-weekly by tail clipping (Cao et al., 2016). A 25 G needle was loosened from its cover carefully to close to the researcher. The needle was left partially in the cover to keep the tip sterile. The rat was gently removed from the cage by grasping it at the tail's base and transferring it to the working surface.

While holding the tail with the non-dominant hand, the distal third of the tail was wiped with a ball of cotton wool moistened with 70% alcohol. In the non-dominant hand, the tail was gently stroked down from the base of the tail to about 1cm from the tip of the tail. Gentle pressure was put on the tail tip by the non-dominant hand to occlude blood flow and also to prevent the rat from moving away. The tip of the tail was placed on a working surface and with the dominant hand, a needle prick was performed using the 25 G needle about 1-2 mm from the end. The needle was inserted perpendicular to the tail surface. The blood sample was collected from the drop of blood that formed at the skin surface onto the glucometer test strip and the reading was done.

The pressure in the non-dominant hand was released and gentle pressure was applied with a small ball of cotton wool for approximately 10 seconds to stop the flow of blood. The tail tip was monitored for re-bleeding and the rat was returned to its cage. The needle was discarded into the appropriate sharps container (McErlane, 2014). Three (3) rats from each group were used for the bi-weekly fasting plasma glucose test.

3.1.2 Lipid Profile

Plasma lipid profile consisting of total cholesterol (TG), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were analysed with an auto-analyser machine and test kits for animals according to the manufacturer's instructions (Mindray Bio-Medical Electronics Bs-200e, Shenzhen, China).

3.2 ETHICAL CONSIDERATIONS

Ethical Clearance was be obtained from the University of Ghana Institutional Animal Care and Use Committee (UG IACUC) with certificate number UG-IACUC 001/21-22.

3.3 QUALITY CONTROL

Laboratory work was conducted and NMIMR provided the optimum environment for the study with supervision from experienced laboratory personnel.

3.4 DATA MANAGEMENT AND ANALYSIS

Each rat was given identification codes for easy identification. Data was entered and stored on a password protected personal computer. The information gathered was used for this research solely. Data was entered into Microsoft excel 2010 and exported after cleaning into STATA MP Version 14 for statistical analysis. Test for normality of data was done using Kolmogorov-Smirnov and Shapiro Wilk statistic. Basic descriptive statistics was run for all continuous variables (FPG, lipid profile and anthropometrics).

Cumulative weight gain was calculated by deducting the basal weight from the weight measured every week and expressed in grams. The total weight gained was calculated by the difference between the basal weight and the final weight in the 17th week.

A one-way ANOVA was conducted to compare means across two groups. Duncan's numerous range tests for post hoc examination was conducted after the ANOVA to measure specific differences between pairs of means. A linear mixed model was used to evaluate the effect of the various sweeteners over time on body weight and BMI. Statistical Significance will be set at $p \leq 0.05$. Correlation was done to measure the linear relationship between variables.

CHAPTER FOUR

4.0 RESULTS

4.1 EFFECT OF NATURAL SWEETENERS ON ANTHROPOMETRIC MEASUREMENTS

The following results show the effect of intake of four natural sweeteners white sugar, brown sugar, honey and stevia on the anthropometric measurements of the rats. These included percentage body weight and BMI.

4.1.1 Effect of natural sweeteners on percentage body weight

Figure 4.1 outlines the mean percentage body weight of rats. There was a general increase in body weight of rats across all treatment groups compared to the control. Taking into consideration the single sweeteners at a time, the means of high doses of white sugar (66.11±7.59), honey (70.12±4.02) and stevia (72.66±10.54) respectively were significantly higher($P<0.05$) compared to the control.

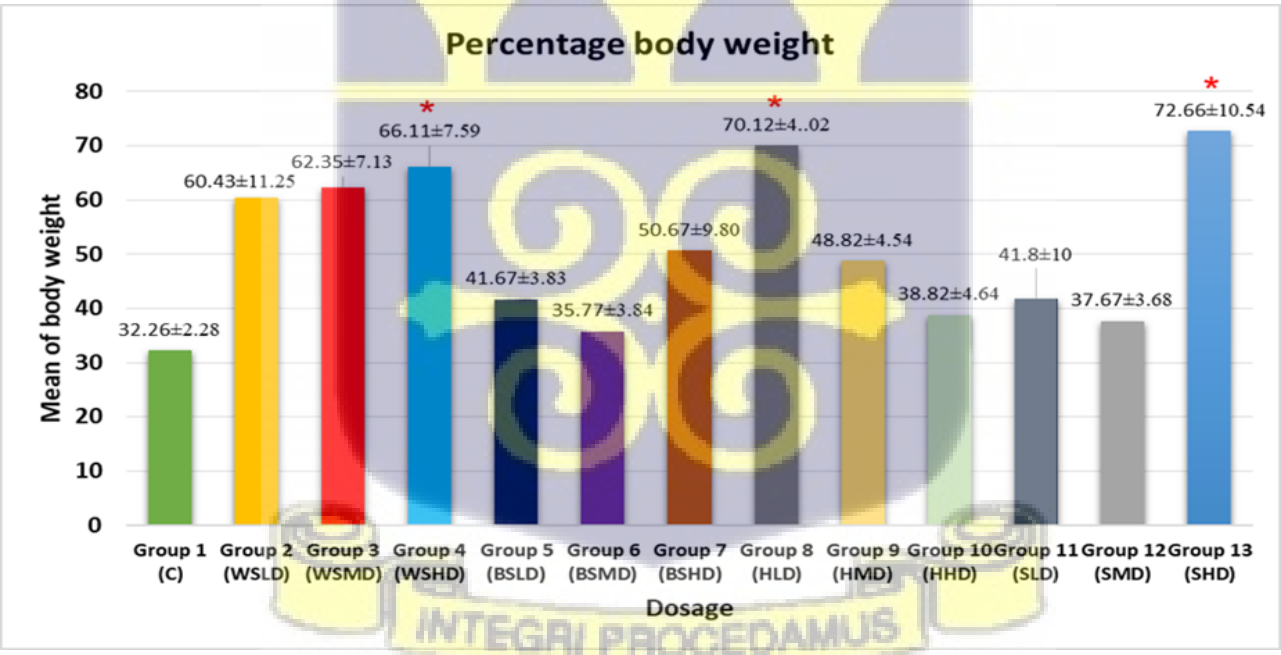

Figure 4.1: *The effects of natural sweeteners at different doses on the body weight of experimental animals. C= Control, WSLD: white sugar low dose, WSMD: white sugar medium dose, WSHD: white sugar high dose, BSLD: brown sugar low dose, BSMD: brown sugar medium dose, BSHD: brown sugar high dose, HLD: honey low dose, HMD: honey medium dose, HHD: honey high dose, SLD: stevia low dose, SMD: stevia medium dose, SHD: stevia high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

4.1.2 Effect of natural sweeteners on BMI of rats

Table 4.1 outlines the effect of white sugar, brown sugar, honey and stevia at different doses on the BMI of experimental animals. A decrease was observed in the treatment groups except for Group 7 (BSLD) which had a higher mean compared to the control. However, these differences observed were not statistically significant.

Table 4.1: Effect of natural sweeteners on BMI of rats

BMI (g/cm^2)	Mean ± SEM	ST	ST Pvalue	SD	SD P-value
Group 1	27.74±0.17			LD	0.40
Group 2	27.23±0.30	WS	0.31		
Group 3	27.5±0.20				
Group 4	27.7±0.22				
Group 5	27.69±0.34	BS	0.40	MD	0.47
Group 6	27.71±0.27				
Group 7	28.02±0.19				
Group 8	27.74±0.18	H	0.09		0.30
Group 9	27.45±0.12				
Group 10	27.17±0.20			HD	
Group 11	27.32±0.16	S	0.48		
Group 12	27.24±0.25				
Group 13	27.74±0.45				

One-way ANOVA, Duncan's numerous range tests for post hoc. *BMI: body mass index, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant at ≤ 0.05.*

4.2 EFFECT OF NATURAL SWEETENERS ON FASTING PLASMA GLUCOSE

Table 4.2 shows the effect of four natural sweeteners (white sugar, brown sugar, honey and stevia) at different doses on glucose levels of rats. There was no significant difference in the means white sugar, brown sugar and honey. However, ANOVA showed a significant difference among groups of stevia (P=0.05) with post hoc analysis showing a significant increase in medium dose of stevia (G1-G12- P=0.03) compared to the control.

Table 4.2: Effect of natural sweeteners on Fasting Plasma Glucose levels of rats

GLUC (mmol/dl)	Mean ± SEM	ST	ST P-value	SD	SD P-value
Group 1	2.20±0.53	WS	0.05	LD	0.30
Group 2	1.93±0.69				
Group 3	4.32±0.51				
Group 4	3.48±0.85				
Group 5	2.86±0.52	BS	0.37	MD	0.01
Group 6	2.73±0.31				G1-G12=0.03
Group 7	1.57±0.48				
Group 8	3.17±0.40	H	0.67		
Group 9	2.87±0.24				
Group 10	2.6±0.67			HD	0.07
Group 11	3.51±0.85	S	0.05		
Group 12	4.67±0.42*		G1-G12=0.05		
Group 13	4.51±0.88				

One-way ANOVA, Duncan's numerous range tests for post hoc. *GLUC: Glucose, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

4.3 EFFECT OF NATURAL SWEETENERS ON LIPID PROFILE

The following results shows the effect of natural sweeteners on atherogenic risk index by measuring concentrations of lipid profile of experimental animals. These included Total Cholesterol (TC), High Density Lipoprotein cholesterol (HDL-C), Triglyceride (TG), Low Density Lipoprotein cholesterol (LDL-C), Very Low-Density Lipoprotein cholesterol (VLDL-C).

4.3.1 Effect of natural sweeteners on TC levels of rats

Table 4.3 results shows the effect of white sugar, brown sugar, honey and stevia on the total cholesterol levels of the experimental rats. A general decrease was observed in the Total Cholesterol levels of rats across all treatment groups. However, the decrease observed had no statistical significance.

Table 4.3: Effect of natural sweeteners on TC levels of rats

TC(mmol/dl)	Mean □ SEM	ST	ST P-value	SD	SD P-value
Group 1	2.27±0.17	WS	0.62	LD	0.79
Group 2	2.24±0.16				
Group 3	2.35±0.14				
Group 4	2.07±0.12				
Group 5	2.17±0.15	BS	0.14	MD	0.15
Group 6	2.37±0.08				
Group 7	1.94±0.06				
Group 8	2.25±0.038	H	0.18		
Group 9	1.93±0.13				
Group 10	2.052±0.10			HD	0.43
Group 11	2.04±0.16	S	0.73		
Group 12	2.15±0.10				
Group 13	2.16±0.13				

One-way ANOVA, Duncan's numerous range tests for post hoc. TC: Glucose, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant at ≤0.05.*

4.3.2 Effect of natural sweeteners on TG levels of rats.

Figure 4.2 results show the effect of white sugar, brown sugar, honey and stevia on triglyceride levels (TG) of experimental rats. From the TG, the ANOVA showed a significant difference in means across the various groups ($P<0.01$) and post hoc showed significances between the following groups: G1-G12, G1-G13, G5-G9, G5-G12, G5-G13, G8-G12, and G8-G13 (all $P<0.05$).

Considering the types of sweeteners, the groups of white sugar were significant when ANOVA was done and post hoc analysis showed the differences between G1-G2 ($P=0.05$) and G1-G3 ($P=0.06$). There was no significant difference in the means of groups of brown sugar. In groups of honey, ANOVA was significant ($P=0.00$) and post hoc analysis showed a significant difference between G1-G9 ($P=0.01$) with mean of G9 higher than the Control; and G8-G9 ($P=0.02$) and G9 higher than G8. Similarly, ANOVA showed a significant in groups of stevia with post hoc showing a significant difference between G1-G12 ($P=0.01$) with G12 being higher than the Control and G1-G13 ($P=0.01$); similarly, G13 higher than the Control.

Pulling all the low doses together and performing ANOVA showed a significant difference ($P=0.01$) and post hoc analysis showed a significant difference between G1-G11 (SLD) ($P=0.02$) with levels of G11 being higher. All medium doses had a significant difference ($P=0.01$) with post hoc analysis showing a significant difference between G1-G9 ($P=0.02$) and G1-G12 ($P=0.00$). Lastly, there was a significant difference between the means of all high doses ($P=0.001$) and post hoc showed a significant difference between G1-G13 ($P=0.01$) and G7-G13 ($P=0.03$).

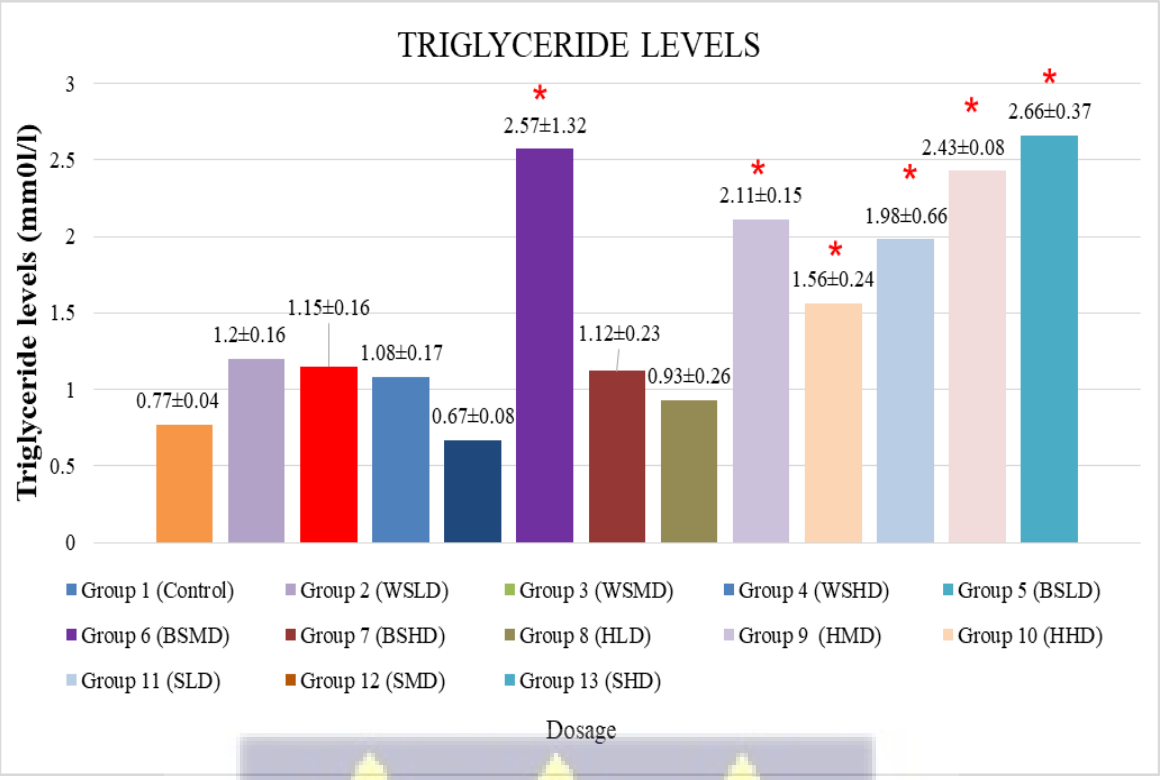

Figure 4.2: *The effects of natural sweeteners at different doses on the triglyceride levels of experimental animals. WSLD: white sugar low dose, WSMD: white sugar medium dose, WSHD: white sugar high dose, BSLD: brown sugar low dose, BSMD: brown sugar medium dose, BSHD: brown sugar high dose, HLD: honey low dose, HMD: honey medium dose, HHD: honey high dose, SLD: stevia low dose, SMD: stevia medium dose, SHD: stevia high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤0.05.*

4.3.2 Effect of Natural Sweeteners on High Density Lipoprotein Cholesterol levels of rats

Table 4.4 shows the effect white sugar, brown sugar, honey and stevia on High Density Lipoprotein Cholesterol (HDL-C) levels of experimental rats. A general increase was observed in the HDL-c levels of rats across all treatment groups compared to the control. However, the decrease observed had no statistical significance.

Table 4.4: Effect of Natural Sweeteners on HDL-c levels of rats

HDL-c (mmol/dl)	Mean ± SEM	ST	ST P-value	SD	SD P-value
Group 1	1.29±0.16	WS	0.33	LD	0.26
Group 2	1.32±0.10				
Group 3	1.34±0.12				
Group 4	1.01±0.15				
Group 5	1.45±0.11	BS	0.51	MD	0.41
Group 6	1.53±0.07				
Group 7	1.42±0.06				
Group 8	1.72±0.18	H	0.17		
Group 9	1.41±0.05				
Group 10	1.43±0.07			HD	0.04
Group 11	1.425±0.1101	S	0.38		G4-G13=0.03
Group 12	1.53±0.09				
Group 13	1.55±0.08				

One-way ANOVA, Duncan's numerous range tests for post hoc. *HDL-c: High Density Lipoprotein Cholesterol, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

4.0.1 Effect of Natural Sweeteners on LDL-C levels of rats

Table 4.5 shows the effect white sugar, brown sugar, honey and stevia on LDL-C levels of the experimental rats. ANOVA showed a significant difference in means across the various groups (0.00) and post hoc showed significance between the following groups: G3-G7, G3-G9 and G3-G11 (all P<0.05). The following types of sweeteners; BS, H and S groups had significant differences (p =0.02, p=0.01 and p =0.01,

respectively), and post hoc showed significant differences between G1-G7 (P=0.02), G1-G9 (P=0.01), G1-G10 (P=0.01), G1-G11 (P=0.01) and G1-G13 (P=0.03). Control values were higher than all test groups. There were significant differences across all the dosage groups (P=0.02) and (P=0.01) and post hoc showed statistical differences between G1-G11 (P=0.04), G3-G9 (P=0.03), G1-G7 (P=0.02), G1-G10 (P=0.04) and G1-G13 (P=0.03) respectively. LDL-C levels were lower in the test groups compared to the control.

Table 4.5: Effect of Natural Sweeteners on LDL-C levels of rats

LDL-c (mmol/dl)	Mean □ SEM	ST	ST P-value	SD	SD P-value
Group 1	0.52±0.05	WS	0.49	LD	
Group 2	0.47±0.05				
Group 3	0.54±0.06				
Group 4	0.43±0.03				
Group 5	0.41±0.04	BS	0.02	MD	0.019
Group 6	0.48±0.02		G1-G7=0.03		G3-G9=0.03
Group 7	0.35±0.03				
Group 8	0.43±0.01	H	0.01		
Group 9	0.35±0.03		G1-G9=0.01		
Group 10	0.36±0.02		G1-G10=0.01	HD	0.015
Group 11	0.34±0.03	S	0.01		G1-G7=0.02
Group 12	0.39±0.02		G1-G11=0.02		G1-G10=0.04
Group 13	0.36±0.03		G1-G13=0.03		G1-G13=0.03

One-way ANOVA, Duncan's numerous range tests for post hoc. *LDL: Low Density Lipoprotein Cholesterol, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

4.3.3 Effect of Natural Sweeteners on VLDL-C levels of rats

Table 4.6 shows the effect of white sugar, brown sugar, honey and stevia on Very Low-Density Lipoprotein Cholesterol (VLDL-c) levels of rats. VLDL-c in all the treatment groups were statistically significant (P=0.01). There were significant differences in the means of types of sweeteners in only honey (p=0.001) and stevia (p <0.00) and post hoc showed significant differences between G1-G9 (P=0.00), G8-G9 (P=0.00),

G1-G11 (P=0.00), G1-G12 (P<0.00), G1-G13 (P<0.00) and G11-G13 (P=0.03). Test group values were higher than the Control group. With the dosage groups, there were significant differences across the LD (P=0.00) and HD (P=0.02) groups and post hoc revealing a significant difference between G1-G11 (P=0.00), G2-G11 (P=0.04), G5-G11 (P=0.00), G8-G11 (P=0.00) and G1-G7 (P=0.04) respectively.

Table 4.6: Effect of Natural Sweeteners on VLDL-C levels of rats

VLDL-c (mmol/dl)	Mean ± SEM	ST	ST P-value	SD	SD P-value
Group 1	0.17±0.01	WS	0.34	LD	0.00
Group 2	0.25±0.03				G1-G11=0.00
Group 3	0.23±0.03				G2-G11=0.04
Group 4	0.23±0.03				G5-G11=0.00
					G8-G11=0.00
Group 5	0.17±0.01	BS	0.14	MD	0.17
Group 6	0.51±0.26				
Group 7	0.67±0.24				
Group 8	0.18±0.05	H	0.00		
Group 9	0.45±0.03		G1-G9=0.00		
Group 10	0.31±0.05		G8-G9=0.00	HD	0.02
Group 11	0.38±0.01	S	<0.00		G1-G7=0.04
Group 12	0.48±0.01		G1-G11=0.00		
Group 13	0.53±0.06		G1-G12<0.00		
			G1-G13<0.00		
			G11-G13=0.03		

One-way ANOVA, Duncan's numerous range tests for post hoc. *VLDL-c: Very Low-Density Lipoprotein Cholesterol, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

4.4 EFFECT OF NATURAL SWEETENERS ON LIPID RATIO FORMULAS

The following results show the effect of the consumption of white sugar, brown sugar, honey and stevia on atherogenic risk of the rats by using lipid ratio formulas. These included Castelli risk index I (CRI I), Castelli risk index II (CRI II), Atherogenic Index of Plasma (AIP), Atherogenic Coefficient (AC).

4.4.1 Effect of natural sweeteners on Castelli risk index I (CRI I) levels of rats

From the CRI I**,** ANOVA showed a significant difference in means across the various groups (P<0.00) and post hoc showed significance between the following groups: G4-G5, G4-G7, G4-G8, G4-G4, G5-G10, G4-G11, G4-G12, and G4-G13. A statistical significance was observed in the means of types of sweeteners in BS (P=0.01), honey (P=0.00) and stevia (P<0.00) and post hoc showed significant differences between G1-G5 (P=0.03), G1- G7 (P=0.03), G1-G9 (P=0.00), G1-G10 (P=0.01), G1-G11 (P=0.00) and G1-G12 (P=0.00), respectively, all showing decreases with respect to the Control. There was no significant difference in the means of groups of brown sugar (P>0.05). All the dosage grouped had significant differences LD (P=0.02), MD (P=0.00), HD (P=0.00) and post hoc showed significant differences between G1-G8 (P=0.03), G1-G9 (P=0.00), G1-G12 (P=0.01), G3-G9 (P=0.01), G3-G12 (P=0.03), G4-G7 (P=0.01), G4-10 (P=0.01) and G4-G13 (P=0.01)

(Table 4.7).Table 4.7: Effect of natural sweeteners on Castelli risk index I (CRI-I) levels of rats

CRI-I	Mean ±SEM	ST	ST P-value	SD	SD P-value
Group 1	1.85±0.12	WS	0.27	L D	0.02
Group 2	1.74±0.19				G1-G8=0.03
Group 3	1.79±0.11				
Group 4	2.30±0.35				
Group 5	1.50±0.05	BS	0.01	MD	0.00
Group 6	1.55±0.04		G1-G5=0.03		G1-G9=0.00
Group 7	1.38±0.08		G1-G7=0.03		G1-G12=0.01
Group 8	1.35±0.09	H	0.00		G3-G9=0.01
Group 9	1.36±0.08		G1-G9=0.00		G3-G12=0.03
Group 10	1.43±0.03		HD		0.00
			G1-G10=0.01		G4-G7=0.01
Group 11	1.43±0.02	S	<0.00		G4-10=0.01
Group 12	1.41±0.03		G1-G11=0.00		G4-G13=0.00
Group 13	1.38±0.01		G1-G12=0.00		
			G1-G13=0.00		

One-way ANOVA, Duncan's numerous range tests for post hoc. *CRI-I: Castelli risk index I, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.0*4.4.2

4.4.2 Effect of natural sweeteners on Castelli risk index II (CRI-II) levels of rats

From the CRI-II, ANOVA showed a significant difference in means across the various groups and post hoc showed significant differences (P<0.00) between the following groups: G1-G3, G1-G9, G1- G11, G1-G13, G4-G5, G4-G6, G4 -G7, G4 -G8, G4-G9, G4 -G10, G4-G11, G4 and G12, and G4 and G13.

A statistical significance was observed in the means of BS (P=0.00), honey (p<0.00) and stevia (p<0.000) and post hoc showed significant differences between G1-G5 (P=0.01), G1-G6 (P=0.04), G1-G7 (P=0.00), G1-G8 (P=0.00), G1-G9 (P<0.00), G1-G10 (P=0.00), G1-G11 (P<0.00), G1-G12 (P<0.00) and G1-G13 (P<0.00), respectively. However, there was no significant difference in the means of groups of white sugar (p>0.05). All the dosage grouped had significant differences LD (p=0.00), MD (p<0.00), HD (p=0.00) and post hoc showed significant differences between G1-G5 (P=0.04), G1-G8 (P=0.01), G1-G11 (P=0.00), G2-G11 (P=0.04), G1-G6 (P=0.01), G1-G9 (P<0.00), G1-G12 (P=0.00), G3-G9 (P=0.00), G3-G12 (P=0.00), G1-G13 (P=0.03), G4-G7 (P=0.00), G4-G10 (P=0.01) and G4-G13 (P=0.00). The general trend showed a decrease in Castelli index II compared to the control

(Table 4.8)Table 4.8: Effect of natural sweeteners on Castelli risk index II (CRI-II) levels of rats

CRI-II	Mean (SEM)	ST	ST P-value	SD	SD P-value
Group 1	0.41±0.02	WS	0.45	LD	0.00
Group 2	0.37±0.05				G1-G5=0.04
Group 3	0.40±0.01				G1-G8=0.01
Group 4	0.49±0.08	BS	0.00		G1-G11=0.00
					G2-G11=0.04
Group 5	0.28±0.03		G1-G5=0.01	MD	<0.01
Group 6	0.32±0.01		G1-G6=0.04		G1-G6=0.01
Group 7	0.25±0.02		G1-G7=0.00		G1-G9<0.01
Group 8	0.26±0.01	H	<0.00		G1-G12=0.00
Group 9	0.25±0.02		G1-G8=0.00		G3-G9=0.00
					G3-G12=0.00
Group 10	0.25±0.01		G1-G9<0.00	HD	0.00
			G1-G10=0.00		G1-G13=0.03
Group 11	0.24±0.01	S	<0.00		G4-G7=0.004
Group 12	0.26±0.01		G1-G11-<0.00		G4-G10=0.006
Group 13	0.23±0.01		G1-G12 <0.00		G4-G13=0.001
			G1-G13<0.00		

One-way ANOVA, Duncan's numerous range tests for post hoc. *CRI-II: Castelli risk index II, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

4.4.3 Effect of natural sweeteners on atherogenic index of plasma (AIP)

From the AIP, ANOVA showed a significant difference in means across the various groups and post hoc analysis showed a significance of P<0.000 between the following groups: G1-G9, G1-G10, G1-G11, G1-G12, G1-G13, G2-G13, G3-G9, G3-G12, G4-G13, G5-G9, G5 –G11, G5 –G12, G5-G13.

A statistical significance was observed in the means of honey (p<0.00) and stevia (p <0.00) and post hoc analysis showed significant differences between G1-G9 (P<0.00), G1-G11 (P=0.00), G8-G9 (P=0.00), G8-G10 (P=0.01), G1-G11 (P<0.00), G1-G12 (P<0.00) and G1-G13 (P<0.00) respectively. However, there was no significant difference in the means of groups of white sugar and brown sugar groups (p>0.05). All the dosage grouped had significant differences LD (p<0.000), MD (p=0.000), HD (p<0.000) and post hoc showed significant differences between the following groups; G1-G11 (P=0.00), G2-G11 (P=0.02), G5-G11 (P=0.00), G8-G11 (P=0.00), G1-G9 (P=0.00), G1-G12 (P=0.00), G3-G9 (P=0.01), G3-G12 (P=0.01), G1-G10 (P=0.00), G1-G13 (P<0.00), G4-G13 (P=0.00) and G7-G13 (P=0.01) **(Figure 4. 3)**

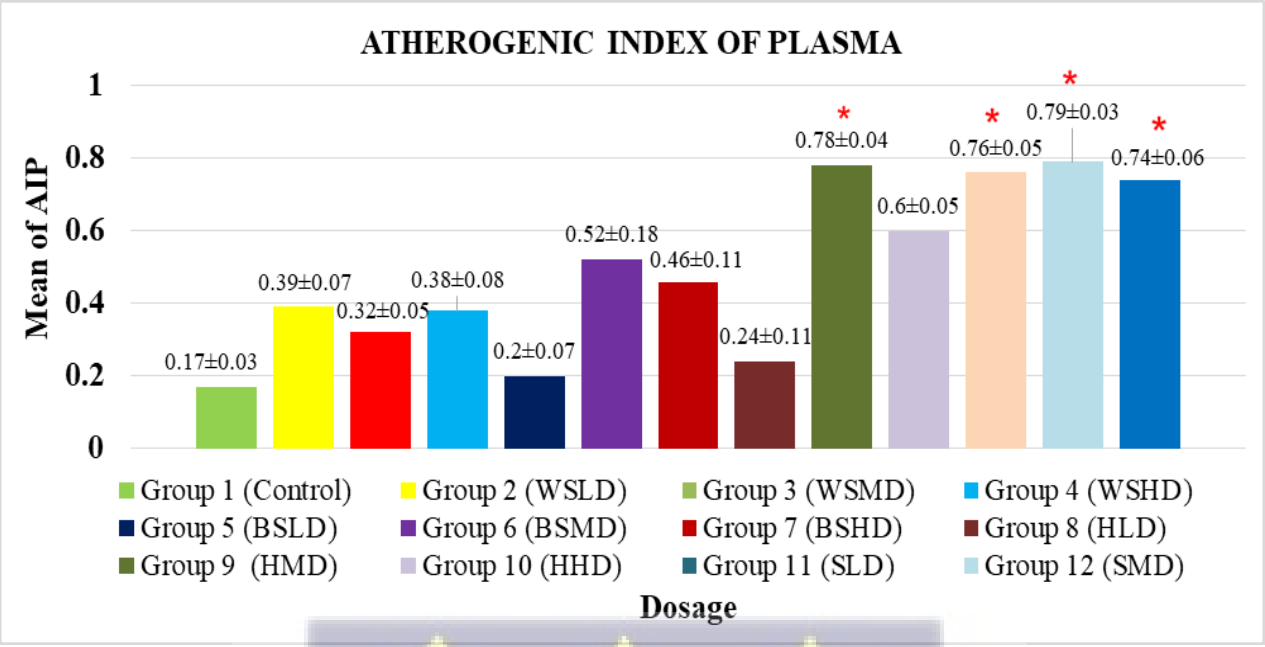

Figure 4.3: *The effects of natural sweeteners at different doses on the atherogenic index of plasma of experimental animals. WSLD: white sugar low dose, WSMD: white sugar medium dose, WSHD: white sugar high dose, BSLD: brown sugar low dose, BSMD: brown sugar medium dose, BSHD: brown sugar high dose, HLD: honey low dose, HMD: honey medium dose, HHD: honey high dose, SLD: stevia low dose, SMD: stevia medium dose, SHD: stevia high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

4.4.4 Effect of natural sweeteners on the atherogenic coefficient (AC) of levels of rats

From the Coefficient of AIP, ANOVA showed a significant difference in means across the various groups and post hoc showed significance (P<0.00) between the following groups: G4-G5, G4-G6, G4 - G7, G4 - G8, G4-G9, G4 -G10, G4-G11, G4 and G12, and G4 and G13.

A significant difference was observed in the means BS (P=0.01), honey (p=0.00) and stevia (p =0.00) and post hoc showed significant differences between G1-G5 (P=0.049), G1-G7 (P=0.01), G1-G8 (P=0.01), G1-G9 (P=0.01), G1-G10 (P=0.02), G1-G11 (P=0.01), G1-G12 (P=0.00) and G1-G13 (P=0.00) respectively. However, there was no significant difference in the means of groups of white sugar (p>0.05).

All the dosage grouped had significant differences LD (p=0.02), MD (p=0.00), HD (p=0.00) and post hoc showed significant differences between G1-G8 (P=0.04), G1-G9 (P=0.01), G1- G12 (P=0.01), G3-G9 (P=0.01), G3-G12 (P=0.03), G4-G7 (P=0.01), G4-G10 (P=0.01) and G4-G13 (P=0.01) **(Table 4.9).**

Table 4.9 Effect of natural sweeteners on the atherogenic coefficient (AC) of levels of rats

AC	Mean± (SEM)	ST	ST P-value	SD	SD P-value
Group 1	0.83±0.11	WS	0.26	LD	0.02
Group 2	0.7405±0.19				G1-G8=0.04
Group 3	0.7929±0.11				
Group 4	1.305±0.35	BS	0.01		
			G1-G5=0.04		
Group 5	0.50±0.05		G1-G7=0.01	MD	0.0012
Group 6	0.55±0.04				
					G1-G9=0.01
Group 7	0.38±0.08				G1-G12=0.01
Group 8	0.35±0.09	H	0.00		G3-G9=0.01
Group 9	0.36±0.08		G1-G8=0.01		G3-G12=0.03
			G1-G9=0.01		
Group 10	0.43±0.033		G1-G10=0.02	HD	0.00
					G4-G7=0.01
Group 11	0.42±0.02	S	0.000		G4-G10=0.01
Group 12	0.41±0.03		G1-G11=0.01		G4-G13=0.01
Group 13	0.38±0.01		G1-G12=0.00		
			G1-G13=0.00		

One-way ANOVA, Duncan's numerous range tests for post hoc. *AC: Atherogenic coefficient, ST: sweetener type, SD: sweetener dosage, WS: white sugar, BS: brown sugar, H: honey, S: stevia, LD: low dose, MD: medium dose, HD: high dose. Data is presented as mean ± standard error of means (SEM). P is significant* at ≤ 0.05.*

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

In this present study, the effect of natural sweeteners on atherogenic risk index was evaluated using Sprague Dawley rats. Hyperlipidemia is a common feature in atherosclerosis, the complications of which lead to ischemic heart disease, myocardial infarction and stroke (Bray et al., 2014).

Added sugar is still a significant issue that affects people all over the world and increases the risk factors for atherosclerosis (Nwachuku et al., 2017). The addition of sweeteners to foods during production, or right before food is consumed makes them an essential part of our diet (Carocho et al., 2017). Because sugar has a sweet flavor, many people eat too much of it (González-Montemayor et al., 2019). Global obesity rates have increased because of excessive sugar consumption (Malik and Hu, 2012). Many people's and families' quality of life has been negatively impacted by the rise in chronic illnesses brought on by being overweight or obese (Nwachuku et al., 2017). Because of the potential health risks, consumers are more cautious about using these sweeteners thus, choose some natural sweeteners over others (Nwachuku et al., 2017). Much information regarding the impact of sugars on nutrition and health are contradictory. It is unknown which natural sweeteners are healthier than others (Roman et al., 2013). Different effects of caloric sweeteners on weight gain, HbA1c, FBG, and lipid profile have been seen in animal experiments employing healthy Sprague-Dawley rats (Nemoseck et al., 2011; Atangwho et al., 2020). It was necessary to investigate anecdotal claims further after the investigations produced inconclusive results.

Body weight

The current study showed a continuous weight gain across the treatment groups, however, there was significant increase in weight among the groups of high doses of white sugar, honey and stevia compared to the control. No significant trends were seen between consumption of sweeteners and BMI. The effect of honey and sugar on body weight can be attributed to their high caloric content. Consuming high calories over a period may contribute to weight gain (Saraiva et al., 2020). White sugar and honey both contain simple carbohydrates, which will have an equivalent impact on energy balance when taken in similar quantities (Fitch & Keim, 2012). Therefore, it makes sense that honey and white sugar supplied in the same amounts of carbohydrates may have each contributed to weight growth equally. These results are consistent with a study by Atangwho et al. (2017) where honey and sugar fed rats had an increased body weight after 13 weeks. In contrast, after 33 days of therapy, rats fed sucrose gained more weight than those fed honey, according to (Nemoseck et al., 2011). This may be as a result of the study being done within a short period of time.

Findings with respect to stevia could be as a result of the tendency of stevia lowering fullness and increasing hunger prior to a meal thereby causing an increased caloric intake from the meal consumed (Becker et al., 2020). This agrees with Becker et al., (2020) who observed a significant difference between stevia consumption and increased appetite which contributed to weight gain in high fat induced mice. In contrast, a study by Duram Aguero et al., (2015) using overweight students, reported normal weight and nutritional status after consuming food and beverages containing stevia as sweetener for one week. Although few human and animal studies have illustrated that Stevia has shown beneficial effects on food intake and weight loss, there are also other clinical trials that have not found a significant effect on body weight (Masoumi et al., 2020). Therefore, further studies are needed to examine the effectiveness of stevia in weight control (Ashwell, 2015).

Glucose

Nutritive sweeteners (NS) are known to elevate blood glucose levels after consumption where as non-caloric sweeteners are expected to regulate glucose levels after consumption because of their low caloric content compared to nutritive sweeteners (NNS) (O'Donnell & Kearsley, 2012). As a result, one would anticipate a postprandial glycemic response after consuming sucrose, but not NNS (Morales-Ríos et al., 2022). Interestingly, in the present study, white sugar, brown sugar and honey did not show significant influence on fasting blood glucose of rat but evidence from stevia on the other hand revealed that, the consumption of medium doses increased glucose levels compared to the control. This could be associated to the imbalances in gut microbiota stevia metabolism elicits thereby affecting insulin signaling which may affect glucose tolerance (stamataki et al., 2020; vamanu et al., 2019). However, this contradicts studies by Abdel Al et al., (2021) and Ajami et al., (2020) which found significant decrease in FPG in stevia fed rats due to the positive effect stevia hs on gut microbiota. This implies inconsistencies; therefore, further research is needed.

Atangwho et al. (2017), in their study found that there was no significant difference in the FPG levels of honey and sucrose fed rats which is in with the current study. Similarly, a previous study on rats fed honey revealed no significant difference in the effect on reducing blood glucose levels (Erejuwa et al., 2016). Depending on its source, table sugar and honey both contain a similar quantity of glucose and fructose in addition to other ingredients. This suggests, that their consumption over time will have a comparable impact on FBG, especially considering that the rats in this study received the same quantity of carbohydrate. However, data with respect to stevia may need further clinical studies for confirmation

Lipid Profile

Numerous cardiovascular disorders have been linked to high blood triglyceride and cholesterol levels, which are exacerbated by a high LDL-to-HDL cholesterol ratio (Nordestgaard & Varbo, 2014). This is due to the possibility that high levels of LDL-cholesterol, also known as "bad" cholesterol, could induce a buildup of cholesterol in the arteries, which could promote atherosclerosis (Badimon & Vilahur, 2012). A higher blood lipid profile was associated with greater sugar consumption. Although it is claimed that sugar replacements like stevia have little impact on lipid profiles, the mechanism by which it affects lipid metabolism appears to vary in research.

This study showed no significant difference in TC and HDL-C levels in all the treatment groups. However, levels of TG of rats were significantly increased in the treatment groups except for groups of brown sugar which showed no significant difference compared to the control. Additionally, LDLc and VLDLc levels in this study were significantly decreased in rats fed with brown sugar, honey and stevia compared to the control group.

Data with respect to caloric sweeteners on TC and HDL levels could be attributed to the duration of the study. A longer study may have resulted in a significant difference among the treatment groups, revealing the exact impact natural sweeteners had on TC and HDL of the rats. This is consistent with a study in honey-fed rats that reported no significant variation in HDL-C and TC levels between the treatment group and control group after a 10-week feeding period (Mohammadimanesh et al., 2019). Furthermore, Nemoseck et al., (2011) found no significant change in the TC and HDL-C levels of honey and sucrose fed rats after 33 days.

Findings with regards to TG levels in this study can also be linked to the high caloric content of sugar and honey, as well as the imbalances in gut microbiota caused by stevia which may lead to several metabolic diseases.

This agrees with a study by Alangwu et al., (2011) which found a significant increase in TG levels in honey and sucrose fed rats. Data on stevia however contradicts a studies don buy Abdel Al et al., (2021), Ahmed et al., (2018) and Ruti & Nandini, (2016) which reported significant decrease in TG levels in stevia fed rats. The increase and decrease seen in triglycerides with regards to this study compared to other research however, implies that, there are inconsistencies in research with regards to the effects of stevia on triglyceride levels (Onakpoya et al, 2015).

Findings on LDL and VLDL levels in this study agrees with a study by Mushtaq et al., (2011), which reported improved cholesterol levels in obese individuals after consuming 40g of honey over a period of time. In addition, Alagwu et al. (2011), reported that the administration of pure honey reduced LDL levels rats compared to the control group. However, the study of Alagwu et al. (2011) showed elevated VLDL levels in rats which contradicts the findings of the current study. Also results observed with respect to stevia agrees with the findings of Ritu & Nandini (2016) which revealed a reduction in LDL levels in diabetic patients after consuming 1g/day stevia for 60 days. Similarly, a study conducted by Ahmed et al. (2018), reported a significant decrease in LDL and VLDL levels in stevia fed hyperlipidemic at different dose levels for 8 weeks (Ahmed et al., 2018).

According to this study, brown sugar, honey, and stevia all had a similar impact on LDL and VLDL cholesterol levels. This could be attributed to the antioxidant, polyphenols, and trace elements in honey as well as the anti-hyperlipidemia property of stevia, which contributes to their cardio-protective properties. According to White (2013), antioxidants in honey are beneficial because they prevent the development of atherogenic plaques in addition to decreasing blood cholesterol and low-density lipoprotein levels. Numerous trace elements, minerals, and vitamins found in honey and stevia are also responsible for atherogenic plaque inhibition. Copper and zinc are two trace metals that inhibit the growth of atherogenic plaques. Vitamins E, C, B, and A are vitamins found in honey and stevia that help prevent oxidative stress and the development of atherosclerotic plaque (Nwachuku et al., 2017)

Lipid Ratios

The general trend of the effects of natural sweeteners on Castelli I, Castelli II, and Coefficient of AIP showed a significant decrease of brown sugar, honey and stevia. This implies that the consumption of these sweeteners may result in a decreased risk of atherosclerosis. This could be attributed to decreased LDLc and VLDLc levels. This is because, higher levels of LDL-C cause an accumulation of lipid particles in the artery walls resulting in atherosclerosis (Badimon & Vilahur, 2012; Ray et al., 2020). Thus, decreased levels in LDLc and VLDLc may imply a lower risk of atherosclerosis (Barquera et al., 2015). However, the Atherogenic index of plasma in this study showed a significant increase in atherogenic risk among medium and high doses of honey as well as all doses of stevia, with stevia having the greatest impact. This could be attributed to the elevated triglyceride levels observed in this study which has been highlighted as a major biomarker of atherogenic risk. This agrees with a study by Nwachuku et al., (2015) which found a significant increase in atherogenic risk in honey and sugar fed using the AIP lipid ratio formula.

Furthermore, the differences observed in forecasting atherogenic risk using the AIP compared to Castelli I, Castelli II, Coefficient of AIP could be attributed to the fact that, the AIP has been introduced as a better marker for CAD since studies have shown that it has the ability to reflect the exact mechanisms associated with an individual's lipid profile and their risk of atherosclerosis than the other conventional ratios in clinical studies (Cure et al, 2018; Niroumand et al., 2015).

5.2 CONCLUSION

This study highlighted that, stevia regardless of the quantity, would cause a higher risk of atherosclerosis by increasing triglyceride levels, bodyweight and fasting plasma glucose more than the other natural sweeteners, thus, may not be a better sugar alternative.

Overall, it was demonstrated that, high intake of natural sweeteners may exert different effects on metabolism, which may ultimately determine their suitability for different people due to differences in their constituent sugars, nutrients and diverse chemical structures. As such, it is advisable that natural sweeteners are taken in moderation and with caution by avoiding excessive consumption.

5.3 CONTRIBUTION TO KNOWLEDGE

- This study will provide evidenced-based information which will address the general debate and undocumented claims on natural sweetness, to assist individuals in their choices.
- Furthermore, Dieticians and nutritionists may use this information to help them create the best interventions for people with conditions that are related to diet and nutrition.
- The study's findings may also help consumers, policymakers, and other healthcare professionals make knowledgeable choices when using natural sweeteners and can assist in the development of dietary recommendations and nutritional policies on the use of added sugars.

5.3 RECOMMENDATION

1. These findings should be extended to a human study.

2. Further research should be conducted on the effect of stevia on atherogenic risk indices, especially on body weight, glucose and triglyceride levels in order to understand its mechanisms of inducing cardiovascular risks.

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APPENDIX A

Calculation of white sugar Dose

- White sugar: 25 g of white sugar was equivalent to 100 kcal

For a 70 kg adult requiring 2000 kcal, medium dose = 25 g/70 kg = 0.36 g/kg body weight

Thus, for a 200 g (0.2 kg) rat, the required dosage = 0.2 kg x 0.36 g/ kg = 0.072

0.07 g of the white sugar was dissolved in 2 ml of distilled water.

A stock solution was prepared at 0.035 g/ml for low-dose white sugar.

Moderate (10 % of total energy intake) = 0.07 g/ml

High dose (15 % of total energy intake) = 0.1 g/ml

Calculation of Brown sugar Dose

- Brown sugar: 25.6 g of brown sugar was equivalent to 100 kcal

For a 70 kg adult requiring 2000 kcal, medium dose = 25.6 g/70 kg = 0.37 g/kg body weight

Thus, for a 200 g (0.2 kg) rat, the required dosage = 0.2 kg x 0.37 g/ kg = 0.073

0.073 g of the brown sugar was dissolved in 2 ml of distilled water.

A stock solution was prepared at 0.036 g/ml for low-dose white sugar.

Moderate (10 % of total energy intake) = 0.073 g/ml

High dose (15 % of total energy intake) = 0.11 g/ml

Calculation of honey Dose

- Honey: Approximately 32 g of honey was equivalent to 100 kcal

For a 70 kg adult requiring 2000 kcal, medium dose = 32 g/70 kg = 0.47 g/kg body weight

Thus, for a 200 g (0.2 kg) rat, the required dosage = 0.2 kg x 0.47 g/ kg = 0.094

0.094 g of the white sugar was dissolved in 2 ml of distilled water.

A stock solution was prepared at 0.047 g/ml for low-dose white sugar.

Moderate (10 % of total energy intake) = 0.094 g/ml

High dose (15 % of total energy intake) = 0.14 g/ml

Calculation of stevia Dose

- 1 pack of stevia is equivalent to 2 tsp of sugar

1 pack of stevia = 2g Low

dose = 3 g of stevia

Moderate dose = 6 g of stevia

High dose = 9 g of stevia

For a 70 kg adult, stevia low dose = 3 g/70kg = 0.042

Thus, for a 200 g (0.2 kg) rat, the required dosage = 0.2 kg x 0.042 g/ kg = 0.008

0.008 g of the white sugar was dissolved in 2 ml of distilled water.

A stock solution was prepared at 0.004 g/ml for low-dose white sugar.

Moderate (10 % of total energy intake) = 0.008 g/ml

High dose (15 % of total energy intake) = 0.013 g/ml

UNIVERSITY OF GHANA

University of Ghana Institutional Animal Care and Use Committee (UG-IACUC)

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Office Location: Department of Animal Experimentation Building, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana

11/04/2022

ETHICAL CLEARANCE
(UG-IACUC 001/21-22)

Your protocol for an ethical clearance has been reviewed by the University of Ghana Institutional Animal Care and Use Committee and has been approved as follows:

TITLE OF PROTOCOL: Action of nutritive and non-nutritive sweeteners on insulin signaling pathway and cardiometabolic risk factors using experimental animal models

PRINCIPAL INVESTIGATOR: Ruth Tenkoramaa Owu

Please note that the final review report must be submitted to the Committee at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to UG-IACUC for review and approval prior to implementation.

Please report all serious adverse events related to this study to UG-IACUC within seven days verbally and in writing within fourteen days.

This certificate is valid till 10st April, 2023. You are to submit annual reports for continuing review.

Signature of Chairperson
Prof. Major (Rtd.) George A. Asare