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Influence of Dried Lemon, Ginger and Cumin in Weight Reduction and Some Biochemical Parameters in Rats Suffering from Obesity

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ABSTRACT

The present study was established to study the effect of dried lemon, ginger and cumin on the nutritional parameters, lipid parameters, serum glucose, and liver enzymes of rats suffering from obesity. Also this study performed to determine the content of lemon, ginger and cumin of phenolic and Flavones as a natural anti-oxidant, recently many experiments performed to assess the role of natural antioxidants in the treatment of obesity. The rats (n=30) were distributed into two main groups, the first main group (n=6 rats) fed on basal diet as a control negative group. The second main group (n=24 rats) received high fat diet for 6 weeks to induce obesity in rats. After these periods, the mean value of body weight gain% was estimated in the two main groups, also blood samples were collected from all rats to estimate the levels of cholesterol and triglycerides, then the high fat diet group was divided into (4) subgroups (n=6 rats for each), the first subgroup fed on high fat diet as a control positive group. Subgroups 3, 4 and 5 and were fed on high fat diet supplemented with 200mg/kg of dried lemon, ginger and cumin, respectively. The results cleared that, addition of dried lemon, ginger and cumin, respectively to the high fat diet decreased the body weights, in addition to significant decrease in the mean values total lipids profile, serum glucose level, liver enzymes in all treated groups, compared to the positive control groups, while high-density lipoprotein (HDLc) increased. It was concluded that, the dried lemon showed the best effect on body weights, lipid profile, liver functions, and glucose level of obese rats fed on high fat diet. It could also be argued that dried lemon, ginger and cumin are rich in antioxidants such as phenols and Flavones which plays an important role in reducing the level of lipids profile, serum glucose, and liver enzymes and are considered natural anti-obesity.

Keywords*:* lemon, ginger and cumin, phenolic, flavonoids obesity, rats, lipid profile, glucose, liver enzymes.

INTRODUCTION

Obesity is the most important nutritional disease in develop countries. Obesity is among the most important factors of morbidity and mortality. Obesity accompanied by many diseases including diabetes, hyperlipidemia, hypertension, and cardiovascular diseases (Mazlom *et al.*, 2009). Nowadays use of medicinal plants has become prevalent in the treatment of many diseases. (Kianbakht *et al.*, 2010). Cumin, as one of these medicinal plants, contains more than 100 different chemicals, including essential fatty acids and volatile oils (Mohiti-Ardekani *et al.*, 2011). Cumin may have decreasing effects of blood lipids and weight (Andallu and Rarnya ., 2010). Citrus fruits contain basic nutrient compounds such as vitamins, minerals, pectin's, dietary fibers, and bioactive compounds including flavonoids and carotenoids, (Gorinstein *et al.*, 2013). Citrus fruits exhibit important bioactivities, including antioxidant, anti-inflammatory, anti-obesity, anti-cardiovascular and antitumor abilities (Tanaka *et al.*, 2012). Citrus regulated the lipid and triglyceride (Jung *et al.*, 2011). Tangerine peel extracts reduced the plasma and hepatic cholesterol levels of rats (Bok *et al.*, 1999). Ginger (Zingiber officinale Roscoe, Zingiberacae) is one of the most commonly used spices around the world. (Ali *et al.*, 2008) , and demonstrated to have various pharmacological activities such as antiemetic, antiulcer antiinflammatory, antioxidant, anti-platelet, glucose and lipid lowering, cardiovascular and anticancer activities (Nicoll and Henein., 2009). Ginger is used medicinally for its hepatoprotective and anti-oxidant (Abdel-Azeem *et al.*, 2013), antidiabetic and antihyperlipidemic (ElRokh *et al.*, 2010), and anti-obesity effects (Mahmoud, 2013).Phenolic compounds have been proven to be successful in attenuating hypercholesterolemia (Rehrah *et al.*, 2007)Moreover, these substances are known by their protective agents in diseases involving oxidative stress (Chenni *et al.*, 2007). In this study, we investigated the effect of dried lemon, ginger and cumin on high-fat diet-induced obesity in rats. In addition to determine its natural antioxidants content and its effects on lipids profile, serum glucose and liver enzymes

MATERIALS AND METHODS

Materials: Dried lemon, ginger and cumin were obtained from local market Cairo, Egypt.

Chemical determination

Determination of phenolic compounds: The total phenolic compounds (TP) in dried lemon ginger and cumin were extracted using methanol solvent at solvent to samples ratio of 10:1. Extraction was carried out using a shaking incubator at room temperature for 24 h followed by filtration through whatman No.1 filter paper. The residue was re-extracted in the same manner and the two filtrates were combined (Sobhy *et al.*, 2009).

Determination of total flavonoids: Total flavonoids content were determined using the method of (Ordon *et al.*, 2006). A volume of 0.5 mL of 2% AlCl3 in ethanol solution was added to 0.5 mL of methanol extract. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/mL.

Experimental animal design: Thirty male albino rats (200 - 210g) were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet adlibitum for adaptation in the animal house of Faculty of Veterinary Medicine, University of Suez Canal. The basal diet consisted of 14 % protein from casein (≥ 80 %), 4% soya oil , 0. 25 % choline chloride, 1 % vitamin mixture, 3.5% salt mixture, 5 % cellulose, 0.18 % L- cystine and the remainder is corn starch up to 100% (Reeves *et al.*, 1993). The vitamin mixture was prepared according to (A.O.A.C., 1975) and the salt mixture was prepared according to (Hegested *et al.*, 1941). After a period of adaptation on basal diet (one

week), the rats (n=30) were divided into two main groups, the first main group (n=6 rats) fed on basal diet and kept as a control negative group. The second main group(n=24 rats) received high fat diet for 6 weeks to induce obesity in rats, the high fat diet consisted of 20% fat (19% beef tallow and 1% soya oil to provide essential fatty acids) according to (Min *et al.,* 2004). Blood samples were collected from all rats to estimate the levels of cholesterol and triglycerides (healthy rats recorded 79.00 ± 4.922 mg/dl cholesterol and 39.722 ± 3.203 mg/dl triglycerides), while the second main group recorded (135.878 ± 4.750 mg/dl cholesterol and 68.251 ± 5.231 mg/dl triglycerides), then the high fat diet group was divided into four subgroups (n=6 rats for each), the first subgroup fed on high fat diet as a control positive group. The other subgroups (3, 4 and 5) fed on high fat diet supplemented with 200mg/kg of dried lemon, ginger and cumin, respectively. At the end of the experiment, the animals were fasted overnight, then the rats were weighed, anaesthetized and sacrificed, then blood samples were collected from the aorta. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters.

Biological Determination: Determination of feed intake, body weight gain and feed efficiency ratio: Feedd Intake (FI) was calculated every other day

Biochemical Determination: Some biochemical analyses were determination, i.e. serum cholesterol (Allain *et al.,* 1974), triglycerides (Foster and Dumns ., 1973), HDL-c (Lopes-Virella *et al.,* 1977), LDL-c and VLDL-c (Fried *et al.,* 1972), glucose (Trinder,1969), aspartate amino transferase (AST) and alanine amino transferase (ALT) (Reitman, and Frankel.,1957),

Statistical analysis: Data was presented as means ± SD statistically analyzed using one way ANOVA test, $p<0.05$ was used to indicate significance (Steel and Torri.,1980).

RESULTS AND DISCUSSION

Phenolic and flavonoid contents.

According to the data shown in the Table (1). The content of total phenolic and total flavonoids in lemon, varying between 48.83 mg GAE/100 g to 46.19mg CE/100 g, was found to be much higher than and cumin - 29.24 mg GAE/ 100g to 28.27 mg CE/100g, respectively. Several investigations have mentioned that the antioxidant potential of plants might be due to their phenolic components (Cook *et al.,* 1996). Flavonoids, a group of polyphenolic compounds with known properties, such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and anti-inflammatory action (Pourmorad *et al* ., 2006) , have been isolated from plants (Omale and Okafor *et al* ., 2008).

Table 1.Total phenolic and, total flavonoids of dried lemon, ginger and cumin.

Plants	Total phenolics, (mg GAE /100 g DW)	Total flavonoids, (mg CE /100 g DW)
Lemon	49.83	46.19
Ginger	47.33	27.36
Cumin	29.24	28.27

Effect of dried lemon, ginger and cumin on food intake, body weight gain % and changes of weight of obese rats.

The effect of dried lemon, ginger and cumin on feed intake, body weight gain% and changes of weight of obese rats are presented in Table (2).

Table 2. Effect of dried lemon, ginger and cumin on feed intake, changes of weight of and body weight gain %

Parameters Groups	Feed intake (g/day)	Initial weight	Final weight	BWG%
Control (-)	19.000 [a b] ± 0.816	172.500 [b] ± 6.455	195.000 [f] ± 8.165	13.035 [d] ± 1.520
Control (+)	17.750 [b] ± 0.957	234.750 [a] ± 6.994	307.500 [a] ± 10.408	30.983 [a] ± 1.440
Lemon(200mg/kg)	18.500 [a b] ± 2.380	239.250 [a] ± 1.258	249.500 [e] ± 2.645	4.281 [f] ± 0.607
Ginger(200mg/kg)	16.750 [b] ± 1.258	236.500 [a] ± 3.109	277.500 [c d] ± 6.455	17.324 [c] ± 1.199
Cumin(200mg/kg)	18.500 [a b] ± 1.732	236.500 [a] ± 6.557	257.250 [e] ± 7.365	8.773 [e] ± 0.666

Feed intake (g/day for each rat).

The mean value of feed intake in healthy group fed on basal diet (control –ve group) showed non significant differences compared with obese group fed on high fat diet containing 20% fat (control +ve group). Feed intake in all obese groups which were treated with dried lemon, ginger and cumin had non-significant differences of mean value at ($p<0.05$), compared with the normal group (control –ve group).

Weight Changes of Obese Rats during the Experimental Period (g).

Table (2) showed the follow-up development in weight of normal and obese rats treated with dried lemon, ginger and cumin during the experiment. Data in this table showed significant decrease ($p<0.05$) in the weight of normal group (control –ve group) in both of the initial and final of the experimental period, as compared to obese group (control +ve group) and all obese treated groups with dried lemon, ginger and cumin. Feeding obese groups on high fat diet containing

20% fat and treated with 200mg/kg of dried lemon, ginger and cumin, respectively led to significant decrease ($p<0.05$) in the weight at the final of the experiment, as compared to the positive control group.

Body Weight Gain % (BWG %): Body weight gain % of obese rats fed on diet containing20% fat (control +ve) increased significantly $p<0.05$, as compared to the negative control group fed on basal diet. On the other side, comparing all treated groups with control +ve group demonstrated significant decrease. Treated group with cumin resulted in the highest decrease in BWG%, as compared to (control +ve) and other treated groups. Body weight gain was significantly reduced by feeding with the diet containing lemon polyphenols (Yoshiko *et al.,* 2008). Treatment with dried rhizomes of ginger produced a significant reduction in elevated lipid levels, body weight, hyperglycemia and hyperinsulinemia (Mahmoud and ELnour. 2013)

Effect of dried lemon, ginger and cumin on Lipid Fractions of Obese Rats.

The mean values of serum cholesterol, triglycerides, LDL-c and VLDL-c (mg/dl) significantly increased $P< 0.05$ for control positive group, in comparison with control negative group as showm in table (3). The percentage of increase in cholesterol value was about 74.79 %, while HDL-c value (mg/dl) for control positive group decreased than that of the control negative group by about 48.26 %. Addition of lemon, ginger and cumin resulted in a significant reduction in cholesterol values. Rats which received high fat diets with the previous concentrations of lemon, ginger and cumin had lower mean values of triglycerides, LDL-c and VLDL-c compared with control positive group. On the other hand, the same treated groups of rats had higher mean values of HDL-c than that of the control positive group. The best result for lipid fractions was noticed in the group of rats fed on high fat diet containing cumin(200mg/kg), followed by group that treated with Lemon(200mg/kg) and finally group of rats treated ginger(200mg/kg) (Table 3). Our results are in agreement with many studies which showed that, oral lemon juice administration resulted in significant decrease in serum total cholesterol, triglyceride (TG) and LDL-cholesterol levels when compared with the control group with a commensurate significant increase in the HDL-cholesterol (Olukanni *et al.,* 2013). The serum TG levels of the mice fed the LP (lemon polyphenols) diet were significantly decreased compared to those of the mice fed the LF and HF diet. (Yoshiko *et al.,* 2008). Cinnamon and ginger in doses 200 and 400 mg kg when given orally to obese diabetic rats significantly lowered the high levels of serum TC and TG in a dose- and also induced a significant ($P <0.05$) increase in serum HDL and decreased in LDL (Mostafa and Hamed., 2014). C. cyminum treatment also resulted in a significant reduction in plasma and tissue cholesterol, phospholipids, free fatty acids and triglycerides (Dhandapani *et al.,* 2002).

Table 3. Effect of dried lemon, ginger and cumin on lipid fractions of obese rats.

Parameters / Groups	Cholesterol	Triglycerides	HDL-c	LDL-c	VLDL-c
			mg/dl		
Control (-)	84.682 ^{e} ± 5.377	41.250 ^{f} ± 2.179	44.488 ^{a} ± 4.228	31.944 ^{h} ± 0.815	8.250 ^{f} ± 0.435
Control (+)	148.013 ^{a} ± 5.502	79.665 ^{a} ± 6.008	23.020 ^{d} ± 2.264	109.059 ^{a} ± 2.420	15.932 ^{a} ± 1.201
Lemon(200mg/kg)	89.210 ^{e} ± 2.075	42.180 ^{f} ± 2.517	37.368 ^{b} ± 2.137	43.405 ^{g} ± 0.566	8.436 ^{f} ± 0.503
Ginger(200mg/kg)	103.534 ^{d} ± 3.443	53.215 ^{e} ± 2.061	36.010 $^{b c}$ ± 2.770	56.881 ^{e} ± 0.261	10.643 ^{e} ± 0.412
Cumin(200mg/kg)	100.521 ^{d} ± 4.549	56.520 $^{d e}$ ± 6.149	37.987 ^{b} ± 2.504	51.230 ^{f} ± 1.181	11.303 $^{d e}$ ± 1.230

Effect of dried lemon, ginger and cumin on some liver enzymes and serum glucose of obese rats. Concerning aspartate and alanine amine transaminase (AST and and cumin were added to the high fat diet of obese rats a significant decrease of AST and ALT values were noticed in comparison to control positive group. Our results are in agreement with many studies which showed that. Mandarin fruit improved the metabolic function of liver and restored the antioxidant enzymes in diabetic rats (Sugiura *et al.,* 2006). Naringin prevented the increase in hepatic enzyme activities (AST, ALP, and ALT) and reduced the accumulation of lipid deposition and fibrosis in the liver of high-carbohydrate, high-fat-diet–fed obese rats (Ashraful *et al.,* 2014). Previous studies indicated that the administration of aqueous extract of *ginger* to rats, orally and intraperitoneally, at two different levels of doses, significantly decreased the activities of some serum enzymes such as aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) (Alnaqeeb *et al.,* 2003). *Ginger* and silymarin reduced serum ALT, AST, and ALP indicating membrane stabilization and antioxidant properties of *ginger* (Bhandari *et al.,* 2003). Results obtained from other study revealed that the values of serum AST and ALT were significantly decreased in rats treated with LTG and ginger 100 mg compared with epileptic group treated with lamotrigine(Ameneh *et al.,* 2014). (Aruna *et al.,* 2005) Indicate that cumin can decrease the lipid levels in

alcohol and thermally oxidized oil induced hepatotoxicity.

Table 4. Effect of dried lemon, ginger and cumin on some liver enzymes and serum glucose of obese rats.

Parameters / Groups	AST u/l	ALT u/l	Glucose mg/dl
Control (-)	46.047 [d] ± 4.035	21.440 [f] ± 2.677	90.427 [e] ± 3.461
Control (+)	76.810 [a] ± 4.833	43.648 [a] ± 3.460	151.636 [a] ± 5.318
Lemon(200mg/kg)	49.934 [d] ± 3.148	24.886 [e f] ± 2.177	93.718 [e] ± 1.983
Ginger(200mg/kg)	56.192 [c] ± 3.590	29.374 [c d] ± 2.903	104.311 [d] ± 4.531
Cumin(200mg/ kg)	57.051 [c] ± 4.238	31.413 [b c] ± 2.601	116.358 [c] ± 6.988

Also, it could be noticed that, the mean values of serum glucose levels (mg/dl) for all treated groups were decreased significantly, as compared to the positive control group, but the finest results were for groups of rats that fed on diet contained 200mg/kg of dried lemon Table (4).

These results are in agreement with much previous study. Glucose levels were substantially reduced in ginger- treated diabetic groups (Al-Noory *et al.,* 2013). Ginger root supplementation significantly lowers blood glucose and levels. When combined with dietary and lifestyle interventions it may be an effective intervention for managing Type 2 diabetes mellitus (James *et al.,* 2015). Consumption of ginger produced a significant antihyperglycemic effect in experimentally induced diabetic rats (Sultan *et al.,* 2014). Treatment with cumin decreased a blood glucose level. This may be through stimulation of surviving β-cells to produce insulin. In addition, it is known that the antioxidant effect of cumin suppressed apoptosis and exerted beneficial effects on pancreas β-cells (Gehan *et al.,* 2016). The researchers attributed the antihyperglycemic and hypoglycemic effects to flavonoids present in cumin, most likely through potentiation of insulin secretion. Based on animal study using diabetic models, cumin (Dhandapani *et al.,* 2002) or a methanolic extract of cumin (Jagtap *et al.,* 2010) resulted in a reduction in blood glucose and glycosylated hemoglobin, and improved serum insulin content when compared to diabetic control rats. C. citratus at a dose of 200 mg/kg body weight decrease the blood glucose level. (Adegbegi *et al.,* 2015).

CONCLUSION

In conclusion, consumption of dried lemon, ginger and cumin at certain levels 200mg/kg in this study may be useful for treatment of obesity because their lowers body weight, lipid profile, liver functions, and serum glucose level Further studies are recommended to determine the medicinal effect of other different fractions of dried lemon, ginger and cumin extract. Also should be noted to the importance of antioxidants in of these herbs and their relation to the treatment of obesity and improving the lipids, sugar and liver enzymes

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تأثير الليمون والزنجبيل والكمون المجفف في تخفيض الوزن وبعض المعاملات البيوكيميائية في الفئران التى تعانى من السمنة

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وقد أجريت هذه الدراسة لمعرفة تأثير الليمون والزنجبيل والكمون المجفف والتى تحتوى على نسبة عالية من مضادات الاكسدة مثل الفلافونات والفينولات على الفئران المصابة بالسمنة وقياس ذلك التاثير عن طريق تقدير بعض القياسات الكيميائية مثل مستوى الليبيدات ومستوى الجلوكوز وانزيمات الكبد وكذلك عن طريق قياس بعض المعاملات الغذائية لدى الفئران مثل معدل الزيادة فى الوزن ومستوى الماخوذ يوميا من العلف .وقمنا ايضا بقياس مستوى الفيتولات والفلافونات فى الليمون والزنجبيل والكمون المجفف . وقد اجريت الدراسة على عدد (٣٠) فار تم تقسيمهم الى مجموعتين رئيسيتين المجموعة الاولى وعددها (٦) فئران واستخدمت كمجموعة ضابطة سالبة وقد تغذت على الغذاء الاساسي والمجموعة الثانية وعددها (٢٤) فار تم تغذيتها على الغذاء العالى فى محتواه من الدهون لمدة ٦ اسابيع وذلك لزيادة وزن الفئران وقدر مستوى الزيادة فى الوزن لدى هذه المجموعة بعد ال ٦ اسبيع وكذلك تم اخذ عينات من الدم لتحديد مستوى الليبيدات فى هذه المجموعة للتاكد من زيادة الوزن . بعد ذلك تم تقسيم هذه المجموعة الرئيسية الى (٤) مجموعات فرعية كل مجموعة عددها (٦) فئران الاولى تلقت الغذاء العالى فى محتواه من الدهون بدون اى معالجات وتم استخدامها كمجموعة ضابطة ايجابية بينما المجموعات الفرعية الثلاث ٣ , ٤ , ٥ تلقت الغذاء العالى فى محتواه من الدهون والذى تم تدعيمه ب ٢٠٠ملجم من الليمون والزنجبيل والكمون المجفف على التوالى كعلاج لزيادة الوزن . وقد اشارت النتائج الى أن إضافة والليمون والزنجبيل والكمون المجفف ادات الى انخفاض ملحوظ فى الوزن لدى الفئران المصابة بالسمنة ،بالإضافة إلى انخفاض ملحوظ في متوسط قيم الكولسترول في الدم والدهون الثلاثية والبروتين الدهن يمن خفض الكثافة (LDL-C) ،البروتين الدهني من خفض الكثافة جدا (VLDL-ج)، والجلوكوز و انزيمات الكبد (ALT), (AST) في جميع المجموعات المعالجة ،مقارنة مع مجموعة الموجبة ،فيحين أن البروتين الدهني عالي الكثافة (HDLC) قد ارتفع.ومن خلال هذه النتائج يمكننا القول ان الليمون المجفف أظهر أفضل النتائج فى تأثيره على وزن الجسم، الدهون، وظائف الكبد، ومستوى الجلوكوز في الفئران التى تعانى من السمنة والتى تغذت على الغذاء العالى فى محتواه من الدهون . ويمكن أيضا القول بأن الليمون والزنجبيل والكمون المجفف غنية بالمواد المضادة للأكسدة مثلا لفينولات الفلافونات والتى تلعب دوراهامافي خفض مستوى الدهون والإنزيمات فى الكبد والجلوكوز وهى بذلك تعتبر مواد طبيعية تساعد فى مكافحة السمنة .