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Comparative biochemical study on the effect of Ginger, Orlistat or Chitosan on Obesity in experimental animals

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ABSTRACT

Obesity is characterized by the expanded mass of adipose tissue, which is accompanied by fat accumulation. Adipose tissue is imperative for energy balance, as indicated by the metabolic necessities of the life form. This study made a comparison between commercially used anti-obesity drugs (Orlistat and Chitosan) and ginger as a natural weight management plant, on rats fed a high-fat diet (HFD) in order to explore some side effects of the drugs. Fifty albino rats were classified into five groups: control, HFD, HFD supplemented with dietary ginger, HFD supplemented with Orlistat and HFD supplemented with Chitosan. Results showed that all different treatments had a significant effect on reducing the body weight and lipid profile. Ginger supplementation increased high-density lipoprotein (HDL)-cholesterol compared with other treatments; it also did not change total bilirubin and pancreatic lipase activity, but Orlistat and Chitosan lowered the concentrations. A HFD changed levels of hepatic mRNA expression of glucose transporter-2 and pyruvate kinase, which were then counteracted by ginger, Orlistat and Chitosan. In conclusion, Orlistat and Chitosan reduce body weight by inhibiting pancreatic lipase, whereas ginger has a greater capability in reducing body weight

without affecting the bilirubin concentration or inhibiting the pancreatic lipase level, with a positive effect on increasing HDL-cholesterol and peroxisomal catalase levels, suggesting that ginger has excellent potential against HFD-induced obesity.

Key words: High fat diet, Obesity, Ginger, Orlistat, Chitosan

1. INTRODUCTION

Obesity is a widespread chronic metabolic disease in the world, which is primarily induced by an energy imbalance resulting in irregular or extreme body fat accumulation (Huang *et al*., 2015). Hyperlipidemia is associated with biochemical alterations in the blood, accompanied by an increased concentration of lipids (Mishra *et al*., 2016). Anti-obesity drugs reduce the body weight by lowering food absorption or consumption or by elevating energy expenditure (Cooke and Bloom, 2006). Lifestyle adjustments, such as exercise and diet, are vital for both preventing and managing obesity; using drug therapy may be important if diet and exercise are useless for obese people with a body mass index (BMI) ≥30 kg/m^2 (Glazer, 2001).

Several medicines have been discovered to control obesity. However, anti-obesity drugs that were accepted and sold were prevented due to their dangerous side effects. For example, dexfenfluramine and fenfluramine were prevented due to their side effects on the heart. The European Medicines Agency withdrew numerous anti-obesity drugs, such as diethylpropion, phentermine and mazindol, because of their hazardous side effects (Kaukua *et al*., 2003).

Orlistat (Xenical) is the drug accepted by the Food and Drug Administration to control weight loss. It is a lipase inhibitor that lowers the absorption of dietary fat by about 30% and is shown to be valuable in both weight maintenance and weight loss (Codo˜ner-Franch *et al*., 2011). Orlistat was permitted in 1998 and is still the only drug available to prevent and control increased body weight. The given dose is a 120-mg capsule three times daily. The valuable efficiency on body weight is enough to recover various cardiac parameters, such as blood pressure, blood glucose levels and lipid profiles. The side effects of Orlistat include faecal incontinence, diarrhea, flatulence, oily spotting, dyspepsia and bloating (Torgerson *et al*., 2004).

Chitosan is mostly a deacetylated polymer of N-acetylglucosamine derived from the polysaccharide chitin. It occurs in a few dietary supplements as a nutritional fibre with high biocompatibility (Liu *et al*., 2011). Regarding its slight biodegradability and toxicity, Chitosan possesses substantial application and can be used successfully in the pharmaceutical industry (Kim *et al*., 2014). Moreover, Chitosan possesses anti-ulcer, wound-healing, hypocholesterolaemic, anti-acid, anti-tumour and haemostatic properties. Recently, it has been used in the pharmaceutical industry as a matrix molecule for tissue engineering applications and drug delivery (Huang *et al*., 2015).

Plant supplements and diet-based treatments to prevent obesity are the most public alternative medicine strategies (Zhang *et al*., 2012). The crude extracts and compounds isolated from plants are considered as natural products that are able encourage weight loss and prevent obesity and used in controlling increased fat accumulation. This is due to their content of active components with dissimilar anti-obesity and anti-oxidant properties on body metabolism and fat oxidation (Hasani-Ranjbar *et al*., 2013).

Ginger is the rhizome of *Zingiber officinale* that is used around the world in different recipes as a spice. It is native to Asia, Africa and other tropical regions (Akhani *et al*., 2004). The ginger plant has deep roots and vertical, upright stems, with nodal rhizomes and numerous divided swells (Kang and Park, 2012). Ginger has a spicy taste and an attractive odour; when dried to a powder, its colour ranges from yellowish brown to yellowish white (Arablou and Aryaeian, 2014). The dehydrated constituents of gingerols are the shogaols that are accountable for the pungency of dried ginger. During the thermal process, gingerols are converted to shogaols, and the rate of degradation depends on the environmental pH (Ali *et al*., 2008). Gingerol, a major pungent integral of ginger, has pharmacological characteristics, as anti-obesity, antioxidant and antiinflammatory (Suk *et al*., 2015).

This study examines some side effects of the commercially used anti-obesity drugs (Orlistat and Chitosan) and compares them with ginger as a natural weight management plant, using rats fed a high-fat diet (HFD). Biochemical and genetic investigations were conducted for this purpose.

2. MATERIALS AND METHODS

Chemicals

Orlistat (as capsules, each capsule containing 120 mg Orlistat) and Chitosan (medium molecular weight) were obtained from Sigma-Aldrich Pharmaceutical Industries, USA. Ginger was obtained from local markets, peeled, and minced, dried in air and ground; the powder was then mixed with the food to prepare diets containing 5% ginger according to Nirmala et al. (2010).

Animals

Fifty male albino rats, weighing 140–145 g, were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA, Giza, Egypt). Rats were kept one per cage for 3 days for adaptation before starting the experiment, in a temperature-controlled room (25 ± 5 °C), with relative humidity (50 ± 10%) and a 12-hour light/dark cycle. The experiment was approved by the Ethical Committee of VACSERA, approval number MI (782).Egyptian Research Ethics Committees.

Rats were assigned to five groups (10 rats/group) as follows:

Group 1: Control group (C): Animals were fed a basic diet, for six weeks.

Group 2: High-fat diet group (HFD): Served as the positive control; rats were fed a HFD (containing 55% corn oil) according to Jacobs (1983), for six weeks.

Group 3: Ginger group (HFD+Gin): Rats were fed a HFD containing 5% dried ginger powder according to Polasa et al. (2013), for six weeks.

Group 4: Orlistat group (HFD+Or): Rats were fed a HFD and received a therapeutic dose of orlistat (200 mg/kg diet) according to Nishioka et al. (2003), for six weeks.

Group 5: Chitosan group (HFD+Ch): Rats were fed a HFD supplemented with dietary Chitosan (2 g/kg diet) according to Anandan et al. (2013), for six weeks.

No animal death occurred throughout the experimental period. The HFD increased the animals' weight as observed by recording the body weight of each animal weekly; food consumption was also recorded. At the end of the experimental period (six weeks), animals were made to fast overnight and allowed free access to water. They were sacrificed under anesthesia with diethyl ether, and blood samples were collected and centrifuged to split the serum, then kept at -80℃ for biochemical determinations. Livers were directly removed and weighed and kept on ice.

Biochemical analysis

Serum triglycerides (TGs), total lipids (TLs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and serum total bilirubin were determined by commercial kits provided by Bio-diagnostic Company, Egypt. Pancreatic lipase activity was evaluated by colorimetric methods according to Tsuzuki et al. (2004).

Determination of peroxisomal liver catalase activity

Catalase activity in peroxisomes was determined according to Leighton et al. (1968). Livers were homogenized in isolation medium 1 [0.25 M sucrose, 10 mM MOPS buffer, pH 7.4, 1 mM EGTA and 0.1 mM PMSF]. The mitochondrial fraction was isolated by differential centrifugation. Peroxisomes were collected and laden on Nycodenz gradients, then were kept at a constant temperature of 4°C. Purified peroxisomes were diluted and centrifuged for 30 minutes to eliminate soluble proteins escaped from broken particles. Fasting serum leptin and adiponectin were analyzed using several commercial rat enzyme-linked immunosorbent assay kits of (R&D, Inc., USA), according to the instructions of the manufacturer. Liver peroxisomes from rats were isolated and detected according to the method described by Goush and Hajra (1986). Livers were homogenized in 0.25 M sucrose with 10mM Tris-HCl. The mitochondrial (t) fraction was gained by liver homogenate centrifugation and suspended in the same buffer in such a way that 1 ml of the suspension obtained from 1g liver (mg protein/ml).

Gene expression of pyruvate kinase (PK) and glucose transporter-2 (GLUT-2)

About 100 mg of liver tissue samples was used for total RNA extraction, using QIAzol lysis reagent (QIAGEN Inc., Valencia, CA, USA), following the instructions of the manufacturer. Add 5 mm stainless steel beads in the microtubes, and allocate the collection microtube stand in dry ice containing box. Eliminate the liver tissue from RNA Stabilization Reagent or from cold storage. Rotate the Tissue Lyser stand to let total homogenization, homogenize again for 5 min at 25 Hz. Put the collection microtube rack on the bench top for 3 minutes at room temperature. Centrifuge again at 6000 rpm (approximately 5600 x g) for 4 min.

Semi-quantitative polymerase chain reaction PCR

Gene expressions concerned with metabolism were confirmed by semi-quantitative polymerase chain reaction using primers. The genes tested were GLUT-2, using a primer sequence (5'–3'): forward: AAGGTCAAAGCCATGTTGG and reverse: GGAGACCTTCTGCTCAGTGG; and PK, using a primer sequence (5'–3'): forward: ATTGCTGTGACTGGATCTGC and reverse:

CCCGCATGATGTTGGTATAG, according to Ismail et al. (2013).β-actin expression (accession number: V01217) mRNA was tested using specific primer sequences: forward: ATGTACGTAGCCATCCAGGC and reverse: TCCACACAGAGTACTTGCGC.

Statistical analysis

Analysis was done using SPSS, version 20, and GraphPad Prism analytical software (8.0) for Windows, using one-way analysis of variance. Data were expressed as mean ± standard deviation (SD).

3. RESULTS

In Table (1), body weight gain in HFD group was more pronounced compared to controls. Additionally, comparing tested group showed a significant reduction ($P < 0.05$) in weight gain as compared to the HFD group, where as the lowest weight reduction was detected in HFD+Or group. There was a significant increase in food intake in all the tested groups compared with the control group; results showed that the HFD +Or group had the highest food intake.

Table 1 Effects of ginger, Orlistat and Chitosan treatments on food intake and final body weight in all rat groups Means have different letters are significantly different at $p< 0.05$ in each row

Groups	C	HFD	HFD+Gin	HFD +Or	HFD+Ch
Initial Body Weight (g)	145.2	145	145.1	145.1	145.2
Final Body Weight (g)	208 ± 10^{a}	290 ± 18^{b}	179 ± 13^{c}	155 ± 15^{d}	162 ± 14^{d}
Food Intake (g/week)	188 ± 15^{a}	210 ± 22^{b}	215 ± 18^{b}	239 ± 13^{c}	221 ± 12^{b}

Table (2) shows the higher values of TLs, cholesterol and TGs appeared in the HFD group. A significant ($p < 0.05$) reduction was seen in TLs, TGs, TC and LDL-C levels in the tested groups HFD+Gin, HFD+Or and HFD+Ch in contrast to the HFD group; whereas a significant ($p < 0.05$) elevation in HDL-C was detected in the HFD+Gin group when compared with the HFD group. No significant difference was noticed in LDL-C between groups HFD+Or and HFD+Ch.

Table 2 Effects of ginger, Orlistat and Chitosan treatments on Lipid profile parameters in all experimental rat groups Means have different letters are significantly different at $p < 0.05$ in each row.

Groups	C	HFD	HFD+Gin	HFD +Or	HFD+Ch
Total lipids (mg/dl)	410 ± 23.5^{a}	822 ± 31.6^{b}	630 ± 22.7^{c}	430 ± 18.9^{d}	580 ± 22.7^{e}
Triglycerides(mg/dl)	155 ± 13.5^{a}	260 ± 16.6^{b}	210 ± 12.7^{c}	170 ± 14.5^{d}	172 ± 15.2^{d}
Cholesterol(mg/dl)	38.5 ± 8.8^{a}	99.5 ± 5.8^{b}	45.8 ± 9.3^{c}	61.6 ± 9.4^{d}	66.4 ± 6.9^{e}
HDL-Cholesterol (mg/dl)	5.2 ± 0.43^{a}	7.3 ± 0.99^{b}	8.7 ± 0.74^{c}	4.2 ± 0.82^{d}	6.5 ± 0.33^{e}
LDL-Cholestrol (mg/dl)	14.8 ± 1.5^{a}	17.4 ± 1.6^{b}	10.3 ± 1.2^{c}	15.4 ± 1.6^{a}	15.7 ± 1.8^{a}

Table (3) shows that the bile content of rats fed a HFD was significantly ($p < 0.05$) higher than that in all the other groups. All treatments could reduce the bile secretion level as compared to HFD; ginger treatment did not alter the total bilirubin level as compared to the control, whereas treatment with Chitosan and Orlistat reduced bile secretion. Results of the serum pancreatic lipase levels showed that a HFD increased the enzyme levels significantly ($p < 0.05$) as compared to the control group; although all treatments reduced the enzyme level, it was noticed that Orlistat treatment reduced serum pancreatic lipase significantly ($p < 0.05$) as compared to the control and all the other tested groups. Additionally, results highlight that a HFD and ginger treatment did not alter peroxisomal catalase activity, while the lowest enzyme activity was seen in both Orlistat- and Chitosan-supplemented groups when compared with the other tested groups. Results of leptin and adiponectin hormone levels represented a significant elevation ($p < 0.05$) in the leptin level was seen in rats fed a HFD compared to control one, whereas the levels were lower in the other treatment groups. However, comparing Orlistat and Chitosan treatments showed no significant difference between these two groups. In contrast, a HFD brought about a significant lowering ($p < 0.05$) in the adiponectin level compared with all the groups; this effect was counteracted by different treatments.

Table 3 Effects of ginger, Orlistat and Chitosan treatments Total Bilirubin, Pancreatic lipase, Peroxisomal catalase, Leptin, Adeponectin in all experimental rat groups Means have different letters are significantly different at p < 0.05 in each row.

Groups	C	HFD	HFD+Gin	HFD +Or	HFD+Ch
Total Bilirubin (mg/dl)	1.8±0.5[a]	2.9±0.7[b]	2.1±0.2[c]	0.9±0.7[d]	1.3±0.9[e]
Pancreatic Lipase (ng/dl)	19.5±1.8[a]	45.7±4.2[b]	23.8±5.9c	15.4±4.2[d]	17.7±5.7[e]
Peroxisomal catalase	412±15.6[a]	405±16.2[a]	415±18.3[a]	220±12.7[b]	214±13.3[b]
Leptin (mg/dl)	2.2±0.8a	5.8±1.1[b]	4.3±1.2[c]	3.9±0.9[d]	3.8±0.7[d]
Adeponectin(mg/dl)	4.9±1.1[a]	2.2±0.92[b]	2.8±0.99[c]	3.9±0.79[d]	3.8±0.98[d]

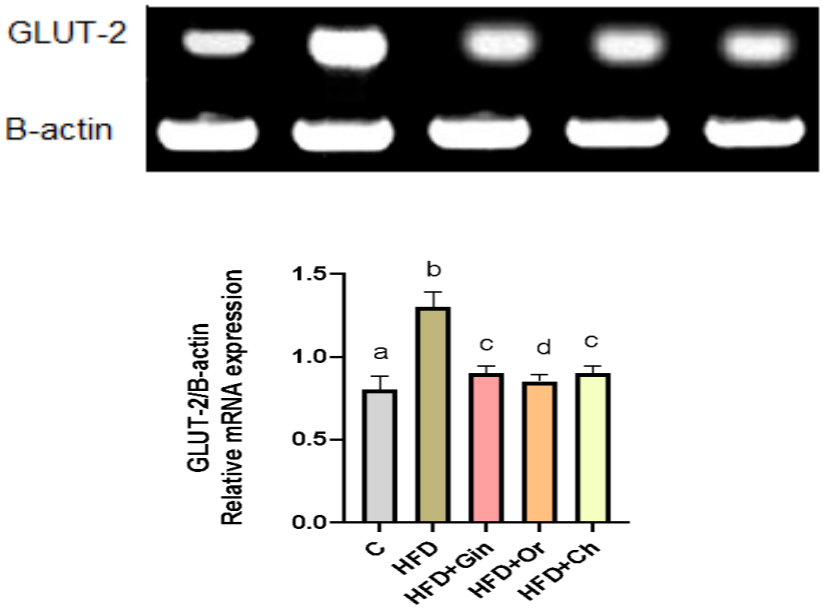

Figure 1 Effect of different treatments on GLUT-2/B-actin expression. Data are shown as mean ± SD; means have different letters as significantly different at P < 0.05

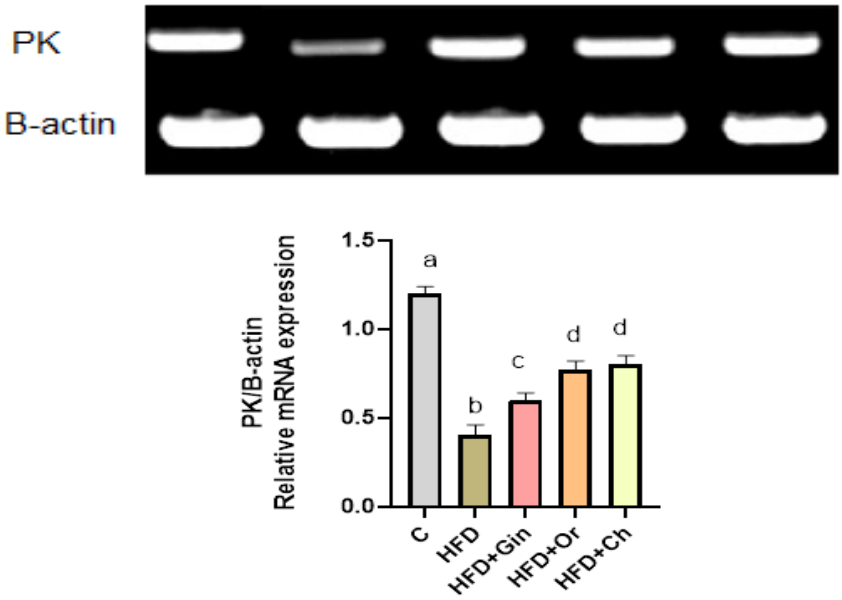

Figure 2 Effect of different treatments on PK/B-actin expression. Data are shown as mean ± SD; means have different letters as significantly different at P < 0.05

Figure (1) shows the effect of different treatments on GLUT-2 mRNA gene expression in hepatic tissues of rats fed a HFD. A HFD motivated mRNA expression of GLUT-2; on the other hand, ginger, Orlistat and Chitosan suppressed this stimulus. The highest effect of inhibition was seen in Orlistat-fed rats (HFD-Or). Moreover, a HFD inhibited the level of PK mRNA gene expression significantly ($p < 0.05$), while other different treatments – ginger, Orlistat and Chitosan – increased the expression of the enzyme, as shown in figure (2).

4. DISCUSSION

Obesity is a metabolic illness as a result of energy inequality where energy consumption is more than energy expenditure. The use of herbs to manage obesity in recent times has been attracting attention (Amin and Nagy, 2009; Verma and Bhoyar, 2017). The anti-obesity drugs that are clinically approved are linked with lethal side effects and are not suited for extended usage (Krentz *et al*., 2016). The progress of obesity after a HFD administration would be clarified by capability of the HFD to cause positive-energy equilibrium, leads to elevated deposition of visceral fat (Xu *et al*., 2008). The increase in body weight was accompanied by elevated TG and TC levels in the serum.

The effect of ginger in reducing body weight may due toits inhibitory effect on dietary fat absorption by inhibiting its hydrolysis (Akinyemi *et al*., 2014). Results showed a significant elevation in the food intake of rats fed an Orlistat-supplemented diet, reflecting an increased appetite. Orlistat affects the salivary glands' structure, and this accounts for the high food consumption in the Orlistat-supplemented rat group. The high food intake seen in the ginger-treated group may be due to its appetite-stimulating properties, as described by Rong et al. (2009). That study described ginger as being helpful in avoiding postoperative nausea and vomiting. Chitosan is used in some dietary supplements as a dietary fibre due to its high biocompatibility and non-toxicity (Luo *et al*., 2015).

Recently, several human and animal trials have examined the effect of ginger consumption on glycaemia, metabolic status, blood pressure and blood lipids (Pavela *et al*., 2016). Ginger treatment reduced serum cholesterol; this is in agreement with the study by Chrubasek et al. (2005), who observed the same changes in the serum total cholesterol in hyperlipidaemic rats. Additionally, a study by Fuhrman et al. (2000) defined the presence of several chemical components in ginger that are accountable for the reduction of high serum lipid levels and of blood pressure in patients with hyperlipidaemia.

Orlistat is a reversible lipase inhibitor that acts by inhibiting the absorption of dietary fats, thus leading to decreased body weight and total adipose tissue levels. The transfer rate of dietary fatty acids from the lumen of the small bowel to the brush border cells and then to the lymph is reduced by decreased dietary lipid hydrolysis. Fatty acids are transported to the lymph, metabolized in the hepatocytes and then transferred to the plasma, before being incorporated into cell membranes. Various surveys have demonstrated that Chitosan acts as a drug-delivery carrier or a dietary fibre against hypertension, hypercholesterolaemia and obesity (Misawa *et al*., 2015).

A reduction of lipid parameters in Chitosan-administered rats was observed as compared with the HFD group. Evidence from animal studies and human subjects shows that Chitosan is helpful in weight management and in reducing serum fats by different mechanisms involving the enhancement of faecal fat excretion or decreasing appetite (Cnubben *et al*., 2016). Similarly, Kang et al. (2012) stated that Chitosan is effective in lowering body weight gain, serum cholesterol and serum TGs, and alleviating lipid accumulation in obese rats.

Our study results indicated a substantial difference in leptin and adiponectin levels in rats fed a HFD as compared with non-obese control rats. Leptin is a satiety hormone, which regulates energy homoeostasis, appetite and glucose/lipid metabolism. Levels of plasma leptin associate with body fat content; the growth of routine fat droplets and an increased size are associated with an increase in leptin secretion. Obesity is characterized by hyperleptinemia, whereas leptin levels decrease considerably during weight loss (Egan *et al*., 2016). A HFD induced a significant reduction in the adiponectin level as compared with all the other groups. Since adiposity is correlated positively with the plasma leptin concentration in rodents and humans, the reduced plasma leptin level found in the non-treated group can be attributed to reduced visceral adiposity after treatments (Denver *et al*., 2011).

In this study, hypoadiponectinemia induced by HFD was counteracted by different treatments, this was in agreement with Zhou et al. (2014). It was clear from the adiponectin results of HFD rats that an increase in body weight is correlated with hypoadiponectinemia, because adiponectin is inversely correlated with BMI (Satoh *et al*., 2010). Chitosan increases faecal fat excretion by a mechanism involving binding with lipids and bile acids or by the suppression of pancreatic lipase activity, thus lowering intestinal fat absorption and elevating faecal fat excretion (Egan *et al*., 2016).

Ginger interferes with catalase activity and increases its level; this may due to the presence of active ingredients of ginger, such as zingerone, gingerol, zingiberene, paradol and shogaols that are reported to have anti-oxidant activity (Kim and Rajapakse, 2005). The results of the present study revealed that HFD stimulated mRNA expression of GLUT-2, which is suppressed by ginger, Orlistat

and Chitosan. The Orlistat-fed rat group showed the most potent inhibitory effect. GLUT-2 is a transmembrane carrier protein that enables glucose transport through the hepatic plasma membrane. It adjusts the route of glucose among organs and the blood, and is accountable for the reabsorption of renal glucose (Riu *et al*., 2003). PK is a glycolytic enzyme that is suppressed in HFD-induced obesity (Iizuka *et al*., 2008). Moreover, ginger, Orlistat and Chitosan treatments counteracted this suppression. No significant difference was observed in with either Orlistat or Chitosan on PK results. Dietary ginger could lower the final body weight and epididymal adipose tissue mass without affecting the energy efficiency ratio and energy intake, consistent with previous studies. Chiu et al. (2015) recommended that the administration of Chitosan improved HFD-enhanced lipogenesis in rats by the activation of AMPK and lipogenesis-associated gene inhibition.

5. CONCLUSION

Comparing the administration of two anti-obesity drugs (Orlistat and Chitosan) with dietary supplementation by ginger, which is a plant used traditionally for weight management, we concluded that ginger has useful potential on the inhibition of HFD-induced obesity and can lessen body weight with no effect on pancreatic lipase or bilirubin levels. It also modulates leptin and adiponectin. In contrast, Orlistat and Chitosan prevent fat absorption by inhibiting pancreatic lipase and peroxisomal catalase activities

Conflict of interest

The author declares that no conflict of interest

Financial resources of the study

None

REFERENCE

1. Akhani SP, Vishwakarma SL, Goyal RK. Anti-diabetic activity of Zingiber officinale in streptozotocin-induced type I diabetic rats. Journal of pharmacy and Pharmacology. 2004; 56(1):101-5.
2. Akinyemi AJ, Ademiluyi AO, Oboh G. Inhibition of angiotensin-1-converting enzyme activity by two varieties of ginger (Zingiber officinale) in rats fed a high cholesterol diet. Journal of medicinal food. 2014; 17(3):317-23.
3. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food and chemical Toxicology. 2008; 46(2):409-20.
4. Amin KA, Nagy MA. Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. Diabetology & metabolic syndrome. 2009; 1(1):17.
5. Anandan R, Ganesan B, Obulesu T, Mathew S, Asha KK, Lakshmanan PT, Zynudheen AA. Antiaging effect of dietary chitosan supplementation on glutathione-dependent antioxidant system in young and aged rats. Cell Stress and Chaperones. 2013; 18(1):121-5.
6. Arablou T, Aryaeian N. The effect of ginger on glycemia and lipid profile: a brief review. Razi Journal of Medical Sciences. 2014; 21(125):94-103.
7. Chiu CY, Chan IL, Yang TH, Liu SH, Chiang MT. Supplementation of chitosan alleviates high-fat diet-enhanced lipogenesis in rats via adenosine monophosphate (AMP)-activated protein kinase activation and inhibition of lipogenesis-associated genes. Journal of agricultural and food chemistry. 2015; 63(11):2979-88.
8. Chrubasik S, Pittler MH, Roufogalis BD. Zingiber isrhizoma: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine. 2005; 12(9):684-701.
9. Cnubben NH, Tel SL, Hemmes MA, Langenkamp-Brand A, Grossouw D, Jansen HT, de Bie BT. A single oral dose of a polyglucosamine influences the bioavailability of [9-14 C]-Oleic acid in adult female Göttingen mini pigs. BMC obesity. 2016; 3(1):18.
10. Codoner-Franch P, Valls-Belles V, Arilla-Codoner A, Alonso-Iglesias E. Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress. Translational Research. 2011; 158(6):369-84.
11. Cooke D, Bloom S. The obesity pipeline: current strategies in the development of anti-obesity drugs. Nature Reviews Drug Discovery. 2006; 5(11):919.
12. Denver RJ, Bonett RM, Boorse GC. Evolution of leptin structure and function. Neuro endocrinology. 2011; 94(1):21-38.
13. Egan ÁM, O'Doherty JV, Vigors S, Sweeney T. Prawn shell chitosan exhibits anti-obesogenic potential through alterations to appetite, affecting feeding behaviour and satiety signals in vivo. PloS one. 2016; 22; 11(2):e0149820.
14. Fuhrman B, Rosenblat M, Hayek T, Coleman R, Aviram M. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of

atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice. The Journal of nutrition. 2000; 130(5):1124-31.
15. Ghosh MK, Hajra AK. A rapid method for the isolation of peroxisomes from rat liver. Analytical biochemistry. 1986; 159(1):169-74.
16. Glazer G. Long-term pharmacotherapy of obesity 2000: a review of efficacy and safety. Archives of Internal Medicine. 2001; 161(15):1814-24.
17. Hasani-Ranjbar S, Jouyandeh Z, Abdollahi M. A systematic review of anti-obesity medicinal plants-an update. Journal of Diabetes & Metabolic Disorders. 2013; 12(1):28.
18. Huang L, Chen J, Cao P, Pan H, Ding C, Xiao T, Zhang P, Guo J, Su Z. Anti-obese effect of glucosamine and chitosan oligosaccharide in high-fat diet-induced obese rats. Marine drugs. 2015; 13(5):2732-56.
19. Iizuka K, Horikawa Y. ChREBP: a glucose-activated transcription factor involved in the development of metabolic syndrome. Endocrine journal. 2008: 55(4): 617-24.
20. Ismail TA, Soliman MM, Ismail SA. Adiponectin regulation in type 2 diabetic rats: Effects of insulin, metformin and dexamethasone. American Journal of Pharmacology and Toxicology. 2013; 8(4):197.
21. Jacobs LR. Effect of short-term dietary fat on cell growth in rat gastrointestinal mucosa and pancreas. The American journal of clinical nutrition. 1983; 37(3):361-7.
22. Kang JG, Park CY. Anti-obesity drugs: a review about their effects and safety. Diabetes & metabolism journal. 2012; 36(1):13-25.
23. Kaukua J, Pekkarinen T, Sane T, Mustajoki P. Health-related quality of life in obese outpatients losing weight with very-low-energy diet and behaviour modification—a 2-y follow-up study. International journal of obesity. 2003; 27(10):1233.
24. Kim GW, Lin JE, Blomain ES, Waldman SA. Antiobesity pharmacotherapy: new drugs and emerging targets. Clinical Pharmacology & Therapeutics. 2014; 95(1):53-66.
25. Kim SK, Rajapakse N. Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review. Carbohydrate polymers. 2005; 62(4):357-68.
26. Krentz AJ, Fujioka K, Hompesch M. Evolution of pharmacological obesity treatments: focus on adverse side-effect profiles. Diabetes, Obesity and Metabolism. 2016; 18(6):558-70.
27. Leighton F, Poole B, Beaufay H, Baudhuin P, Coffey JW, Fowler S, De Duve C. The large-scale separation of peroxisomes, mitochondria, and lysosomes from the livers of rats injected with Triton WR-1339: improved isolation procedures, automated analysis, biochemical and morphological properties of fractions. The Journal of cell biology. 1968; 37(2):482-513.
28. Liu X, Yang F, Song T, Zeng A, Wang Q, Sun Z, Shen J. Effects of chitosan, O-carboxymethyl chitosan and N-[(2-hydroxy-3-N, N-dimethylhexadecyl ammonium) propyl] chitosan chloride on lipid metabolism enzymes and low-density-lipoprotein receptor in a murine diet-induced obesity. Carbohydrate polymers. 2011; 85(2):334-40.
29. Luo, X., Zeng, J., Liu, S. and Zhang, L., 2015. An effective and recyclable adsorbent for the removal of heavy metal ions from aqueous system: magnetic chitosan/cellulose microspheres. Bioresource technology, 194, pp.403-406.
30. Misawa K, Hashizume K, Yamamoto M, Minegishi Y, Hase T, Shimotoyodome A. Ginger extract prevents high-fat diet-induced obesity in mice via activation of the peroxisome proliferator-activated receptor δ pathway. The Journal of nutritional biochemistry. 2015; 26(10):1058-67.
31. Mishra AK, Dubey V, Ghosh AR. Obesity: an overview of possible role (s) of gut hormones, lipid sensing and gut microbiota. Metabolism. 2016; 65(1):48-65.
32. Nirmala K, Krishna TP, Polasa K. Modulation of xenobiotic metabolism in ginger (Zingiber officinale Roscoe) fed rats. Int J Nutri Metab. 2010; 2:56-62.
33. Nishioka T, Hafkamp AM, Havinga R, van Lierop PP, Velvis H, Verkade HJ. Orlistat treatment increases fecal bilirubin excretion and decreases plasma bilirubin concentrations in hyperbilirubinemic Gunn rats. The Journal of pediatrics. 2003 Sep 1; 143(3):327-34.
34. Pavela G, Lewis DW, Locher J, Allison DB. Socioeconomic status, risk of obesity, and the importance of Albert J. Stunkard. Current obesity reports. 2016; 5(1):132-39.
35. Riu E, Ferre T, Hidalgo A, Mas A, Franckhauser S, Otaegui P, Bosch F. Over expression of c-myc in the liver prevents obesity and insulin resistance. The FASEB Journal. 2003; 17(12):1715-17.
36. Rong X, Peng G, Suzuki T, Yang Q, Yamahara J, Li Y. A 35-day gavage safety assessment of ginger in rats. Regulatory Toxicology and Pharmacology. 2009; 54(2):118-23.
37. Satoh H, Fujii S, Tsutsui H. The high-molecular weight (HMW) form of adiponectin is a significant surrogate marker for the prediction of type 2 diabetes mellitus in the Japanese population. Nutrition, Metabolism and Cardiovascular Diseases. 2010; 20(3):e9-10.
38. Suk S, Seo SG, Yu JG, Yang H, Jeong E, Jang YJ, Yaghmoor SS, Ahmed Y, Yousef JM, Abualnaja KO, Al-Malki AL. A Bioactive Constituent of Ginger, 6-Shogaol, Prevents Adipogenesis and Stimulates Lipolysis in 3 T 3-L 1 Adipocytes. Journal of food biochemistry. 2016; 40(1):84-90.
39. Torgerson JS, Hauptman J, Boldrin MN, Sjöström L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to

lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes care. 2004; 27(1):155-61.

40. Tsuzuki W, Ue A, Nagao A, Endo M, Abe M. Inhibitory effect of lysophosphatidylcholine on pancreatic lipase-mediated hydrolysis in lipid emulsion. Biochimicaet Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2004; 1684(1-3):1-7.
41. Verma J, Bhoyar B. A comprehensive review of Ayurvedic herbal drugs and procedures in the management of childhood obesity. Medical Science, 2017, 21(84), 104-113
42. Xu RY, Wan YP, Tang QY, Wu J, Cai W. The effects of high fat on central appetite genes in Wistar rats: A microarray analysis. Clinica Chimica Acta. 2008; 397(1-2):96-100.
43. Zhang HL, Zhong XB, Tao Y, Wu SH, Su ZQ. Effects of chitosan and water-soluble chitosan micro-and nanoparticles in obese rats fed a high-fat diet. International journal of nanomedicine. 2012; 7:4069.
44. Zhou J, Zhang L, Zhang J, Wan X. Aqueous extract of post-fermented tea reverts the hepatic steatosis of hyperlipidemia rat by regulating the lipogenic genes expression and hepatic fatty acid composition. BMC complementary and alternative medicine. 2014; 14(1):263.