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ORIGINAL RESEARCH

The Effectiveness of *Nigella sativa* and Ginger as Appetite Suppressants: An Experimental Study on Healthy Wistar Rats

Lubna Al Asoom[1], Maha A Alassaf[2], Najd S AlSulaiman[3], Dhuha N Boumarah[4], Aldana M Almubireek[2], Gaeda K Alkaltham[2], Hussain A Alhawaj[5], Taleb Alkhamis[5], Nazish Rafique[1], Ahmed Alsunni[1], Rabia Latif[1], Seham Alsaif[1], Dana Almohazey[6], Sayed AbdulAzeez[7], J Francis Borgio[7]

[1]Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [2]King Fahd hospital of the University, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [3]Department of Internal Medicine, King Fahd Hospital of the University, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [4]Department of Surgery, King Fahd Hospital of the University, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [5]Animal House, Environmental Health Department, Institute for Research and Medical Consultation, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [6]Department of Stem Cell Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [7]Department of Genetic Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Correspondence: Lubna Al Asoom, Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal University, P.O Box 2004, Dammam, 31541, Saudi Arabia, Tel +966505846344, Email lasoom@iau.edu.sa

Background: Obesity is a global pandemic that is associated with high morbidity and mortality. Natural herbs are commonly used for weight reduction and appetite suppression. Therefore, we aim to investigate the role and mechanism of *Nigella sativa* (NS) and ginger on weight reduction and appetite regulation.

Methods: This experimental study was performed at Imam Abdulrahman Bin Faisal University. Twenty-five female rats were distributed into 5 groups: NS (oral 1000mg/kg), Ginger (500 mg/kg), NS-ginger (both interventions), a positive control (intraperitoneal 50 µg/kg Liraglutide), and a negative control. Each intervention was given for 9 weeks. Food intake and body weight were assessed weekly. Serum lipid profile and peptides involved in appetite control (cholecystokinin (CCK), glucagon-like peptide 1(GLP-1), gastric inhibitory polypeptide (GIP), ghrelin, peptide YY, and orexin) were assayed at the end of the experiment.

Results: None of the interventions showed a statistically significant difference regarding food consumption or weight gain ($p > 0.05$). However, the three interventions significantly reduced total cholesterol (TC), NS and NS-ginger significantly increased HDL, NS increased ghrelin and ginger increased orexin.

Conclusion: The present dose and duration of NS, ginger, or in combination did not demonstrate a significant change in body weight or food consumption in comparison to the negative or positive controls. However, NS or ginger has improved the lipid profile by reducing TC and increasing HDL. In addition, NS or ginger can influence some of the peptides involved in appetite regulation such as the increase in ghrelin induced by NS and the reduction of orexin induced by ginger. We believe that these latter effects are novel and might indicate a promising effect of these natural products on appetite regulation.

Keywords: *Nigella sativa*, ginger, body weight, appetite, Liraglutide

Introduction

Obesity is a significant global health issue with an alarming increase in rates and concerns over the years. In 2016, 650 million individuals worldwide were obese as reported by the World Health Organization.[1] It is well known that obesity brings about major consequences, in particular, cardiovascular diseases, diabetes, and malignancies.[2] The markedly increasing prevalence of obesity in the Kingdom of Saudi Arabia is among the highest worldwide. By the year 2022, 60% of the Saudi population is expected to become obese if the present trend continues.[3] There are many risk factors influencing obesity; while some cannot be counteracted

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ie genetic factors, others are modifiable such as maintaining a healthy diet regimens and increasing physical activity.[4] Yet, all the efforts spent by the health institutions towards increasing the awareness of adopting healthy lifestyle of diet and exercise fail to show a significant modification of the international trends of obesity.[5]

New interventions have been introduced as an attempt to solve this major international health threat such as the surgical bariatric procedures or the medical anti-obesity drugs. These anti-obesity interventions are either highly invasive, required cautious follow-up, and might precipitate long-term adverse effects. In addition, these interventions are eligible only for obese individuals with BMI ≥30 kg/m^2 and are not available as a prophylaxis for vulnerable subjects.[6] Therefore, there is an urgent need for a safe and practical intervention that can be used as treatment as well as prevention of obesity.

Interestingly, natural herbs have long been used in different parts of the world, not only as food condiments, but also for their various nutritional constituents and health benefits.[7,8] Herbs are widely used to help in the reduction of body weight in multiple culture. In a cross-sectional study of 422 overweight and obese participants in Taif, Saudi Arabia, revealed that 98.1% of the participants used herbal medicines to lose weight. They reported the use of multiple herbs in different combinations, doses and frequencies haphazardly with no scientific basis or as recommended by the folk healers.[9] Among these is a widely used seed called *Nigella sativa* (NS), which belongs to the Ranunculaceae family.[10] *Nigella sativa* (NS) contains fixed oil, volatile oil, proteins, alkaloids, saponin and essential oils. The fixed oil and volatile oil contain fatty acids mainly oleic acid, linoleic acid, palmitic acid, and thymoquinone.[11] Multiple studies showed multitherapeutic effects of NS in relation to metabolic syndrome such as the improvement of blood glucose level,[7] lipid profile,[12] cardiac function[13] and blood pressure.[14] Moreover, a decrease in body weight and waist circumference or central obesity was observed in NS experimental and clinical trials.[15,16] However, the mechanism of NS-weight lowering effect was not investigated.[17]

Another traditionally popular substance is a spice and medicinal plant called *Zingiber officinale* Roscoe, commonly known as ginger. Ginger is used in traditional medicine for a variety of illnesses, as it demonstrates anti-inflammatory, antioxidant, anti-platelet, anti-emetic, anti-ulcer, anti-cancer, antihyperglycemic and antilipidemic properties.[18,19] The major constituents of ginger are gingerols and shogaols, respectively, to which its medicinal properties are attributed.[20] According to several studies, these two bioactive constituents exhibited anti-obesity effects in both animal experiments and human trials.[21] Although the exact mechanisms through which ginger exhibits its anti-obesity effects have not been definitively established, studies have suggested its ability to increase thermogenesis and energy expenditure, promote the action of hormone-sensitive lipase enzyme, and attenuate appetite and food craving.[22] Therefore, we believe that the study of the effect of these medicinal herbs on the body weight and the appetite should be in alignment with the investigation of the peptides and hormones involved physiologically in the regulation of appetite and energy balance, especially that such investigations have yielded the discovery of an FDA approved anti-obesity drug: Liraglutide (Saxenda). Liraglutide (Saxenda) is a glucagon-like peptide-1 (GLP-1) analog, recently used as an adjunctive to lifestyle therapy for weight management in obese or overweight adults with comorbid conditions.[23] Liraglutide (Saxenda) or the intrinsic GLP-1 has an inhibitory central effect on the appetite center in the hypothalamus that leads to a reduction of food cravings and activation of satiety, thereby causing reduction of caloric intake.[24] However, Liraglutide (Saxenda) has several limitations including high cost, side effects and invasive mode of administration.[25]

Besides the GLP-1, there are multiple other central and peripheral peptides that influence the appetite. In the hypothalamus, there are two-order nuclei: the first one is the arcuate nucleus and it contains two classes of neurons; one secretes neuropeptide Y which is a major appetite stimulator and the other secretes melanocortins that suppress the desire for food.[26] Neuropeptide Y and melanocortins act on second-order nuclei which is located in the lateral hypothalamic area and the paraventricular nucleus. The lateral hypothalamic area is an appetite stimulation area through the release of a chemical messenger known as orexins. Lateral hypothalamic area and the release of orexins are stimulated by neuropeptide Y and inhibited by melanocortins. Conversely, the paraventricular nucleus releases corticotropin-releasing hormones (CRH) that suppress appetite. Melanocortins are stimulators of the paraventricular nucleus, while neuropeptide Y suppresses it.[27] Moreover, the first-order neurons are influenced by the levels of other hormones in the blood that play a long-term role in energy balance, such as leptin and insulin.[28]

Other hormones play a short-term effect in energy balance, such as ghrelin and peptide YY3-36. Ghrelin is released by the stomach before food intake; hence, it is an appetite stimulator. After the response of the body to ghrelin by eating, peptide YY3-36 is released by the small and large intestine to suppress appetite during that time. Both ghrelin and peptide YY3-36 affect only the Neuropeptide-Y secreting hormones and have not been found to influence pro-opiomelanocortin secreting neurons.[29] Furthermore, nucleus tractus solitarius in the brainstem also plays a role as a satiety center in the short term. It receives signals

from the hypothalamus and the gastrointestinal tract in response to food intake such as the simulation by cholecystokinin which is released from the duodenal mucosa and other gastric hormones such as GLP-1 and oxyntomodulin.[30]

Therefore, in light with the current knowledge, we plan to inspect the individual and synergistic effects of the long-term administration of NS and gingers on body weight reduction and appetite regulation in comparison to the well-studied anti-obesity agent Liraglutide (Saxenda), and to investigate the response of multiple hormones and peptides of the central and peripheral appetite regulation ie cholecystokinin (CCk), glucagon-like peptide 1(GLP-1), gastric inhibitory polypeptide (GIP), ghrelin, peptide YY, and orexin to the aforementioned interventions.

Materials and Methods

The current study was conducted in accordance with the National Institute of Health guidelines and the ethical regulations of Imam Abdulrahman Bin Faisal University. Ethical approval was provided by the Institutional Review board at Imam Abdulrahman Bin Faisal University, with the reference number IRB-UGS-2020-01-385. Furthermore, the study was reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). All experimental work was carried out in the animal experimental unit at Imam Abdulrahman Bin Faisal University.

Animals

Twenty-five healthy adult female Wistar rats, weighing 200–250 g, were included in the study. They were obtained from the animal house at Imam Abdulrahman Bin Faisal University in Dammam, Saudi Arabia. The sample size was calculated using G-power software where the calculation was based on the means and standard deviations of a previous experiment performed in the physiology laboratory to study the effect of NS intake on the body weight of the rats (controls 325 ± 6 g, NS-treated group 294 ± 8 g). Effect size was 0.91, $\alpha = 0.05$, and $\beta = 0.85$,[31] The sample size was also similar to previously published experiments, with consideration of minimum requirements.[32]

Experimental Protocol

Before being divided into groups, all rats were kept for one week without intervention for acclimatization. Assessment of food intake and body weight was carried out during this week. Rats were housed at an ambient temperature of 25 ± 2 °C, relative humidity, and on a 12-hour light/dark cycle. Individually housed rats had ad libitum access to tap water and laboratory diet throughout the experimental period. Then, they were divided randomly into five equal groups taking into consideration that the groups are equivalent in regard to the initial body weight. Table 1 describes the five different experimental groups along with the doses of the interventions. The NS group was fed five days/week with a dose of 1000 mg/kg, according to previous work, but has been adjusted with an additional 200 mg/kg since the treatment was carried on during the weekdays only. Fresh seeds of NS, harvested from Qassim, were grinded mechanically, and then a suspension was prepared by dissolving 10 g of NS in 100 mL distilled water.[33] Ginger powder was obtained from a local market and prepared in the same manner. A dose of 500 mg/kg for 5 days/week for 9 weeks, according to Wang et al, was given to the ginger group rats.[34] A mixed solution of NS and ginger was given to a separate group of rats to observe for any synergistic effect. A suspension, consisting of 10 g of NS and 5 g of ginger dissolved in 100 mL distilled water, was given, with a dose of 1000 mg/kg of NS and 500 mg/kg of ginger, five days/week for 9 weeks. All doses were administered at around 1 pm through feeding needles. The chemical composition of Qassim black seeds

Table 1 Description of the Experimental Groups and Their Interventions

Group No.	Intervention	No. of Rats	Intervention Dose and Duration
1	Negative control group	5	Basic diet and distilled water-5 days/week for 9 weeks
2	*Nigella sativa* (NS)	5	1000 mg/kg-5 days/week for 9 weeks
3	Ginger	5	500 mg/kg-5 days/week for 9 weeks
4	NS and Ginger	5	1000 mg/kg NS and 500 mg/kg ginger- 5 days/week for 9 weeks
5	Liraglutide (*Saxenda*®) (Positive control)	5	50 μg/kg- 5days/week for 9 weeks

and ginger was analyzed in detail by Ali et al[35] and Abdullahi et al.[36] As for the positive control group, a 50 µg/kg dose of Liraglutide (*Saxenda*®) was injected, intraperitoneally, for five days/week during the light cycle. This dose was described in several studies as the safest effective dose.[37,38] Rats which served as negative controls received a basic diet and an equivalent volume of distilled water throughout the study period using the same feeding technique. The amount of food intake was assessed every three days and body weight was recorded using the laboratory scale weekly for the whole period of the experiment.

Collection of Blood Samples

After nine weeks of regular feeding and weekly weight measurements, blood extraction was performed to assess the effect of the various treatments ie NS, ginger, and Saxenda on hormones and peptides involved in the control of appetite. All rats were fasting for 12–14 hours, and the procedure of blood collection was started at 9:00 am and continued for 2 hours. After anesthetizing the rats with Sevoflurane, a standard midline laparotomy was performed and a transperitoneal approach was followed to expose the inferior vena cava and collect blood. The blood samples were centrifuged in (Sigma 3–16 KL, Germany) with a speed of 3500 revolutions per minute. Serum was separated and stored at-80°C until analysis.

Enzyme-Linked Immunosorbent Assay (ELISA) Tests

Several serum levels of hormones and peptides were analyzed using ELISA kits. These include gastric inhibitory polypeptide (GIP) ELISA kit (catalogue number CEA882Ra), Glucagon-like peptide 1 (GLP-1) ELISA kit (catalogue number E-EL-R3007), ghrelin ELISA kit (catalogue number E-EL-R0842), peptide YY ELISA kit (catalogue number E-EL-R0720), orexin A ELISA kit (catalogue number CEA607Ra), cholecystokinin (CCK) ELISA kit (catalogue number CEA802Ra), cholesterol estimation kit (catalogue number E-BC-K004- M), high-density lipoprotein cholesterol (HDL) colorimetric assay kit (catalogue number E-BC-K206-S) and triglyceride colorimetric assay kit (catalogue number E-BC-K261-M). The kits were obtained from Elabscience, United States.

Statistical Analysis

The collected data was statistically analyzed using the Statistical Package for the Social Science (SPSS) program version 26 and expressed as mean ± SD. Analysis of variance and LSD post hoc tests were used to compare the parameters between the different groups and particularly to compare the intervention groups to the control group. A P-value <0.05 was considered statistically significant. Pearson correlation was used to find out the association of body weight and food consumption and the serum level of peptides involved in appetite regulation.

Results

The original number of rats included in the experiment was 25 rats (5 rats per group). However, two rats from the negative control group died at week six and week seven due to trauma by the feeding needle.

Body Weight

The body weight of all five rat groups was comparable at the beginning and the end of the experiment. In another word, there was no significant difference in the initial and final body weight among the five rat groups. The weight gain pattern was also similar among the five rat groups throughout the experiment. Figure 1 shows the weekly progression of body weight during the study period, expressed as mean ± SD of each group. As the body weight data follow normal distribution (Shapiro–Wilk test >0.05), a mixed between-within subjects' analysis was conducted. Although there was a significant difference in the body weight over time, it did not differ significantly between the groups ($p > 0.05$).

Food Intake

The food consumption of all the rat groups was measured twice a week, Sundays and Wednesdays. Figure 2 shows the mean value of the weekly food consumption of each rat-group throughout the study expressed as the mean food consumption ±SD. As the food consumption value does not follow the normal distribution (Shapiro–Wilk test <0.05), Kruskal–Wallis Test was conducted. The weekly effect of each intervention on the food consumption of each group of the study did not reveal a significant difference ($p > 0.05$).

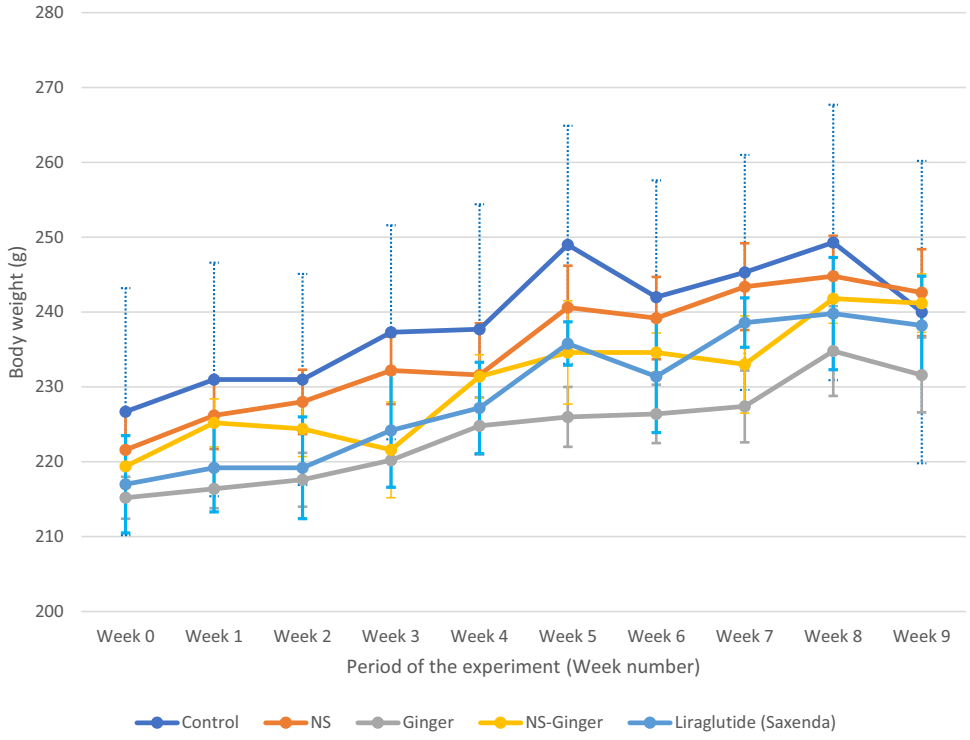

Figure 1 Mean body weight progression of each rat-group during the period of the experiment.

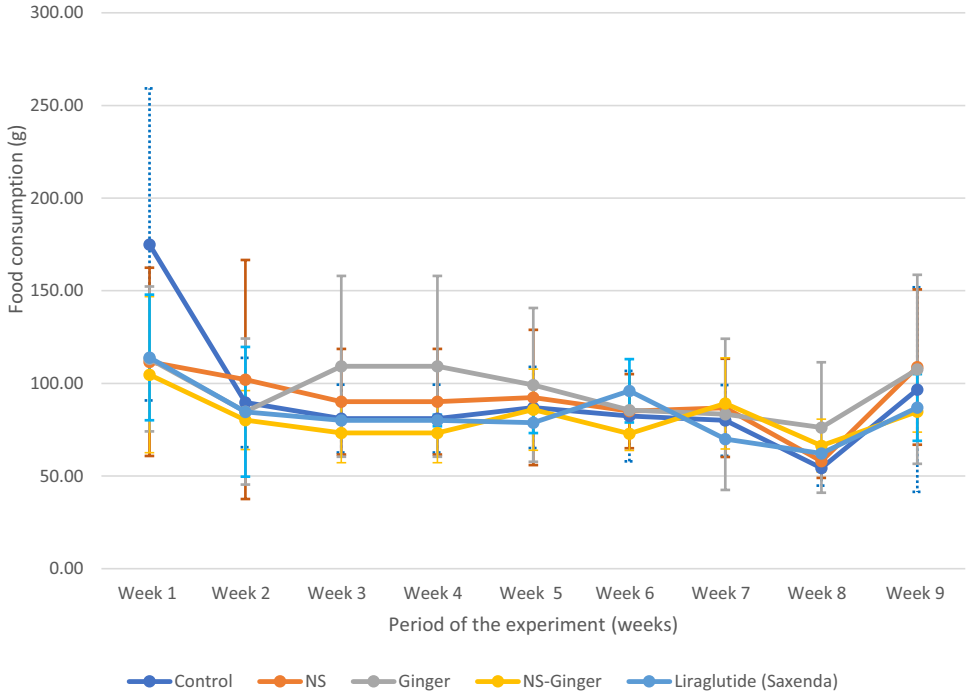

Figure 2 Mean food consumption (g/week) of each rat-group during the period of the experiment.

Appetite Suppressants and Anti-Obesity Agents

Three interventions: NS, ginger and positive control significantly reduced the total cholesterol (Table 2). High-density lipoprotein increased significantly in NS and NS-ginger than the negative control. Furthermore, significant increase in ghrelin and reduction of orexin was observed in the NS and ginger, respectively.

Table 2 Comparison of Lipid Profile, Hormones and Peptides Involved in the Regulation of the Appetite Among All the Groups Using ANOVA and LSD Post Hoc Test

	Control N = 3	NS N = 5	Ginger N = 5	NS-Ginger N = 5	Liraglutide (Saxenda) N = 5
CCK	434.8±209.3	360.3±84.6	332.6±115.8	485.3±94.5	391.5±111.8
Total cholesterol	1.8±0.4	1.2±0.3* P=0.028	1.2±0.4* P=0.021	1.4±0.5	1.1±0.1** P=0.008
HDL	1.3±0.1	2.7±0.5* P=0.008	1.5±0.6	2.5±0.6* P=0.021	1.8±0.9
Triglycerides	0.6±0.2	0.4±0.2	0.3±0.2	0.6±0.2	0.4±0.1
GLP-1	354.2±355.3	702.1±463.9	425.8±523.1	167.1±34.9	142.7±53.6
Ghrelin	3.0±0.0	3.3±0.1* P=0.034	3.1±0.3	3.3±0.2 P=0.055	3.2±0.2
GIP	5. 8±4.2	3.8±0.6	4.4±0.8	3. 8±1.3	3.9±0.1
Peptide YY	93.5±17. 8	78.6±21.5	66.5±23.1	63.3±16.1	72.0±22.1
Orexin	721.0±141.9	604.2±95.5	475.7±152.0* P=0.022	591.5±96.3	615.5±162.0

Notes: *P<0.05, **P<0.01.
Abbreviations: CCK, cholecystokinin; HDL, high density lipoprotein; GLP-1, glucagon like peptide-1; GIP, gastric inhibitory polypeptide.

Discussion

The current experimental work aimed to test the effects of selected natural herbs, namely ginger and NS, on body weight and food consumption in comparison to a negative control group that received an equal amount of distilled water and a positive control group that received the anti-obesity agent Liraglutide (Saxenda). Despite the continuation of these interventions for nine weeks, there was no significant effect on body weight or food consumption by any of the substances used in comparison to controls. Moreover, there was no synergistic effect demonstrated by the combination of NS and ginger on neither body weight nor food consumption.

NS is an easily attainable and commonly used medicinal herb in Middle Eastern and North African traditional medicine.[39] Among its many documented nutritional benefits is its positive effect on weight loss.[40] However, this effect remains controversial in the literature, as some studies exhibited the weight-reducing effects of NS, while others showed no significant changes, as in ours.[41] Among the studies that demonstrated diminished weight gain in growing rats is the one conducted in Morocco on 24 Wistar Kyoto rats which were allocated to two groups: control and NS-treated groups. The control group received 2 mL/kg of distilled water, whereas the second group received NS-fixed oil orally for 12 weeks. Results revealed that NS-rats had significantly lower weights compared to their control counterparts. This effect was demonstrated at 6-weeks onward.[42] This significant reduction in weight gain could be attributed to the form of NS used (ie NS-fixed oil) since thymoquinone, an active constituent of NS, is more abundant in NS-oil compared to other forms of the substance. Meddah et al also demonstrated NS-weight reducing effects on normal Sprague Dawley rats subjected to oral glucose tolerance test (OGTT) for glycemic control assessment. NS was administered orally at a dose of 0.2g/kg of dry aqueous extract (an amount equivalent to 2g/kg of original seed powder) daily for 6 weeks. Compared to the control group which received distilled water of a dose equivalent to that of NS, the average weight of rats was significantly lower in the NS-group from 2-weeks onward.[43] The significant effect seen with Meddah et al's experiment could be attributed to the larger dose of NS used in their study. Similarly, in clinical studies, some authors demonstrated a weight lowering effect of NS such as Safi et al, who tested the effect of 8 weeks administration of 1000 mg NS-oil capsules on 39 women aged 25–55 years with BMI 27–35 kg/m^2 and demonstrated a significant body weight reduction.[15] Similarly, Mostafa et al[44] showed significant reduction in body weight and BMI in 117 prediabetic male and female subjects after six months of 900 mg/day NS-oil capsules. However, Bamosa et al[7] reported no change in body weight or BMI in 94 diabetic male and female patients after 3 months treatment with 1, 2, or 3 g/day NS-powder capsules.

Therefore, the dose and preparation of NS seems to be a critical factor in inducing the weight lowering effect. NS-oil as reflected by multiple studies is more potent body weight lowering agent than NS crude powder.

Regarding the effect of NS on food consumption, the literature remains relatively scarce in reporting this. An experimental study by Le et al[45] addressed the effect of NS petroleum ether extract on 14 normal male Sprague-Dawley rats for a period of 4-weeks. The sample was divided into two groups, with the first receiving petroleum ether extract equivalent to 2g/kg/day of the original seed powder by daily intragastric gavage, while the other (control) group received an equivalent amount of tapwater. The petroleum ether extract of NS induced a significant transient weight loss initially and a sustained reduction in food intake but not water. This effect was not demonstrated by the NS powder preparation used in our study, and there is very little information in the literature on what may or may not be effective in NS preparation to elicit this change.

On the other hand, the NS dose and preparation used in our experiment was sufficient to elicit a statistically significant reduction in total cholesterol and an increment in HDL. Multiple studies consistently demonstrated a lipid reduction effect of long-term ingestion of NS for about 6–12 week. In clinical trial, Badar et al showed a significant reduction of total cholesterol, LDL, and significant elevation of HDL after 6 months of daily 2g/day of powdered NS in capsules.[46] Similarly, Moustafa et al[47] reported significant reduction in total cholesterol, LDL, and triglycerides in diabetic patients treated with 1350 NS-oil capsules for three months. Therefore, the effect of NS on the lipid profile seems to be more consistent than its effect on body weight despite the use of different preparations of NS ie raw powdered or extracted oil.

The exact mechanism of NS on the metabolism and the energy balance is unclear yet. Our trial to explore the possible effect of NS on the peptides influencing the appetite demonstrated an unexpected finding of a significant increment of ghrelin. Ghrelin is known as the hunger hormone. It is secreted by the stomach during fasting state and encourages food intake. It plays a key role in insulin resistance and diabetes. It favors the reduction of energy expenditure, thermogenesis, and lipolysis.[48] Therefore, the significant elevation of ghrelin in the NS-rats is inconsistent with the reduction in lipid profile and this might be explained as a rebound physiological compensation to the possible negative energy balance induced by NS. Will higher dosage of NS-powder or NS-oil overcome this compensatory mechanism and succeed in obtaining negative energy balance and lowering body weight? The answer to this question needs further studies with higher dosage of NS-powder or NS-oil while exploring the same peptides and pathways of appetite control and energy balance.

The other natural herb that we used in this study is Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) which is one of the widely available and most used spices in the world, for its abundant beneficial and therapeutic properties.[49] Multiple studies in the literature have reported a body weight reduction effect of ginger with enhancement of energy metabolism. Among these studies is the one performed by Sayed et al[50] on fifteen adult male Wistar rats, which were distributed into 3 equal groups; control, and two experimental groups which received ginger water in 25% and 50% of their drinking water, respectively. After one month, both ginger groups showed significant lowering of body weight gain in comparison to the control group. However, there was no difference in the average food consumption across all the groups. This difference between the control group and the ginger-treated groups in regard reduced weight gain was not evident in our study results, perhaps due to the different preparation methods of ginger. Moreover, a 30-day experiment was performed on 40 male albino rats divided into 4 groups: a control group of rats on a basic diet (G1), a high-fat diet group (G2), an orlistat treated group on a high-fat diet (G3), and lastly a group of high-fat diet supplemented with 5% dried ginger powder (G4). Results illustrated significant reduction of weight gain in the orlistat-treated group G3 and the ginger-treated group G4 when compared to the control group G1 and the high-fat diet group G2. There was also a significant increase of food consumption in G3, when compared to other groups, followed by G4.[49] Another study compared the effect of ginger supplemented with a high-fat diet to its effects with a normal diet. The study included 32 mice, distributed into 4 groups equally. The first two groups received a normal diet with one of them being supplemented with ginger powder 500 mg/kg, while the other two groups were started on high-fat diets with one of them being supplemented with ginger in the same manner. After 16-week period, there was no significant difference in weight gain between the two normal diet groups. However, the group that received a high-fat diet supplemented with ginger had a lower final body weight and weight gain compared to the other high-fat diet group.[51] The aforementioned results suggested that ginger does not affect the amount of food consumption, which is similar to the findings of our experiment. However, when ginger was combined with high-fat diet, it demonstrated an appetite stimulant effect.

Another important finding related to the administration of ginger was the significantly reduced appetite-regulating peptide ie orexin. Orexin is a peptide released centrally in the brain from neurons in the dorsolateral and perifornical areas of the hypothalamus. It has been found to play multiple central autonomic functions related to cardiovascular system and energy regulation. Orexin initiates responses such as increased heart rate, arterial blood pressure and appetite. As a result, orexin is blamed to be involved in the pathophysiology of obesity.[52] Therefore, the reduction of orexin induced by ginger is a desirable response and it is in line with the battle against obesity. Unfortunately, this preferable response was mildly abolished when ginger was combined with NS. This phenomenon – most probably – indicates that these two natural substances provoke different molecular pathways. Nevertheless, multiple higher dosage of either ginger, NS or the combination of both with serial blood sample analysis for the appetite regulating peptides might still be needed to understand the exact physiological influence of ginger and NS on appetite regulation.

Lastly, Liraglutide (Saxenda), which is a GLP-1 analog known for its anti-obesity properties and have repeatedly demonstrated a weight loss effect,[53] was used in this study as an agent for a positive control group. Unfortunately, our positive control rats did not exhibit any significant change in body weight or food consumption. This failure in the induction of weight loss or reduced weight gain in the positive control rats could be attributed to the dose used in this study and the low level of physical activity of the rats. In contrast to our work, Hyde et al who treated normal Sprague-Dawley rats with 1mg/kg injection daily for 22 days were able to demonstrate body weight and food consumption reducing effects of Liraglutide.[54] The reason why we have adopted 500 mg intraperitonially and not higher doses is the repeatedly reported serious side effect of Liraglutide in rats. It has been found that higher doses of Liraglutide can culminate in medullary thyroid carcinoma in rats.[55]

On the other hand, Liberini[56] et al supported the results of our study by demonstrating that Liraglutide did not lead to a reduction of body weight or food intake on rats of both sexes consuming a regular chow diet. However, weight reduction was observed only in male rats on high-fat and high-sugar diets compared to controls from post-natal day 40 to 60.

Furthermore, a number of factors, including caloric intake, basal metabolic rate and physical activity, affect the energy expenditure and subsequently play a pivotal role in modulating the physiology of weight loss. These factors were found different among male and female rats supporting the phenomenon of sexual dimorphism.[57] Biologically, sex hormones have a key role in regulating several physiological parameters, including energy hemostasis. Estrogen, in particular, is known to exert a modulatory effect on energy balance, through deactivation of thermogenesis, resulting in a reduced energy expenditure.[58] In addition to the influence of sex hormones, Leptin, a hormone proven to reduce dietary intake by interacting with hypothalamic receptors, was found to be higher in male rats compared to females. Therefore, it has been suggested that female rats have a tendency to maintain stable body weight despite dietary modifications and restrictions.[59] This concept can be supported by a study conducted by Cortright et al to compare the body response to daily running in male and female rats and showed that female rats were able to maintain their body mass with constant levels of fat and protein throughout a nine-week experimental period, regardless of the induced energy imbalance.[60] Given these differences, we believe that sexual dimorphism might be a contributor to the resultant difference in our observation regarding three interventions effect on body weight and food consumption when compared to other studies conducted on male rats.

Lastly, the restriction in physical activity of the studied rats in this experiment is also another possible explanation of the weight maintenance in the current study despite the long-term administration of the natural herb supplement ie NS or/and ginger or Liraglutide/Saxenda.

Conclusion

In summary, the present experimental study did not demonstrate a significant change in body weight or food consumption with long-term administration of NS or ginger in comparison to the negative control group that received distilled water and the positive control group that received Liraglutide (Saxenda). On the other hand, the three interventions (NS, ginger, Liraglutide) used in this study induced significant reduction of total cholesterol, while NS only induced significant increase in HDL and ghrelin. Furthermore, ginger interestingly reduced orexin which is an appetite-stimulation peptide. To the best of our knowledge, the ginger inhibitory effect on orexin might be a novel and new discovery, and it is in consistent with our main objective of suppressing the appetite and lowering body weight.

Limitations and Recommendations

This study might be limited by the inclusion of female rats only, which deprived the investigators from exploring and comparing the effect of sex on the responses of body weight and appetite control to the adopted interventions ie NS, ginger and Liraglutide/Saxenda. Furthermore, the use of single dose and preparation per intervention restricted the expression of the full range of effect of these substances. Therefore, we recommend further studies to compare the response of male and female Wistar rats to NS, ginger and their combination therapy using multiple doses and preparation in order to definitively determine their effects on body weight and appetite control as well as to establish a consensus regarding the safety profile.

Abbreviations

BMI, Body metabolic index; CCK, Cholecystokinin; CRH, Corticotropin-releasing hormones; ELISA, Enzyme-linked immunosorbent assay; FDA, U.S. Food and Drug Administration; GIP, Gastric inhibitory polypeptide; GLP-1, Glucagon-like peptide-1; HDL, High-density lipoprotein cholesterol; LSD, Least significant difference; NS, *Nigella sativa*; SD, Standard deviation; SPSS, Statistical Package for the Social Science.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Review Board of Imam Abdulrahman Bin Faisal University, with the reference number IRB-UGS-2020-01-385.

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Disclosure

The authors declare that they have no competing interests.

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