UJMR, Vol. 8 No. 2, December, 2023, pp. 136 - 145 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2382.016

Received: 11 November 2023 **Accepted:** 16 December 2023

Changes In Biochemical Components of Obesity In *Wistar* Rats Fed *Mentha Piperita*-Supplemented High-Fat Diet

[1]Idoko, A.S., [1]Madaki, U.A., [1]Nura, L., [2]Sadiq, M.E and [1]Umar, S.

1 Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma

2 Usmanu Danfodiyo University, Department of Biochemistry and Molecular Biology, Sokoto, Nigeria

Corresponding author usmanmadaki04@gmail.com

Abstract

The research is designed to assess changes in biochemical components of obesity in Wistar rats fed peppermint-supplemented high-fat diet. Twenty (20) female Wistar rats of about 117.88±10.82g were divided into four (4) groups of five (5) rats each and randomly assigned to the formulated diets. The groups had similar average weights. Groups A and B were fed standard diet and High-fat diet respectively, while groups C and D were respectively fed 4% peppermint-supplemented standard diet and 4% peppermint-supplemented high-fat diet. The changes in weight and blood glucose level were monitored on weekly basis. At the end of six (6) weeks of the experiment, organ to body weight ratio, serum insulin level and HOMA-IR were determined. Markers of hepatic functions (transaminases, total bilirubin, albumin, globulin, conjugated bilirubin and total protein), markers of renal functions (electrolyte concentrations, urea and creatinine), serum activities of antioxidant enzymes (catalase, glutathione peroxidase, reduced glutathione and superoxide dismutase) and serum concentrations of malondialdehyde (MDA) were also determined. No significant (p≤0.05) differences were observed among the groups in weight gain, organ to body weight ratio, blood glucose levels, activities of antioxidant enzymes and serum MDA concentrations. The group fed 4% peppermint-supplemented high-fat diet had significantly (p≤0.05) higher activities of ALT, AST, total cholesterol and low-density lipoprotein cholesterol but significantly (p≤0.05) lower high-density lipoprotein cholesterol when compared with the group fed standard diet or high-fat diet. Serum creatinine and bicarbonate were significantly (p≤0.05) lower in the group fed 4% peppermint-supplemented high-fat diet compared with the group fed the standard diet. It is therefore concluded that including peppermint leaf powder in the diet for a period of 6 weeks may not have a significant impact on the body weight, organ development and glucose utilisation. However, it is important to note that consumption of high-fat diet supplemented with peppermint might lead to dyslipidemia, liver and kidney dysfunctions without altering oxidative balance in Wistar rats.

Keywords: Obesity, Mentha piperita (peppermint), High-fat diet, lipid profile, Dyslipidemia, Glucose, insulin resistance, antioxidant.

INTRODUCTION

The term "obesity" derives from the Latin word "obesitas," which translates to "stout, fat, or plump." This medical condition arises when the body accumulates excess fat, which can adversely affect human health (World Health Organisation, WHO, 2015). The hallmark of obesity is the deposition of excessive fat in adipose tissue, as well as in other internal organs such as the liver, heart, skeletal muscles, pancreatic islet, and blood (Derdemezis *et al.*, 2011; Singla *et al.*, 2010) Obesity is caused by intricate connections between genetic, behavioral, and environmental factors that are related to lifestyles, economic status, and social standing (WHO, 2020). The widespread prevalence of obesity across age groups, genders, and races makes it a significant global public health challenge (Berke and Morden, 2000; Kavita *et al.*, 2011). Management of obesity entails diverse interventions, including dietary changes, increased physical activity, and use of anti-obesity medications, dietary supplements, and even surgery.

The leaf of *Mentha piperita* has been utilized as a therapeutic agent for various ailments such as the common cold, inflammation of the mouth and pharynx, liver disorders, and gastrointestinal maladies including nausea, vomiting, diarrhea, cramps, flatulence, and dyspepsia. Additionally, it is recognized for its antioxidative, anti-inflammatory, antiviral, antimicrobial, and anticarcinogenic properties, as supported by Muhammad *et al.* (2017), Johari *et al.* (2015), Ferreira *et al.* (2014), Barbalho *et al.* (2011) and Rodriguez-fragoso *et al.* (2008).

This research is therefore designed to study diet supplementation with peppermint leaf powder as an affordable means of controlling obesity and its components in *Wistar* rats fed obesity-causing high-fat diet.

MATERIALS AND METHODS

Feed formulation

Corn starch, rice husk, soya beans meal, palm oil, vitamin premix and D-Methionine were purchased from Dutsin-ma central market while corn starch was prepared from yellow corn. Yellow corn was first soaked in water for 48 hours and then grinded. This was immediately filtered, the filtrate was then allowed to dry. The dried filtrate was collected as corn starch in powdered form. Peppermint was bought from Lambun Sarki Garden at Kofar Marusa area of Katsina town, Katsina State.

Plant materials

Peppermint was identified by a Botanist at the Department of Biological Science, Faculty of life Science, Federal University Dutsin-Ma and assigned a voucher number as FUDMA/PSB/00231.

Experimental Animals:

Twenty (20) female *Wistar r*ats were purchased from National Veterinary Research Institute, Vom, Plateau State, Nigeria. The rats were maintained according to approved standards for keeping experimental animals as approved by the Federal University Dutsin-Ma Ethical Committee on the use of experimental animals and registered as FUDMA/CEA/2023/078.

Preparation of the plant materials:

The leaves of *Mentha piperita* were cleaned and dried at 25℃ and pulverized into powdered using mortar and pestle.

Feed formulation and supplementation

The standard diet was formulated following the method of Idoko *et al*. (2022). Peppermint-supplemented standard diet was formulated by mixing thoroughly 96 g of the standard diet with 4 g of the prepared powdered peppermint, while Peppermint-supplemented high-fat diet was formulated by mixing 96 g of the high-fat diet with 4 g of the prepared powdered peppermint.

Experimental Design

The twenty (20) female *wistar* rats weighing about 117.88 ± 10.82g were divided into four (4) groups of five (5) rats each and randomly assigned to the formulated diets as follows:

A. Rats fed the standard diet
B. Rats fed high-fat diet
C. Rats fed 4% peppermint-supplemented standard diet
D. Rats fed 4% peppermint-supplemented high-fat diet

The rats were maintained on their respective diets *ad libitum* for a period of six (6) weeks.

Evaluation of Changes in Body Weight

Changes in weight were monitored weekly by measuring the experimental animal's body weights using an electronic weighing balance.

Animal Sacrifice and Collection of Blood Sample

At the end of the six (6) weeks experimental period, the animals were weighed and anaesthetized in a jar containing wool soaked in chloroform after measuring the blood glucose level using Accu-Chek glucometer. To obtain serum for analysis, the blood was collected in plain containers and centrifuged following the method Idoko *et al*. (2018)

Assessment of Organ Development

The organs such as liver, kidney and heart were dissected out and weighed immediately. The ratios of the organ to the live body weight were then computed (Milan *et al*., 2017).

Determination of Blood glucose

The blood glucose level during the experimental period was measured bi-weekly using Accu-check glucometer.

Determination of Serum Insulin level

The serum insulin level was determined using Randox assay kits according to manufacturer's instructions.

The homeostasis model assessment (HOMA) index signifies insulin resistance and is calculated as follows.

$$\text{HOMA - IR} = \frac{fasting\ insulin\ \left(\frac{umiu}{ml}\right) \times fasting\ glucose\ (mmol/l)}{22.5}$$

Yin *et al*. (2013).

Determination of anti-dyslipidemia Potentials of peppermint leaf powder

The serum concentrations of total cholesterol (TC), triglyceride (TG) and high-density lipoproteins (HDL) cholesterol concentrations were estimated using commercial Randox assay kits according to manufactures instruction. Low-density Lipoprotein (LDL) was computed as described by Friedewald *et al*. (1972).

Determination of liver function indices

Total protein, Albumin, Globulin, Bilirubin, Conjugated Bilirubin, Liver enzymes such as Alanine transaminase (ALT) and Aspartate transaminase (AST) were determined using their respective assay kits from Randox Laboratory Limited (UK) following the manufacturer's protocol.

Determination of kidney function indices

Creatinine and urea were determined enzymatically using assay kits from Randox Laboratory Limited (United Kingdom) according to the manufacturer's protocol. Electrolytes

such as sodium ion (Na^+), potassium ion (K^+), chloride (Cl^-) and bicarbonate ion (HCO_3^-) concentrations were determined using automated electrolyte machine.

Determination of antioxidant enzymes

Antioxidant enzymes such as Catalase, Superoxide dismutase (SOD), Glutathione peroxide (GPX) and Glutathione reductase were analysed according to the protocols by Randox Laboratories Ltd. United Kingdom.

Statistical Analysis

Results are presented as mean ±standard error of mean (SEM) of 3 determinants. Data was statistically analyzed using one-way analysis of variance (ANOVA) as described by SPSS version 22. Software package at 95% confidence interval.

RESULTS

Changes in Weight in *Wistar* Rats fed Peppermint- Supplemented Standard and High-Fat Diets

The average weekly weights of rats fed peppermint leaf powder- supplemented standard and high-fat diets are presented in Figure 1. There were no significant ($p \leq 0.05$) changes in the weight gains among the groups throughout the experimental period.

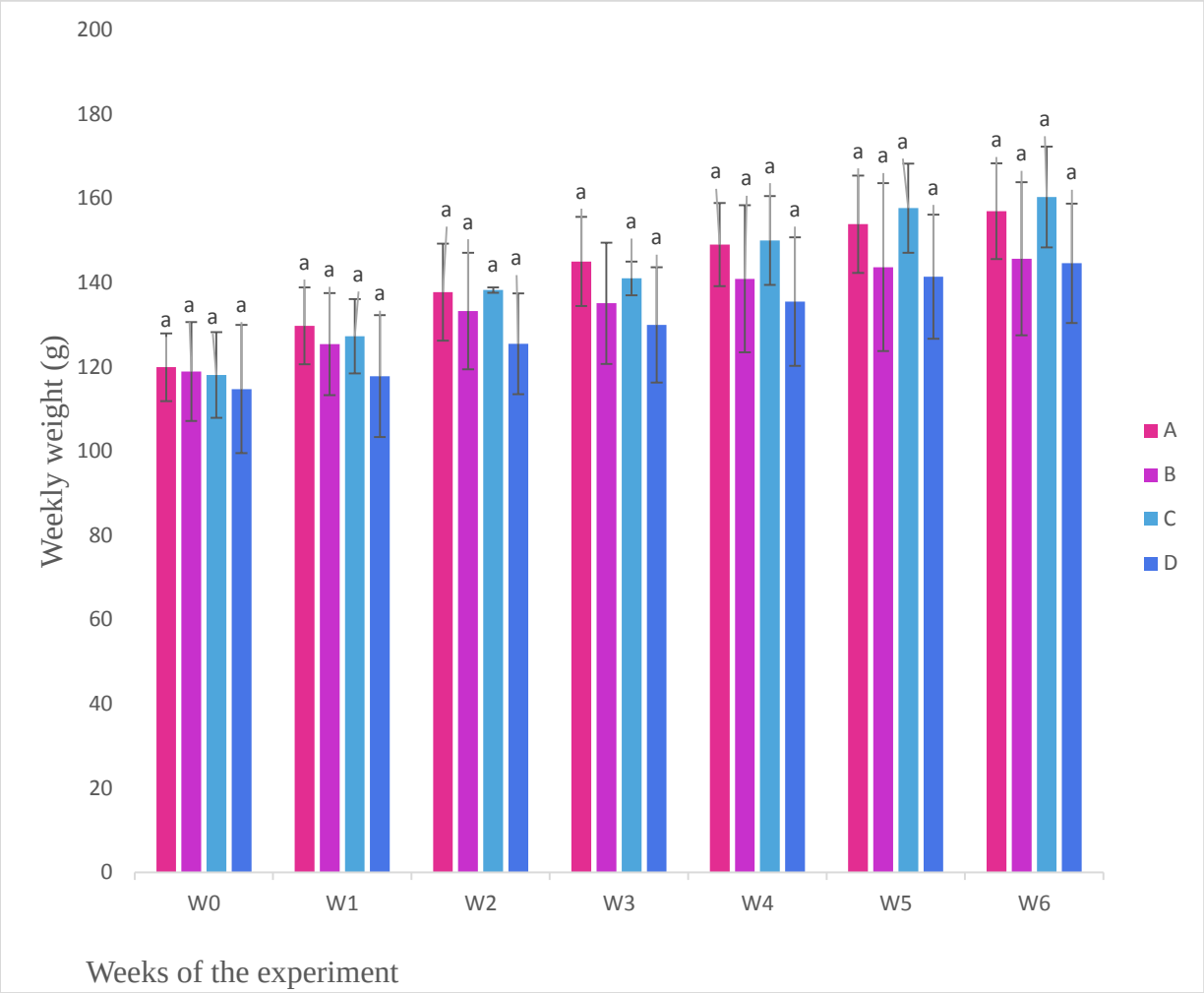

Figure 1: The average weekly weights of rats fed peppermint- supplemented standard and high-fat diets

Key: A= Group fed standard diet
B= Group fed high-fat diet
C= Group fed 4% peppermint-supplemented standard diet
D= Rats fed 4% peppermint-supplemented high-fat diet
W0= Day 1 W1-W6 = Week1 to Week6.

Resistance to Insulin in *Wistar* rats Fed Peppermint-Supplemented Standard and High-Fat Diets

Table 1 shows the determined insulin concentration, blood glucose level and computed HOMA-IR. No significant ($p \leq 0.05$) differences were observed between the groups

fed the peppermint-supplemented diets and the groups fed either standard diet or high-fat diet. The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly ($p \leq 0.05$) different

Table 1: Blood Glucose level, insulin concentration and HOMA-IR in *Wistar* rats Fed Peppermint-Supplemented Standard and High-Fat Diets

GROUP	INSULIN(µiu/ml)	BGL(mmol/l)	IR
A	21.50±2.49[a]	5.60±0.60[a]	5.40±0.89[a]
B	20.60±4.55[a]	5.20±0.36[a]	4.90±1.30[a]
C	18.40±3.61[a]	4.37±0.12[a]	4.27±0.68[a]
D	21.88±4.98[a]	4.60±0.26[a]	5.63±1.92[a]

Key: A=Group fed standard diet B-Group fed high-fat diet
C-=Group fed 4% peppermint-supplemented standard diet
D-=Rats fed 4% peppermint-supplemented high-fat diet
BGL=Blood glucose level, IR-insulin resistance

Lipid Profile levels in *Wistar* Rats Fed Peppermint- Supplemented Standard and High-Fat Diets

The group fed 4% peppermint-supplemented high-fat diet had significantly ($p \leq 0.05$) higher total cholesterol and low-density lipoprotein cholesterol but significantly ($p \leq 0.05$) lower high-density lipoprotein cholesterol when compared with the group fed standard diet or high-fat diet (Table 2). The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different

Table 2: Lipid profile level in *Wistar* rats Fed Peppermint- Supplemented Standard and High-Fat Diets

GROUP	TC(mg/dl)	TG(mg/dl)	HDL-C(mg/dl)	LDL-C(mg/dl)
A	102.67±4.37[a]	49.00±7.23[a]	25.33±3.76[b]	67.67±0.88[a]
B	107.00± 1.53[c]	49.67±3.18[a]	19.33±2.60[b]	77.67±4.33[a]
C	102.00±2.89[a]	35.00±2.00[a]	20.67±3.71[b]	74.00±5.29[a]
D	113.33±2.03[b]	42.67±4.06[a]	14.67±1.45[a]	90.33±2.33[b]

Key: A=Group fed standard diet
B=Group fed high-fat diet
C=Group fed 4% peppermint-supplemented standard diet
D=Rats fed 4% peppermint-supplemented high-fat diet
TC=Total Cholesterol
TG=Triglyceride
HDL-C=High-Density Lipoproteins Cholesterol
LDL-C=Low-Density Lipoproteins Cholesterol

Changes in Liver Function Indices in *Wistar* Rats Fed *peppermint leaf powder* Supplemented Standard and High-Fat Diets.

There were no significant ($p \leq 0.05$) changes observed in the liver function indices among the test groups, and in comparisons with the control (Table 3). The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different

Table 3: liver function indices in *Wistar* Rats Fed Peppermint- Supplemented Standard and High-Fat Diets

Group	TP(g/l)	ALB(g/l)	GLO(g/l)	TB(mg/dl)	CONJ.B(mg/dl)
A	69.33±2.73^a	46.67±0.88^a	22.67±1.86^a	3.20±0.57^a	1.70±0.42^a
B	69.33±1.76^a	42.67±2.33^a	26.67±0.88^a	2.33±0.44^a	0.83±0.17^a
C	67.67±1.20^a	42.67±1.20^a	25.00±1.73^a	2.33±0.44^a	0.83±0.17^a
D	66.00±1.53^a	44.33±0.33^a	21.67±1.86^a	3.33±0.60^a	0.96±0.23^a

Key: A=Group fed standard diet
B=Group fed high-fat diet
C=Group fed 4% peppermint-supplemented standard diet
D=Rats fed 4% peppermint-supplemented high-fat diet
TP=Total Proteins
ALB=Albumin
GLO=Globulin
TB-C=Total Bilirubin
Conj. B= Conjugated bilirubin

Changes in Transaminase Activities in *Wistar* Rats Fed Peppermint-Supplemented Standard and High-Fat Diets

The group fed 4% peppermint-supplemented high-fat diet had significantly ($p \leq 0.05$) higher activities of ALT and AST when compared with the group fed standard. There was no significant difference between the group fed 4% peppermint-supplemented high-fat diet and the group fed high-fat diet in the activities of the enzymes (Table 4). The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different.

Table 4: Transaminase activities in *wistar* rats Fed Peppermint Leaf Powder-Supplemented Standard and High-Fat Diets

GROUP	ALT(U/L)	AST(U/L)
A	4.67±0.33^a	10.33±0.67^a
B	9.33±1.20^b	19.67±2.90^c
C	4.67±0.33^a	9.00±1.15^a
D	8.33±1.45^b	17.67±3.84^c

Key: A=Group fed standard diet
B=Group fed high-fat diet
C=Group fed 4% peppermint-supplemented standard diet
D=Rats fed 4% peppermint-supplemented high-fat diet
ALT=Alanine Aminotransferase
AST=Aspartate Aminotransferase

Changes in Kidney Function Indices in *Wistar* Rats Fed Peppermint Leaf Powder-Supplemented Standard and High-Fat Diets.

The groups fed high-fat diet and 4% peppermint-supplemented high-fat diet had significantly ($p \leq 0.05$) lower serum bicarbonate while only the group fed 4% peppermint-supplemented high-fat diet had significantly ($p \leq 0.05$) lower concentration of creatinine compared with the control. No significant ($p \leq 0.05$) variations were observed in other studied kidney function indices (Table 5). The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different.

Table 5: Kidney function indices in *Wistar* rats Fed Peppermint Leaf Powder- Supplemented Standard and High-Fat Diets

GROUP	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3 (mmol/L)	UREA (mmol/L)	CREATININE (mmol/L)
A	132.67±2.85^a	7.40±0.95^a	107.67±1.86^a	6.33±1.45^a	14.03±3.42^a	146.00±20.55^b
B	137.67±3.76^a	8.77±0.44^a	110.67±1.86^a	4.33±0.33^c	11.33±1.22^a	168.33±1.76^b
C	131.67±1.45^a	6.77±0.28^a	106.00±1.00^a	7.33±1.67^b	9.40±1.05^a	103.00±13.89^b
D	136.33±4.37^a	9.63±0.91^a	111.00±0.58^a	2.67±0.88^b	13.00±1.47^a	68.33±2.03^a

Key: A=Group fed standard diet
B=Group fed high-fat diet
C=Group fed 4% peppermint-supplemented standard diet
D=Rats fed 4% peppermint-supplemented high-fat diet
Na^+=Sodium,K^+=Potassium,Cl^- =Chloride,HCO_3^-=Bicarbonate

Antioxidant levels in *Wistar* Rats Fed Peppermint Leaf Powder- Supplemented Standard and High-Fat Diets

As could be seen in Table 6, there were no significant ($p \leq 0.05$) differences among the groups in both the activities of the studied enzymes and in the concentrations of MDA. The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly ($p \leq 0.05$) different.

Table 6: Antioxidant levels in *Wistar* rats Fed Peppermint Leaf Powder- Supplemented Standard and High-Fat Diets

GROUP	GPX (u/mL)	SOD (u/mL)	CAT (u/mL)	GSH (ug/mL)	MDA (nmol/mL)
A	32.05±17.09^a	19.87±1.98^a	13.93±1.81^a	65.23±16.38^a	189.83±9.44^a
B	51.23 ±12.68^a	19.17±1.74^a	13.07±2.87^a	33.40±9.94^a	176.27±13.76^a
C	36.09±8.08^a	14.00±1.80^a	15.03±4.21^a	66.47±17.75^a	156.50±36.68^a
D	42.36±19.60^a	14.47±3.66^a	9.53±2.32^a	35.37±3.37^a	148.57±9.42^a

A=Group fed standard diet
B=Group fed high-fat diet
C=Group fed 4% peppermint-supplemented standard diet
D=Rats fed 4% peppermint-supplemented high-fat diet
GPX= Glutathione Peroxidase; SOD=Superoxide Dismutase; CAT=Catalase; GSH=Reduced Glutathione; MDA=Melondialdehyde

DISCUSSION

Consumption of high-fat diet for a long period plays a significant causative role in the development of obesity and is associated with increased risk of cardiometabolic diseases (Wang *et al.*, 2020; Shan *et al.*, 2018) since dietary fat is easily stored in form of body fat. Further, it had long been shown that fat is either almost completely used or completely stored in accordance with the energy balance fluctuations. This is unlike carbohydrate which is tightly regulated (Golay and Bobbioni, 1997).

The absence of significant difference among the studied groups even when they consumed similar amount of diet is therefore not in agreement with the reported role of high-fat diet in development of overweight and obesity. The reason for this is unclear but could mean that 6-week feeding on high-fat diet may not lead to manifestations of overweight and organ abnormality. In a study by Zhuang *et al.* (2022), experimental mice were fed for 9 months before the manifestations of the adverse effects of high-fat diet. Similarly, Hasegawa *et al.* (2020) observed manifestation of adverse effects such as overweight on mice after 5 months. It follows from the finding in this research that it may take much longer period than 6 weeks before rats fed high-fat diet could become obese or over weight.

The observed non-significant difference in insulin sensitivity is understandable since there was no difference in weight gain. Weight gain, particularly visceral adiposity is a major cause

ofinsulin resistance as it leads to increased production of inflammatory cytokine which impair insulin signalling (De Mutsert *et al.*, 2018; Hardy *et al.*, 2012). On the other way, weight gain could result from insulin resistance since more insulin is produced in a state of insulin resistance. This increase in insulin favors increased feed intake and fat storage (Ferrario & Finnell, 2023). It could also be that longer period of feeding is necessary before the manifestations of the effects on glucose utilization and insulin sensitivity.

As expected, high-fat diet caused hypercholesterolemia in the rats. The significantly higher cholesterol and LDL-cholesterol, but lower HDL-cholesterol in the group fed 4% peppermint-supplemented high-fat diet is an indication that such supplementation, instead of ameliorating dyslipidemia associated with high-fat diet could rather complicate it. This could have resulted from the lipid and high volatile oil content (Mainasara *et al.*, 2018) of the mint. The addition of the mint to a diet already containing high-fat may have raised the lipid component of the diet beyond acceptable level leading to dyslipidemia. This calls for concern as dyslipidemia is at the root of many metabolic and cardiovascular diseases. It is for this that reducing LDL-C levels had long been recognized as an important means of managing dyslipidemia (Hadaegh *et al.*, 2022). Circulating lipid components, if above the acceptable levels get deposited in the artery wall and subsequently become involved in atherogenic process (Hedayatnia *et al.*, 2020; Proctor & Mamo, 1998). It follows from the finding that supplementation of high-fat diet with peppermint up to 4% may cause more harm than good.

A significant increase in the activities of liver enzymes such as ALT and AST reflects hepatocellular disorders (Lavanaya *et al.*, 2011). The significantly higher ALT and AST levels in the groups fed high-fat diet and 4% peppermint supplemented high-fat diet is therefore an indicator of disruptions in their hepatocellular structures. This is consistent with the dyslipidemia seen in these groups. Dyslipidemia is closely associated with liver diseases and is common in people having non-alcoholic fatty liver disease (NAFLD) and has been used as clinical index of liver damage especially in HBV-positive patients (Shoaib *et al.*, 2023). Furthermore, anti-dyslipidemic drugs have been successfully used in the management of liver diseases (Kathak *et al.*, 2022; Ciffone *et al.*, 2019).

The significantly lower bicarbonate in the groups fed high-fat diet and 4% peppermint-supplemented high-fat diet is consistent with the dyslipidaemia and elevation of serum ALT and AST in the groups, and shows the that the two diets might cause kidney dysfunction. Low bicarbonate also called metabolic acidosis occurs when the kidney fails to adequately remove acid from the body. Low serum bicarbonate is associated with both acute and chronic kidney diseases, hypertension and cardiac dysfunction (Masevicius *et al.*, 2017; Dobre *et al*, 2015; Raphael *et al.*, 2014). Chronic kidney disease predisposes to premature death due to increased risk of cardiovascular diseases such as ischemic heart disease (Shroff *et al.*, 2014) stroke (Masson *et al.*, 2015).

This study shows that the high-fat diet and peppermint-supplemented diets did not result in any oxidative stress. This is not in agreement with Ansari *et al.* (2012) who reported that high-fat diet-induced obese rats were characterized by decreased SOD and CAT activities and glutathione levels as well as increased TBARS which they attributed to accumulated superoxide radicals and hydrogen peroxide. Oxidative stress, dyslipidemia and organ damage are interrelated. It could be proposed from the finding in this research that dyslipidemia and kidney and liver dysfunction seen in this study may not have progressed through oxidative. In other words, it may take much longer period than 6 weeks before oxidative defence system of the rats fed the high-fat and peppermint-supplemented high-fat is overwhelmed.

CONCLUSION

The evaluated peppermint as an anti-obesity supplement in *Wistar* rats fed obesity-causing high-fat diet. Following the findings, it is concluded that including peppermint leaf powder in the diet for a period of 6 weeks does not have a significant impact on the body weight, organ development and glucose utilization. However, it is important to note that consumption of high-fat diet supplemented with peppermint might lead to dyslipidemia, liver and kidney dysfunctions without altering oxidative balance in *Wistar* rat

REFERENCES

Ansari, S. A. & Husain, Q. (2012). Potential applications of enzymes immobilized on/in nano materials: A review. *Biotechnology advances*, **30**(3): 512-523. **[Crossref]**

Barbalho, S.M., Machado, F.M.V.F., Oshiiwa, M., Abreu, M., Guiger, E. L., Tomazela, P. & Goulart, R. A. (2011). Investigation of the effect of peppermint (*Mentha piperita)* on the biochemical and anthropometric profile of university

students. *Ciencia Tecnologia Alimentacion Campinas,* **31**(3): 584-588. [Crossref]

Barbalho, S.M., Damasceno, D.C., Spada, A.P., Silva V.S., Martuchi, K.A., Oshiiwa, M., Machado, F.M. & Mendes, C.G. (2011). Metabolic Profile of Offspring from Diabetic Wistar Rats Treated with *Mentha piperita* (Peppermint). *Evi dence Based Complement Alternative M edicine*. 430237. [Crossref]

Berke, M.E. & Morden, E.N. (2000). Medical management of obesity. *American Family Physician*. **62**(2): 419-26.

Ciffone, N. A. & Copple, T. (2019). Managing dyslipidemia for CVD prevention: A review of recent clinical practice guidelines. *Nurse Practitioner*. **44**: 8-16. [Crossref]

De Mutsert R., Gast, K., Widya, R., Dekoning, E., Jazet, I., Lamb, H., Lecessie, S., De Roos, A., Smit, J., Rosendaal, F., Den, Haidar M. (2018). Associations of Abdominal Subcutaneous and Visceral fat with Insulin Resistance and secretion differ between Men and Women: The Netherlands Epidemiology of obesity. *Clinical Experimental Rheumatology*. **16**(1): 54-63. Epub 2018 Jan 17 PMID: 29338526. [Crossref].

Derdemezis, C.S., Voulgari, P.V., Drosos, A.A. & Kiortsis, D.N. (2011). Obesity, adipose tissue and rheumetoid arthritis: coincidence or more complex and relationship? *Clinical Experimental Rheumatology*. **29**: 112-2. Enup 2011 Sep 1. PMID: 21640051.

Dobre, M., Yang, W. & Pan, Q. (2015). Persistent high serum bicarbonate and the risk of heart failure in patients with chronic kidney disease (CKD): a report from the Chronic Renal Insufficiency Cohort (CRIC) study. *Journal of American Heart Association* **4**(4):e001599. [Crossref]

Ferrario, C. J. & Finnell, J.E. (2023). Beyond the hypothalamus: Roles of insulin as a regulator of neurotransmission, motivation, and feeding. *Neuropsychopharmacology*, **48** (1): 232-233. [Crossref].

Ferreira, P., Cardoso,T., Ferreira, F., Fernandes, Ferreira M., Piper P. & Sousa, M.J. (2014). Mentha piperita essential oil induces apoptosis in yeast associated with both cytosolic and mitochondrial ROS mediated damage. FEMS Yeast Res. Salicylic acid elicitation during cultivation of the peppermint plant improves anti-diabetic effects of its infusions. *Food Function*. **6**(6): 1865-1874. [Crossref]

Friedewald, W. T., Levy, R. I. & Fredrickson, D.S. (1972). Estimation of low-density lipoprotein cholesterol without the use of the preparative ultracentrifuge. *Clinical Chemistry*, **18**(6): 499-. [Crossref]

Golay, A. & Bobbioni, E., (1997). The Role of Dietary Fat in Obesity. *International Journal Obesity related metabolism Disorders*. **21**, S2-S11.

Hadaegh, A., Akbarpour, S., Tohidi, M., Barzegar, N., Hosseinpour-Niazi, S., Azizi, F. & Hadaegh, F. (2022). The role of different lipid measures for incident hypertension during more than 12- year folloe up: Tehran lipid and Glucose study. *British journal of nutrition*, **128**(9): 1700-1710. [Crossref].

Hardy, Olga T., Czech, Michael P. & Corvera, S. (2012). What causes the insulin resistance underlying obesity? *Current opinion in Endocrinology & Diabetes and obesity*, **19**(2): 81-87. [Crossref]

Hasegawa, Yu, Chen, Shin-Yu, Sheng, Lili, Jene, Prasant, Kumar, Kalanetra, Karen M., Mills David A., Wan, Yu-jui Yvonne & Slupsky, Carolyn M. (2020). Long-term effects of western diet consumption in male and female mice. *Scientific Reports*.**10:**14686. [Crossref]

Hedayatnia, M., Asadia, Z., Zare-Feyzabadi, R., Yaghooti-Khorasani, M., Ghazizadeh, H., Ghaffarian-Zirak, R., Nosrati-Tirkani, A., Mohammadi-Bajgiran, M., Rohban, M., Sadabadi, F., Rahimi, H.R., Ghalandari, M., Ghaffari, M.S., Yousefi, A., Pouresmaeili, E., Besharatlou, M.R. & Moohebati, M. (2020). Dyslipidemia and Cardiovascular disease risk among the MASHAD study population. *Lipids in health and Diseases*. **19**(1): 42. PMID: 32178672; PMCID: PMC7075010. [Crossref]

Idoko, A.S., Abdullahi, A., Maibulangu, B.M., Nura, L., Imam, N.U., Muhammed, F. & Umar, S. (2022). *Allium sativum* and *Curcuma longa* Powder Protect against Hepatotoxic and Nephrotoxic Effects of High-fructose Diet. *FUOYE Journal of Pure and Applied Sciences*. 7(1): 59-78. [Crossref]

Idoko, A.S., Zaharaddeen, A.S., Muhammad, F., Nwabuke, G.C., Oyolola, O.S. & Ahmed, H. (2018). Growth response and biochemical changes in Wistar rats fed on mixed spices-supplemented

diet. *FUDMA Journal of Sciences*. **2**(3):34-40.

Johari, N.Z., Ismai,l I.S., Sulaiman, M.R, Abas, F. & Shaari, K. (2015). Acute toxicity and metabolomics analysis of hypocholesterolemic effect of *Menthapiperita* aqueous extract in *Wistar* rats. *International Journal of Applied Research in Natural Products*, **8**(1): 1-11. [Crossref]

Kathak, R.R., Sumon, A. H., Molla, N.H., Hassan, M., Miah, R., Tuba, H. R., Habi, A., Ali, N. (2022). The association of between elevated lipid profile and liver enzymes: a study bon Bangladashi adults. *Scientific Reports* **12**(1): 1711. [Crossref]

Kavita, P., Sanjivani, K., lena, B., Gerard, E.M. & lewrence, J.C. (2011). Nutracetical supplements for weight loss: A systematic review. *Nutritional clinical practice*. **26**:539-552. [Crossref]

Lavanaya, S., Ramesh, M., Kavitha, C. & Malarvizhi, A. (2011). Hematological, biochemical and ionoregulatory responses of Indian major carp Catla catla during chronic sublethal exposure to inorganic arsenic. *Chemosphere*, **82**(7), 977-985. [Crossref]

Mainasara, Muhammad Murtala, Abubakar, Mohd Fadzelly, Waziri, Ahmed Hassana & Musa, Amina Rabe. (2018). Comparison of phytochemical, proximate and mineral composition of fresh and dried peppermint (*Menthapiperita*) leaves: *Journal of Science and Technology* **10**(20): 85-91. [Crossref]

Masevicius, F.D., Rubatto, Birri P.N., Risso, Vazquez, A., Zechner, F.E., Matta, M.F., Valenzuela, Espinoza, E.D., Welsh, S., Guerra, Arias E.F., Furche, M.A., Berddaguer, F.D. & Durbin, A. (2017). Relationship of at admission lactate, unmeasured anions and chloride to the outcome of critically ill patients. *Critical care medicine* **.45**(12): e1233-9. [Crossref]

Masson, P., Webster, A.C, Hang, M., Turner R., Lindley R.I. & Craig, J.C. (2015). Chronic kidney diseases and the risk of stroke: a systematic review and meta-analysis. *Nephrology, Dialysis and transplantation*. **30**(7):1162-9. Doi: 10.1093/ndt/gfv009. [Crossref]

Milan, B., Nazmul, A., Nilanjana, P., Khaled, H., Mohammad, A.R., Masashi, K., Nobutaka, O. & Anwarul, A.A. (2017). Amelioration of arsenic-induced toxic effects in mice by dietary supplementation of *Syzygium cumini* leaf extract. Nagoya. *Journal of Medical Science*, **79**: 169-179. [Crossref].

Muhammad, F., Wiley, J. & Riviere, J.E. (2017). Influence of some plant extracts on the transdermal absorption and penetration of marker penetrants. *Cutaneous and Ocular Toxicology*. **36**(1): 60-66. [Crossref]

Proctor, S.D. & Mamo, J.C. (1998). Retention of fluorescent-labelled chylomicrons remnants within the intima of the arterial wall-evidence that plaque cholesterol may be derived from post-prandial lipoproteins. *Journal of Chinese Investment*. **28**: 497-503. [Crossref]

Raphael, K.L., Zhang, Y., Ying, J. & Greene, T. (2014). Prevalence of and risk factors for reduced serum bicarbonate in chronic kidney disease. *Nephrology (Carlton)*, **9**(10): 648-54. [Crossref]

Rodriguez-Fragoso, L., Reyes-Esparza, J., Burchie, S.W., Herrera-Ruiz, D. & Torres, E. (2008). Risks and benefits of commonly used herbal medicines in Mexico. *Toxicology and Applied Pharmacology*. **227**(1): 125-135. [Crossref]

Shan, Ruiqi, Duan, Wei, Liu, Qi Lei, Jiayue, Gao, Jian, Zhang, Yunlong, Du, Shanshan, Han, Tianshi, Pang, Xiuyu, Sun, Changhao & Wu, Xiaoyan. (2018). *Nutrients*, **10**(1) 77. Published 2018 Jan 12. [Crossref].

Shoaib, M., Saleem, A., Zeb, A., Khan, M.I. & Akhtar, M.F. (2023). Chemical characterization and ameliorating Effect of *Centratherum anthelminticum* extract against polycystic ovary syndrome in Wistar rats. *International Journal of Endocrinology*. pmid: 37483646. [Crossref]

Shroff, G.R., Frederick, P.D. & Herzog, C.A. (2014). Renal failure and acute myocardial in-farction: clinical characteristics in patients with advanced chronic kidney disease, on dialysis, and without chronic kidney disease. A collaborative project of the United States Renal Data System/National Institutes of Health and the National Registry of Myocardial Infarction. *American Heart Journal*. **163**: 399-406. [Crossref]

Singla, P., Bardoloi, A. & Parkash, A.A. (2010). Metabolic effects of obesity: A

review. *World Journal Diabetes*. **1**: 76-88. **[Crossref]**

Wang, L., Wang, H., Zhang, B., Popkin, B.M. & Du, S. (2020). Elevated Fat Intake Increases Body Weight and the Risk of Overweight and Obesity among Chinese Adults: 1991-2015 Trends. *Nutrients*. **12**(11): 3272. **[Crossref]**.

World Health Organisation (2015). Obesity and overweight fact sheet no. 311. www. Int./medicate /factsheet/F5311/en

World health organization (2020). obesity and overweight, available form: https://www.www.int/news-room/fact-sheets/details -obesity- and overweight.

Yin, J., Li, M., Xu, L., Wang, Y., Cheng, H., Zhao, X., Mi, J. (2013). Insulin Resistance determined by Homeostasis Model Assessment (HOMA) and associations with metabolic syndrome among Chinise children and teenagers. *Diabetology and metabolic Syndrome*. **5**(1): 71. DOI: 10.1186/1758-5996-5-71. https://doi.org/10.1186/1758-5996-5-71.

Zhuang, H., Yao, X., Li, H., Li, Q., Yang, C., Wang, C., Xu, D., Xiao, Y., Gao, Y., Gao, J., Bi, M., liu, R., Teng, G. & Liu, L. (2022). Long-term high-fat diet consumption by mice throughout adulthood induces neurobehavioral alterations and hippocampal neural remodelling accompanied by augmented microglial lipid accumulation. *Brain, behaviour and immunity*, **100**: 155-171. https://doi.org/10.1016/j.bbi.2021.11.018