Available online www.unicrossjournals.com

UNICROSS JOURNAL OF SCIENCE AND TECHNOLOGY, UJOST

RESEARCH ARTICLE ISSN:2814-2233

Date Accepted: 30th, May 2022 Pages 1-10

COMPARATIVE EFFECT OF CALORIC (HONEY AND SUCROSE) AND A COMMERCIAL NON-CALORIC (ASPARTAME, SODIUM CYCLAMATE AND ACESULFAME K) SWEETENERS ON SELECTED ANTHROPOMETRIC INDICATORS OF WISTAR RATS

[1]Asuk, A. A., [2]Atangwho, I. J., [1]Ati, B. U., and [3]Ikpa, J. O.

[1]Department of Medical Biochemistry, University of Cross River State

[2]Department of Biochemistry, University of Calabar

[3]Department of Anatomy, University of Cross River State

ABSTRACT

Obesity and overweight are worldwide health problems with their attendant clinical and social implications. Sweetened foods are known to contribute greatly to this rise in the prevalence of obesity and overweight. Hence, this study evaluated the comparative impact of food sweetened with natural honey, sucrose and combined commercial non-caloric sweeteners on bodyweight regulation in an animal model. Forty-eight rats (140-190 g) comprised of 24 males and 24 females, divided into 4 groups of 12 rats each (6 males and 6 females) were used. Group I, the control, received rat chow and water *ad libidum,* while groups II, III and IV received 20%, 0.3% and 20% (w/v) of honey, combined non-caloric sweeteners and sucrose solutions respectively for 10 weeks. Results of anthropometric indices obtained showed that mean weight increase (MWI), body mass index (BMI) and abdominal circumference (AC) were increased significantly ($p < 0.05$) in rats fed sucrose or aspartame-sweetened diets relative to honey diet and the control. Whereas these impacts were not sex-dependent with the honey diet, aspartame and sucrose diets indicated differential effects. Taken together, data from the study suggest that the impact of honey (used as a food sweetener) on bodyweight, may be more beneficial when compared to sucrose and commercial non-nutritive sweeteners.

Keywords: Obesity, Caloric sweetener, non-caloric sweetener, BMI, Abdominal circumference

1. Introduction

Worldwide, the prevalence of overweight and obesity is increasing dramatically. Global estimate data reveal that in 2016, more than 1.9 billion adults were overweight, out of which over 650 million were obese (WHO, 2021). This increased prevalence has serious health implications. Obesity and overweight are well-known risk factors for Type 2 diabetes, cardiovascular disease, obstructive sleep apnea, non-alcoholic steatohepatitis, osteoarthritis and some cancers (Jehan *et al*., 2018). According to Corey and Kaplan (2014) obesity alone is substantially responsible for the increased all-cause mortality, given its causal role in more than 65 demonstrated co-morbidities, including diabetes mellitus, cardiovascular disease, and several forms of cancer.

Obesity however develops only when energy intake consistently exceeds energy expenditure – a positive energy balance. From current reports, it is noted that obesity and overweight and indeed non-communicable diseases which hitherto were problems of industrialized nations are increasingly becoming issues of the developing world, due to a drift in lifestyle and the quest for modernization, urbanization, and globalization of food markets (Islam *et al*., 2014). One such major lifestyle change that contributes immensely to the obesity epidemic is the change in eating habits - increased consumption of energy-dense foods with high levels of calorie-rich sweeteners.

Sweeteners including honey, sugar, and aspartame have always been used to provide sweet flavor to food thereby improving its palatability (Alvarez-Suarez et al., 2010). These sweeteners are comprised mainly of simple carbohydrates - sucrose, glucose, fructose, etc., (except aspartame which is a peptide and non-caloric). Hence, there is a possibly significant contribution of "extra" calories to the diet. Simple carbohydrates also have a high glycemic index which does not only affect the body's glucose, insulin, and lipid response to the food but also affects the appetite and energy intake, hence the bodyweight (Vermunt *et al*., 2003). Consequently, this "extra calorie" can lead to positive energy balance and ultimately obesity as the consumption of more than the needed calories is one of the major causes of overweight and obesity (Atakan et al., 2021).

Sugars in the diet, particularly habitual taking of sugar-sweetened beverages (SSB) have been linked with factors leading to obesity, type II diabetes, and cardiovascular disease (Malik and Hu, 2022). Diets containing high amounts of fructose, either as a monosaccharide or as a component of the disaccharide sucrose, have been shown to produce weight gain (Johnson *et al*., 2017), increase plasma triglyceride concentrations, induce insulin resistance, and glucose intolerance, and contribute pro-oxidative effects in animals (Dornas *et al*., 2015; Kang et al., 2019). Fructose is a primary sugar in honey (Nguyen *et al.*, 2019). However, honey is a complex, natural sweetener, and its metabolic effects have not been thoroughly explored. Besides its naturally sweet taste, it is known to have a low glycaemic index and other medicinal properties. Its benefit to health and its usage have been well known for a long time and to various civilizations (Haslam, 2016). Findings from many studies also showed the ability of honey in controlling overweight and obesity when consumed orally, thus making it a potential anti-obesity agent (Haslam, 2016; Hashim *et al*., 2021). However, the effect of using local honey along with other common sweeteners is still unknown.

There is therefore an urgent need for scientific clarification given the widespread daily use of these sweeteners and the advancing scale of the global obesity epidemic, using a detailed and carefully designed study. Consequently, the present study is designed to investigate and compare the effect of natural honey, table sugar, and a non-caloric sweetener to provide clarification on whether or not indigenous natural honey from the Obudu Cattle Ranch and table sugar as well as common non-caloric sweeteners contribute to the aetiology of obesity.

2. Materials and Methods Sample Collection

Twenty-five (25) litres of honey were purchased at the downhill of Obudu cattle ranch and adjudged to be original honey. The honey was stored at room temperature of 26 ± 2 °C until when required for use. A commercial non-caloric sweetener containing aspartame, sodium cyclamate and acesulfame K and a caloric sweetener, table sugar was purchased in sachets at Jopal supermarket, Igoli, Ogoja, in Cross River State.

Preparation of samples for administration

The honey was filtered with a muslin cloth to remove dead bees and particulates. The filtered honey was cleanly possessing the regular reddish-brown color, typical of good honey. Twenty percent (20 % v/v i.e. 1: 4 portions of honey to water) solution of honey and 25 % w/v (25 g of the sugar in 100 mL volume of solution) of table sugar were prepared and stored safely for oral administration to the test rats. Similarly, 0.3 % w/v solution of a commercial product containing aspartame, sodium cyclamate and acesulfame K in distilled water (i.e., 0.3g/300mg of sweetener in 100 mL volume of solution), was prepared and used in this experiment.

Animals

Forty-eight (48) Wistar rats weighing 140-190 g were obtained from the animal house of the Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus, and kept in well-ventilated laboratory cages. The animals were allowed to acclimatize to the sweeteners for a week before the commencement of the study. They were fed standard rat pellets and drank tap water *ad libitum* at room temperature. Illumination was the natural, regular 12-h light and 12-h dark cycle all through the period of the experiment. Ethical approval for the study was obtained from the Faculty of Basic Medical

Sciences Animal Research Committee of the Cross River University of Technology, Calabar, Nigeria.

Design of experiment and treatment plan

Forty-eight (48) rats comprised of 24 males and 24 females were divided into 4 groups of 12 rats each (6 males and 6 females) treated, thus:

Group I (Control): Given raw chow and water only *ad libitum*

Group II (Honey): 20 % honey solution *ad libitum* for 12 h per day

Group III (Aspartame): 0.3 % (aspartame, sodium cyclamate and acesulfame K) solution *ad libitum* for 12 h per day

Group IV (Sucrose): 20 % sucrose solution *ad libitum* for 12 h per day

The animals were exposed to honey, aspartame, and sucrose solutions orally for 12 h at night – 7.00 pm to 7.00 am. Tap water replaced the test sweeteners for another 12 h in the day – 7.00 am to 7.00 pm. This exposure cycle was maintained through the study period and was adopted from a previous study (Creze et al., 2018). The quantity of sweetener consumed was estimated using a difference in the outset volume of sweetener at 7.00 pm and the volume of leftover at 7.00 am.

Anthropometric measurements

Bodyweight: Bodyweight in grams (g) of both male and female counterparts in groups was measured using an electronic weighing balance with 0.1g precision. The weights were measured once/twice per week throughout the 10-week study period, to observe the step-wise response of bodyweight to the oral exposure to the sweeteners.

Body length and abdominal circumference (AC): The calibrated measuring tape was used to determine the body length in centimetres (cm) of the rats i.e. nostril to the anal length (Novelli et al., 2007). The abdominal circumference (in centimetres) was taken immediately anterior to the forefoot, also using the calibrated measuring tape wound around the abdominal region as described earlier by Novelli et al. (2007).

Body mass index (BMI): Using the bodyweight and body length, the body mass index of each animal was estimated thus: Weight in grams (g) / square of the body length (cm^2) (Novelli et al., 2007). Also, the mean weight increase (%) was calculated according to Rahman et al. (2017) thus;

$$\frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

Statistical analysis

Data obtained from the experiment were statistically analyzed using graph pad prism 8.0 software and presented in graphs. A statistically significant difference was taken at $p < 0.05$.

3. **Results**

The results of the anthropometric data obtained for rats after a 10-week oral exposure to solutions of honey, sucrose, and aspartame are shown in Figures 1, 2, 3, 4 and 5. In Figure 1, the BMI of male rats is expressed. The result showed a significant ($p < 0.05$) increase in the BMI of groups administered both aspartame and table sugar (sucrose) compared with the honey group and the control. The BMI of the group administered honey compared well with the control. This pattern was repeated in the female group as shown in Figure 2, except there was no significant ($p > 0.05$) difference in the BMI of the aspartame group compared with the sucrose group. The initial and final BMI values in the groups administered honey were relatively unchanged in both sexes. The mean weight increase (MWI) for both male and female rats is given in Figure 3. The MWI of male and female rats administered sucrose and aspartame were significantly ($p < 0.05$) increased compared with the honey group and control. The MWI of the male rats administered aspartame and sucrose was significantly ($p < 0.05$) higher than the female rats. However, there was no significant ($p > 0.05$) difference in MWI between the male and female rats administered honey. Abdominal circumference (AC) in male and female rats are given in Figures 4 and 5 respectively. The AC in both males and females administered aspartame and sucrose was significantly ($p < 0.05$) increased compared with the honey group and the control. The sucrose group of females was significantly increased compared with the aspartame group. The honey group compared well with the control in both sexes, however, there was a slight decrease ($p > 0.05$) in AC of males compared with the control.

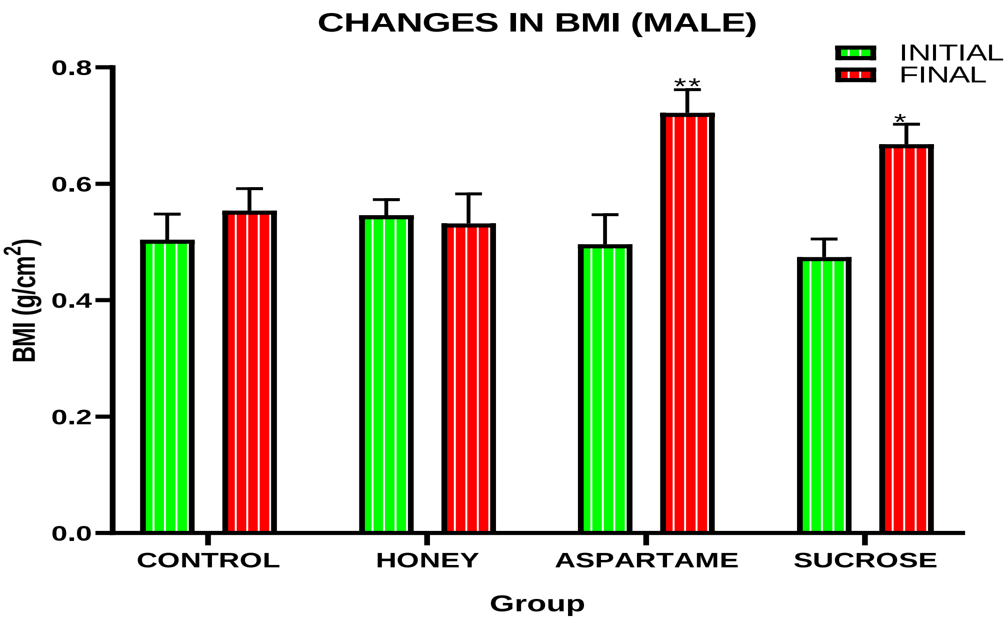

Figure 1. Body mass index (BMI) of male rats administered honey, aspartame and sucrose solutions for 10 weeks. Values are the mean ± SEM (n = 6). * Indicates a significant difference of sucrose at $p < 0.05$ vs honey, aspartame and control. ** indicates a significant difference of aspartame at $p < 0.05$ vs honey, sucrose and control. Aspartame = (aspartame, sodium cyclamate and acesulfame K)

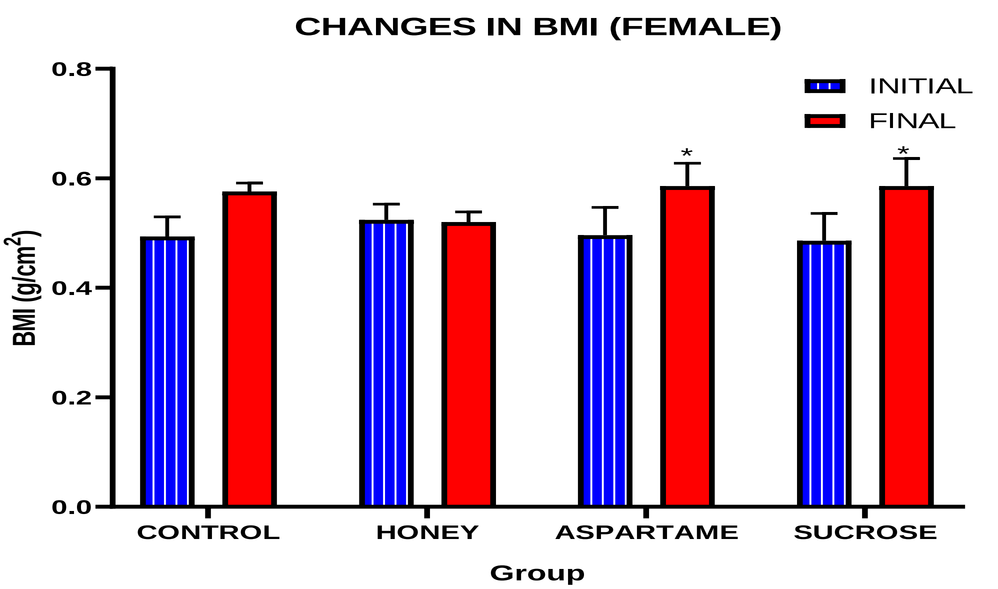

Figure 2. Body mass index (BMI) of female rats administered honey, aspartame and sucrose solutions for 10 weeks. Values are the mean ± SEM (n=6). * Indicates a significant difference in aspartame and sucrose at $p < 0.05$ vs honey and control. Aspartame = (aspartame, sodium cyclamate and acesulfame K)

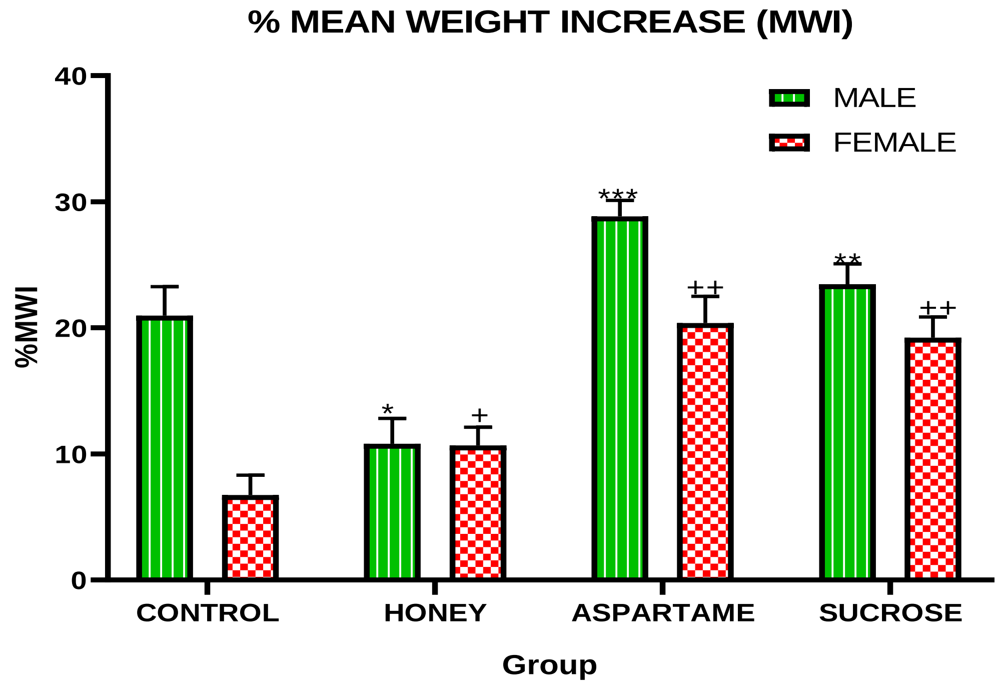

Figure 3. Mean weight increase (MWI) of male and female rats administered honey, aspartame and sucrose solutions for 10 weeks. Values are the mean ± SEM (n=6). *Significant difference in MWI of male rats administered honey at $p < 0.05$ vs sucrose, aspartame and control. **Significant difference in MWI of male rats administered sucrose at $p < 0.05$ vs honey, aspartame and control. *** Significant difference in MWI of male rats administered aspartame at $p < 0.05$ vs honey, sucrose and control. + indicates a significant difference in MWI of female rats administered honey at $p<0.05$vs sucrose, aspartame and control. ++indicates significant difference in MWI of female rats administered sucrose or aspartame at $p<0.05$ vs honey, and control. Aspartame = (aspartame, sodium cyclamate and acesulfame K)

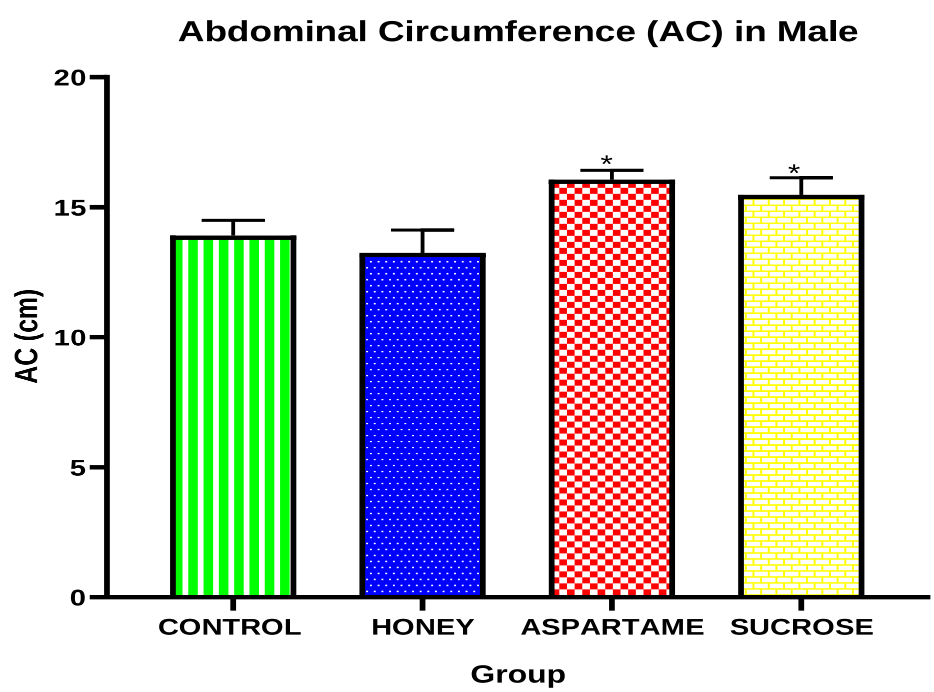

Figure 4. Abdominal circumference (AC) of male rats administered honey, aspartame and sucrose solutions for 10 weeks. Values are the mean ± SEM (n=6). *Indicates a significant difference in aspartame or sucrose at $p<0.05$ vs honey and control. Aspartame = (aspartame, sodium cyclamate and acesulfame K)

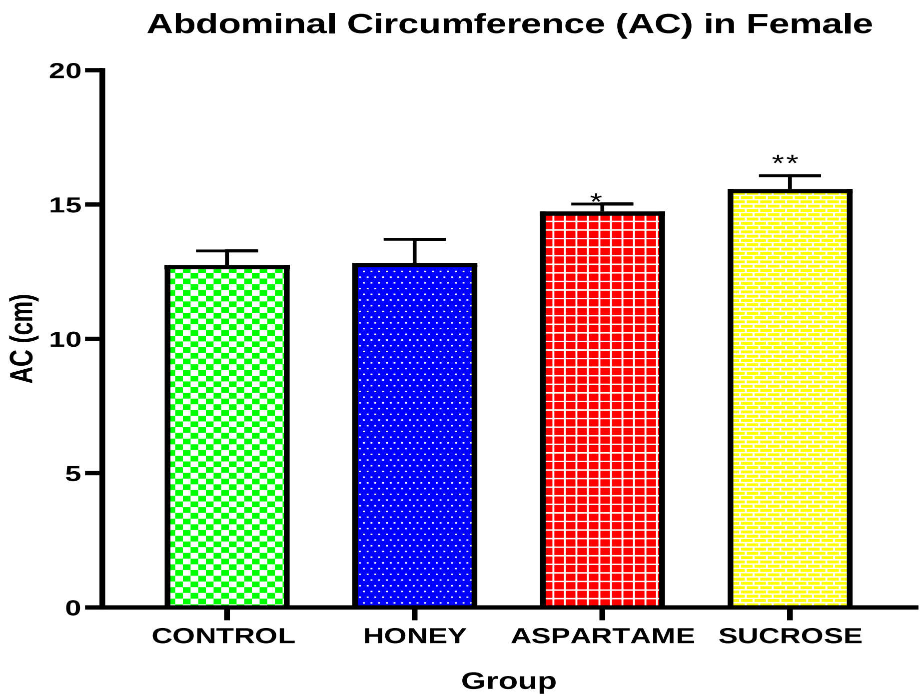

Figure 5. Abdominal circumference (AC) of female rats administered honey, aspartame and sucrose. Values are mean ± SEM (n=6). *Indicates a significant difference of aspartame at $p<0.05$ vs sucrose, honey and control. ** indicates a significant difference of sucrose at $p<0.05$ vs aspartame, honey and control. Aspartame = (aspartame, sodium cyclamate and acesulfame K)

Discussion

Obesity is a global public health issue with high prevalence in all age groups (de Moura e Dias *et al*., 2021). It generates a considerable social and economic impact since it affects people's health and quality of life (de Moura e Dias *et al*., 2021). Thirty-nine (39) million children under the age of 5 were overweight or obese in 2020 (WHO, 2021)

At least one-third of the global population is now overweight or obese and over 60 % of people with obesity live in developing countries in which the prevalence of hypertension and obesity-associated cardio-metabolic disorders is rapidly increasing (Hall *et al*, 2019).

On a global scale, obesity has been ranked as the fifth leading risk factor for global deaths and is projected to be the third leading cause of death by 2030 (Arika *et al*., 2019). Obesity is most accurately defined as excessive accumulation and/or storage of body fat (Hall *et al*., 2019). The most commonly used measure of obesity is body mass index (BMI). In humans, weight in kilograms (kg)/height in square metres (m^2) is used while in rats it is weight in grams (g)/length in square centimetres (cm^2).

The BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and all ages of adults. However, it should be considered a rough guide because it may not correspond to the same degree of fatness in different individuals (WHO, 2021)

This research is premised on the fact that diet can induce obesity (Grzeda *et al*., 2022). Several sweeteners have been implicated in the induction of obesity viz honey, sucrose, and aspartame. In this study, honey was found to exhibit a stable BMI in both sexes when the initial and final BMIs were compared. There was a significant increase in the BMIs of aspartame and sucrose-fed animals compared with both the control and honey groups. The increase was more significant in the males of aspartame-exposed rats. Natural honey is considered a healthy sweetener than table sugar (which is essentially sucrose) and aspartame. There has been the widespread acceptance that the use of table sugar as a food sweetener increases the tendency to weight gain and subsequently, obesity, with its attendant health consequences (Burke et al., 2018; Malik and Hu, 2022).

Honey is the naturally sweet, sticky, and viscous substance mostly produced by *Apis mellifera* bees from the nectar of plants, secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature (Ramli *et al*., 2018).

The composition, flavour, and aroma of the honey depend on the plant sources, climate and environmental conditions. Carbohydrate is the main constituent in dry weight honey with fructose being the highest component at approximately 32–38% followed by glucose as well as other disaccharides and oligosaccharides. Honey also contains organic acids, minerals (Calcium, Magnesium, Phosphorus, and Potassium), trace elements (iron, copper, manganese and zinc), numerous vitamins (especially B-complex and vitamin C), enzymes and proteins (Ajibola *et al*., 2012; Ramli *et al*., 2018). The antioxidant capability of honey is linked to its polyphenol compounds which comprise flavonoids (e.g., quercetin, luteolin, kaempferol, apigenin, chrysin, galangin), phenolic acids, antioxidant enzymes (e.g., glucose oxidase and catalase), ascorbic acid, and carotenoid (Ramli *et al*., 2018). This rich array of nutrients present in honey may have had a significant effect on maintaining a healthy BMI. Prudent dietary intake has been associated with low BMI and waist circumference. Phytochemicals such as flavonoids and minerals like manganese are put together to enhance a low BMI (Vincent et al., 2010). This is corroborated by the fact that administration of honey resulted in a lower percentage of weight gain in rats than in those fed with a sucrose and mixed sugars diet (Ramli *et al*., 2018). This outcome is also supported by our study. The mean weight increase (%) also corresponded with the changes in BMI as aspartame and sucrose gave increased MWI in both males and females, with the males showing a higher MWI. The MWI in both male and female groups administered honey was not altered. It was much lower than that of aspartame and sucrose. This has further demonstrated the anti-obesity potential of honey. Obesity has been associated with leptin and ghrelin regulation (Rahman *et al*., 2017). Aspartame and sucrose may cause leptin and insulin resistance which are associated with obesity, while honey corrects the effect (Rahman *et al*., 2017). The use of antipsychotic drugs which by nature are not caloric but could cause weight gain by increasing the appetite for the intake of food and drink intake may play a similar role here with regards to aspartame and sucrose causing the release of ghrelin or increasing leptin and insulin resistance (Zhang et al., 2016; Rahman *et al*., 2017). Honey may act in a reverse way to these effects being demonstrated by aspartame and sucrose in a very controlled manner. The mechanism of action of these various sweeteners requires further research. However, in a recent report by Hashim *et al*. (2021), the presence of caffeic acid and quercetin in honey reduces body weight and fat mass through the reduction in fatty acid synthase activity.

The abdominal circumference in the male and female rats in this study was increased in the aspartame and sucrose administered rats, while honey was not, compared with the control. Further increase was found in the female sucrose group compared with the aspartame group. Abdominal circumference is a good indicator for determining abdominal fat mass storage (Gerbaix *et al*., 2010). Abdominal circumference is an exact, simple and more reliable determinant of abdominal obesity or excess visceral fat (Muaidi and Ahsan, 2019). It is established that abdominal obesity, assessed by abdominal circumference, predicts obesity and health-related risk. Research finding indicated that abdominal circumference is a sound marker of a health risk than BMI; abdominal circumference is also a strong predictor of abdominal and non-abdominal fat (Muaidi and Ahsan, 2019). Intra-abdominal fat thickness (visceral fat mass) is a significant predictor of metabolic disturbances including; cardiovascular diseases, atherosclerosis, dyslipidaemia, and hypertension (Arika *et al*., 2019). This, therefore, means that taking honey as a sweetener is better than sucrose and aspartame. Taking honey drastically reduces the possibility of being obese as well as the chances of suffering from other metabolic syndromes such as diabetes, cardiovascular diseases and other heart-related diseases.

4. Conclusion

This study has further strengthened support for the health benefits of honey and its use as an anti-obesity agent. It has brought to the fore the importance of the use of locally produced honey as an agent for the prevention of overweight and obesity, irrespective of sex.

5. Acknowledgements

This research work was sponsored by a grant from the 2021 Institution-Based Research (IBR) Fund of the Tertiary Education Trust Fund Institution Based Research (TETFUND). The authors are grateful to TETFUND and the University of Cross River (UNICROSS) for their support.

References

Ajibola, A., Chamunorwa, J. P., & Erlwanger, K. H. (2012). Nutraceutical values of natural honey and its contribution to human health and wealth. *Nutrition & metabolism*, *9*, 61. https://doi.org/10.1186/1743-7075-9-61

Alvarez-Suarez J.M., Tulipani, S., Romandini, S., Bertoli, E., Battino, M. (2010). Contribution of honey in nutrition and human health: a review. *Mediterranian Journal of Nutrition and Metabolism*, 3**,** 15–23. https://doi.org/10.1007/s12349-009-0051-6

Arika, W. M., Kibiti, C. M., Njagi, J. M., & Ngugi, M. P. (2019). Anti-obesity effects of dichloromethane leaf extract of *Gnidia glauca* in high fat diet-induced obese rats. *Heliyon*, *5*(11), e02800. https://doi.org/10.1016/j.heliyon.2019.e02800

Atakan, M. M., Koşar, Ş. N., Güzel, Y., Tin, H. T., & Yan, X. (2021). The Role of Exercise, Diet, and Cytokines in Preventing Obesity and Improving Adipose Tissue. *Nutrients*, *13*(5), 1459. https://doi.org/10.3390/nu13051459

Burke, S. J., Batdorf, H. M., Martin, T. M., Burk, D. H., Noland, R. C., Cooley, C. R., Karlstad, M. D., Johnson, W. D., & Collier, J. J. (2018). Liquid Sucrose Consumption Promotes Obesity and Impairs Glucose Tolerance Without Altering Circulating Insulin Levels. *Obesity (Silver Spring, Md.)*, *26*(7), 1188–1196. https://doi.org/10.1002/oby.22217

Corey, K.E., & Kaplan, L. M. (2014). Obesity and liver disease: the epidemic of the twenty-first century. *Clinics in liver disease*, 18 (1), 1–18. http://dx.doi.org/10.10 16/j.cld.2013.09.019

Crézé, C., Candal, L., Cros, J., Knebel, J. F., Seyssel, K., Stefanoni, N., Schneiter, P., Murray, M. M., Tappy, L., & Toepel, U. (2018). The Impact of Caloric and Non-Caloric Sweeteners on Food Intake and Brain Responses to Food: A Randomized Crossover Controlled Trial in Healthy Humans. *Nutrients*, *10*(5), 615. https://doi.org/10.3390/nu10050615

de Moura E Dias, M., Dos Reis, S. A., da Conceição, L. L., Sediyama, C., Pereira, S. S., de Oliveira, L. L., Gouveia Peluzio, M., Martinez, J. A., & Milagro, F. I. (2021). Diet-induced obesity in animal models: points to consider and influence on metabolic markers. *Diabetology & metabolic syndrome*, *13*(1), 32. https://doi.org/10.1186/s13098-021-00647-2

Dornas, W. C., de Lima, W. G., Pedrosa, M. L., & Silva, M. E. (2015). Health implications of high-fructose intake and current research. *Advances in nutrition (Bethesda, Md.)*, *6*(6), 729–737. https://doi.org/10.3945/an.114.008144

Gerbaix, M., Metz, L., Ringot, E., & Courteix, D. (2010). Visceral fat mass determination in rodent: validation of dual-energy X-ray absorptiometry and anthropometric techniques in fat and lean rats. *Lipids in health and disease*, *9*, 140. https://doi.org/10.1186/1476-511X-9-140

Gręda, E., Matuszewska, J., Ziarniak, K., Gertig-Kolasa, A., Krzyśko-Pieczka, I., Skowrońska, B., & Sliwowska, J. H. (2022). Animal Foetal Models of Obesity and Diabetes - From Laboratory to Clinical Settings. *Frontiers in endocrinology*, *13*, 785674. https://doi.org/10.3389/fendo.2022.785674

Hall, J. E., do Carmo, J. M., da Silva, A. A., Wang, Z., & Hall, M. E. (2019). Obesity, kidney dysfunction and hypertension: mechanistic links. *Nature reviews. Nephrology*, *15*(6), 367–385. https://doi.org/10.1038/s41581-019-0145-4

Hashim, K. N., Chin, K. Y., & Ahmad, F. (2021). The Mechanism of Honey in Reversing Metabolic Syndrome. *Molecules (Basel, Switzerland)*, *26*(4), 808. https://doi.org/10.3390/molecules26040808

Haslam D. (2016). Weight management in obesity - past and present. *International journal of clinical practice*, *70*(3), 206–217. https://doi.org/10.1111/ijcp.12771

Islam, S. M., Purnat, T. D., Phuong, N. T., Mwingira, U., Schacht, K., & Fröschl, G. (2014). Non-communicable diseases (NCDs) in developing countries: a symposium report. *Globalization and health*, *10*, 81. https://doi.org/10.1186/s12992-014-0081-9

Jehan, S., Myers, A. K., Zizi, F., Pandi-Perumal, S. R., Jean-Louis, G., & McFarlane, S. I. (2018). Obesity, obstructive sleep apnea and type 2 diabetes mellitus: Epidemiology and pathophysiologic insights. *Sleep medicine and disorders: international journal*, *2*(3), 52–58.

Johnson, R. J., Sánchez-Lozada, L. G., Andrews, P., & Lanaspa, M. A. (2017). Perspective: A Historical and Scientific Perspective of Sugar and Its Relation with Obesity and Diabetes. *Advances in nutrition (Bethesda, Md.)*, *8*(3), 412–422. https://doi.org/10.3945/an.116.014654

Kang, L. L., Zhang, D. M., Jiao, R. Q., Pan, S. M., Zhao, X. J., Zheng, Y. J., Chen, T. Y., & Kong, L. D. (2019). Pterostilbene Attenuates Fructose-Induced Myocardial Fibrosis by Inhibiting ROS-Driven Pitx2c/miR-15b Pathway. *Oxidative medicine and cellular longevity*, *2019*, 1243215. https://doi.org/10.1155/2019/1243215

Malik, V. S., & Hu, F. B. (2022). The role of sugar-sweetened beverages in the global epidemics of obesity and chronic diseases. *Nature reviews. Endocrinology*, *18*(4), 205–218. https://doi.org/10.1038/s41574-021-00627-6

Muaidi, Q. I., & Ahsan, M. (2019). Measurement of Visceral Fat, Abdominal Circumference and Waist-hip Ratio to Predict Health Risk in Males and Females. *Pakistan journal of biological sciences*, 22 (4), 168-173.

Nguyen, H., Panyoyai, N., Kasapis, S., Pang, E., & Mantri, N. (2019). Honey and Its Role in Relieving Multiple Facets of Atherosclerosis. *Nutrients*, *11*(1), 167. https://doi.org/10.3390/nu11010167

Novelli, E. L., Diniz, Y. S., Galhardi, C. M., Ebaid, G. M., Rodrigues, H. G., Mani, F., Fernandes, A. A., Cicogna, A. C., & Novelli Filho, J. L. (2007). Anthropometrical parameters and markers of obesity in rats. *Laboratory animals*, *41*(1), 111–119. https://doi.org/10.1258/002367707779399518

Rahman, H. A., Sahib, N. G., Saari, N., Abas, F., Ismail, A., Mumtaz, M. W., & Hamid, A. A. (2017). Anti-obesity effect of ethanolic extract from Cosmos caudatus Kunth leaf in lean rats fed a high fat diet. *BMC complementary and alternative medicine*, *17*(1), 122. https://doi.org/10.1186/s12906-017-1640-4

Ramli, N. Z., Chin, K. Y., Zarkasi, K. A., & Ahmad, F. (2018). A Review on the Protective Effects of Honey against Metabolic Syndrome. *Nutrients*, *10*(8), 1009. https://doi.org/10.3390/nu10081009

Vermunt, S. H., Pasman, W. J., Schaafsma, G., & Kardinaal, A. F. (2003). Effects of sugar intake on body weight: a review. *Obesity reviews: an official journal of the International Association for the Study of Obesity*, *4*(2), 91–99. https://doi.org/10.1046/j.1467-789x.2003.00102.x

Vincent, H. K., Bourguignon, C. M., & Taylor, A. G. (2010). Relationship of the dietary phytochemical index to weight gain, oxidative stress and inflammation in overweight young adults. *Journal of human nutrition and dietetics: the official journal of the British Dietetic*

Association, *23*(1), 20–29. https://doi.org/10.1111/j.1365-277X.2009.00987.x

World Health Organisation (2021). *Obesity and overweight*. Geneva: World Health Organisation. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Zhang, J. P., Lencz, T., Zhang, R. X., Nitta, M., Maayan, L., John, M., Robinson, D. G., Fleischhacker, W. W., Kahn, R. S., Ophoff, R. A., Kane, J. M., Malhotra, A. K., & Correll, C. U. (2016). Pharmacogenetic Associations of Antipsychotic Drug-Related Weight Gain: A Systematic Review and Meta-analysis. *Schizophrenia bulletin*, *42*(6), 1418–1437. https://doi.org/10.1093/schbul/sbw058