Biological and Histopathological Studies on Onions and Fenugreek in Obese Rats

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***ABSTRACT*:**
The present work was conducted to study effect of different levels (5%, 10% and 15%) fromonions and fenugreek on liver, kidney, function and blood lipid profile in rats feeding with diets containing high fat content for induction obesity. Fifty five female albino rats weighing of 250±10g were used and divided into elevenequl groups, one was kept as a control-ve group, while the other groups were fed onhigh fat diet for induction obesity and treated with onions , fenugreek and mixturefrom (onions and fenugreek) for 8 weeks. Body weight and food intake were recorded weekly. At the endof the experimental, all rats were weighted for calculation of body weight lose%,feed efficiency ratio. Blood serum samples were used for estimation of the liver and heart functions. Serum analysis showed a significant decrease in cholesterol, triglyceride and LDL in rat groups consumed different concentrations from onions and fenugreek. While HDL was significantly increased in all rats groups comparing with control positive group, serum liver function was significantly decreased. The obtained results concluded that an improvement of all chemical analysis as compared tothe positive control group.
It can be recommended that dietary intake from onions and fenugreek to decrease the side effects ofobesity and to reach healthy conditions. Further study should be conducted to carry out the pathological and safety investigation in concern to consume onions and fenugreek for a long time.

Key words: Obese rats, fenugreek , onions, high fat,HDL,LDL,cholesterol.

دراسات بيولوجية وتشريحية علي البصل والحلبة في الفئران المصابة بالسمنة

الملخص :

استهدف هذا البحث تقييم استخدام نسب مختلفة للبصل والحلبة (٥%، ١٠%،١٠%) علي وظائف الكبد والكلي وصور دهون الدم في الفئران التي تتغذي علي وجبات عالية الدهن تم استخدام ٥٥ فار من اناث الألبينو يترواح وزن كل منهم ٢٥٠±١٠ جرام وتم تقسيمهم الي ١١ مجموعة احداهما المجموعة الضابطة السالبة أما المجموعات الأخري تم تغذيتهم علي وجبات تحتوي علي وجبات تجريبية تحتوي علي وجبات عالية الدهن للتحريض علي السمنة وتم معالجتها بالبصل والحلبةومخلوط من البصل والحلبة.

وسجل وزن الجسم والمتناول أسبوعيا وفي نهاية التجربة تم حساب معدل التغيير في وزن الجسم ومعدل كفاءة الغذاء وقدرت وظائف الكبد والقلب عن طريق سيرم الدم وقد أوضحت النتائج المتحصل عليها. نقص معنوي في كلا من الكولسترول والجليسيريدات الثلاثية واللليبوبروتينات منخفضة الكثافة في كل المجموعات المعالجة بينما ظهر زيادة معنوية في اللليبوبروتينات عالية الكثافة في كل مجموعات الدراسة وحدوث تحسن معنوي في وظائف الكبد.

وتوصي الدراسة بضرورة تناول البصل والحلبة حتي نتجنب الاثار الجانبية والمحافظة علي كفاءة القلب والكبد مع اجراء ابحاث مستقبلية وهستوباثولوجية لمعرفة سلامة الاثر الناتج لاستعمال البصل والحلبة لفترات طويلة.

الكلمات الرئيسية: الفئران السمينة، الحلبة ، البصل،وجبات عالية الدهن، برتينات الدهون مرتفعة الكثافة، بروتينات الدهون منخفضة الكثافة ،الكوليسترول.

INTRODUCTION

Raju*et al*. (2001) found that the altered enzyme activities were significantly restored to control values in both the liver and kidney after *Trigonella* seed powder treatment. The therapeutic role of *Trigonella*seed powder in type-1 diabetes as exemplified in this study can be attributed to the change of glucose and lipid metabolising enzyme activities to normal values, thus stabilizing glucose homeostasis in the liver and kidney. These biochemical effects exerted by *Trigonella* seeds make it a possible new therapeutic in type-1 diabetes.

Thakran*et al*. (2004) showed that liver degenerative and early nephropathic changes in diabetic rats. Ultrastructure of the diabetic liver revealed a reduction in the rough endoplasmic reticulum and swelling of mitochondria in the hepatocytes. TSP treatment to the diabetic rats effectively prevented the alteration in the activities of the two enzymes and partially prevented the structural abnormalities thus suggesting a protective effect of TSP on the liver and kidney of the diabetic rats. The role of TSP in reversing the diabetic state at the cellular level besides the metabolic normalization further proves its potential as an antidiabetic agent.

Arivalagan*et al*.(2013) found that *Trigonellafoenum-graecum*, commonly called fenugreek, is a leguminous plant native to many Asian, Middle Eastern and European countries. Fenugreek oil is very effective in digestion. Identification of fenugreek genotype rich in saponins and fixed oil will be useful for pharmaceutical industries. In the present study, steroidal saponin and fixed oil content was analysed in 46 diverse fenugreek genotypes on dry weight basis .

Kendler(1987) found that garlic and onion have been used for millenia in the traditional medical practice of many cultures to treat cardiovascular and other disorders. Both *Allium* species, their extracts, and the chemical constituents of these plants have been investigated for possible effects on cardiovascular disease risk factors--both definite (hyperlipidemia, hypertension and hyperglycemia) and suspected (platelet aggregation and blood fibrinolytic activity).

Lata*et al*. (1991) found that oral administration of petroleum ether extract of *Allium sativum*, *Allium cepa* and ethylacetate extract of Commiphoramukul in albino rats significantly prevented rise in serum cholesterol and serum triglyceride level, caused by atherogenic diet. All the three agents were also found to confer significant protection against atherogenic diet induced atherosclerosis.

Banq*et al*. (2009) indicated that onion decreased blood glucose, serum lipid levels and reduced renal oxidative stress in STZ-induced diabetic rats and this effect might exert the anti-diabetic effect of onion.

Orie*et al*. (2012) suggested that dietary onion extract is beneficial for improving diabetes by decreasing lipid levels. They also examined differentiation ability of rat white preadipocyte cells using the onion extract and its sulfur-containing components. Cycloalliin, S-methyl-L-cysteine, S-propyl-L- cysteine sulfoxide, dimethyl trisulfide, especially S-methyl-L- cysteine sulfoxide were reported to be

effective in inhibiting formation of oil drop in the cells, suggesting that these compounds may be involved in the anti-obesity effect of the onion extract.

Kim *et al*. (2013) found that the total cholesterol level, low-density lipoprotein cholesterol level and atherogenic index significantly decreased ($P \leq 0.05$). No changes were observed in activities of erythrocyte antioxidant enzymes or levels of lipid peroxidation markers following onion peel extract supplementation. Additionally, no significant difference was found in plasma antioxidant vitamin (retinol, tocopherols, carotenoids, and coenzyme Q10) levels or ex vivo H2O2-provoked oxidative DNA damage after onion peel extract supplementation. The present interventional study provides evidence of the health benefits of onion peel extract and demonstrates its effects in modulating lipid profiles in healthy young Korean women.

MATERIALS AND METHODS

Materials:

1- Casein, all vitamins,minerals,cellulose, L- Cystine and choline chloride were obtained from El-Gomhoriya Company, Cairo, Egypt
2- Beef tallow, sucrose and corn oil were obtained from local market, Cairo,Egypt.
3- Strach was obtained from local market,Cairo,Egypt**.**
4- (*Allium cepa*)for slimming was obtained from the spices shop and *Trigon*ella*foenum*) was obtained from the spices shop and gaind it.

Animals:

Fifty five female albino rats were obtained from The Research Institute Ophthalmology Medical Analysis Department, Giza, Egypt and divided to eleven groups of five rats,each rat housed individually in cylindncal metabolic wire cages.

-group 1 is a negative control group, 2 is a positive control and numbers from 3 to 5were treated with*Trigonellafoenum* (5% - 10% - 15 %) .Groups 6 to 8 were treated with *Allium cepa* (5% - 10% - 15 %) .Groups 9 to 11were treated with amixture of *Allium cepa* and *Trigonellafoenum*(2.5 : 2.5 % - 5 : 5 % - 7.5 :7.5%).

Animals and Experimental Design:

Rats housed individually inwell aerated cages under hygienic laboratory conditions,in animals house of Medical Analysis Department, Giza, Egypt.and fed for 8 weeks on high dietdiet (20 % fat and 20 % protein) for adaptation before the beginning of the experiment according to**AIN (1993).**

Rats were divided into 11 groups , every five rats in each group fed on certain diet for 58 days as following:

Group (1): Rats fed on normal diet as negative control.

Group (2) :Rats fed on high fat diet (20 % fat and 20 % protein) as positive control.

Group (3) :Obeserats fed on high fat (20 % fat and 20% protein) and treated with *Ttrigonellafoenum* (for slimming) 5 % decoction.
Group (4) : Obeserats fed on high fat (20 % fat and 20% protein) and treated with *Ttrigonellafoenum* (for slimming) 10 % decoction.
Group (5) : Obeserats fed on high fat (20 % fat and 20% protein) and treated with *Ttrigonellafoenum* (for slimming) 15 % decoction.
Group (6) : Obeserats fed on high fat (20 % fat and 20% protein) and treated with *Allium cepa*(for slimming) 5 % decoction.
Group (7) : Obese rats fed on high fat (20 % fat and 20% protein) and treated with *Allium cepa*(for slimming) 10 % decoction.
Group (8) : Obeserats fed on high fat (20 % fat and 20% protein) and treated with *Allium cepa*(for slimming) 15 % decoction.
Group (9) : Obese rats fed on high fat (20 % fat and 20% protein) and treated with amixture of *Allium cepa*and *Ttrigonellafoenum* (for slimming)2.5:2.5% decoction.
Group (10): Obeserats fed on high fat (20 % fat and 20% protein) and treated with amixture of *Allium cepa* and *Ttrigonellafoenum* (for slimming) 5 :5 % decoction.
Group (11): Obeserats fed on high fat (20 % fat and 20% protein) and treated with amixture of *Allium cepa* and *Trigonellafoenum* (for slimming) 7.5 :7.5 % decoction.

Methods:

Food intake was calculated daily and rats were weighed weekly. Feeding and growth performance were carried out by determination of feed intake, body weight gain and feed efficiency ratio (FER) according to**Chapman *et al.* (1959)**, using the following formulae:

$$\textbf{FER} = \frac{\textbf{Body weight gain (g)}}{\textbf{Feed Intake (g)}}$$

$$\textbf{(BWG\%)} = \frac{\textbf{Final weight – Initial weight}}{\textbf{Initial weight}} \times 100$$

At the end of the of the experiment period(8weeks), rats were starvedfor 12 hr., then sacrificed under ether for anesthesia. Blood samples were collected into clean dry centrifuge tubes and stored at room temperature for 15 minutes, then put into a refrigerator for 2 hour; after that centrifuged for 10 minutes at 3000 rpm to separatetransferred into dry clean Wasserman tubes using a Pasteur pipette and then kept frozen at (-20C^{o})till analysis.

Biochemical Analysis:

Serum total cholesterol was calorimetrically determined according to **Allain*et al.* (1974)** and triglyceride was determined calorimetrically according to **Wahlefeld (1974).** High Density Lipoprotein cholesterol (HDL-c) was determined calorimetrically according to **Richmond (1973)**. Low Density Lipoprotein

cholesterol (LDL-c) and Very Low Density Lipoprotein cholesterol (VLDL-c) were calculated mathematically according to **Friedewald*et al*. (1972)**:LDL-c = TC-[HDL-c + (TG/5)] VLDL-c = Triglycerides/5.

Serum glucose and serum insulin were estimated according to**Trinder (1969)**. Serum Asparate and Alanine amino transferases (AST and ALT) were determined using enzymatic colorimetric methods**Young(1990)** and**Tietz (1976).**

Statistical Analysis:

Statistical analysis was performed using computer program, Statistical Package for Social science and compared with each other using the suitable tests**SPSS(1998).**

RESULTS AND DISCUSSION

Data present in Table(1) show effect of feeding with different concentrations(5%,15% and 15%) foronions, fenugreek and their mixture of them on food intake, body weight gain% and feed efficiency ratio in obeserats.

The mean value of feed intake of control positive group was18.72gwhile the mean value of control negative group was 16.86g. The obtained results showed that there were non- significant differences expect for 5% ,10% and 15% tested groups as compared to positive groups.

The same table showed the highest mean value of BWG% was95.62 for rats fed on control positive group. While the lowest mean value was15.45g and 25.72g of rats fed on diet containing for15% mixture of onions and fenugreek and 15%for fenugreek. However, feeding rats on 5% onions and onions 10% gave the highest values as compared with the other treatments.

There were a significant decrease in BWG% among all tested groups as compared to positive group.

As for FER, the results show that mean value of all groups were a significantly differences among all tested groups as compared to positive groups expect for rats feeding 5% Which was significant ($P \leq 0.05$).

The mean values of feed intake were almost the same in most of cases, food efficiency ratio showed a pronounced decrease as compared to the negative control. This reduction may be due to the decrease of body weight during the experimental period.

The results agreed with**Gisele *et al* .(2011)**and**Parveen*and Uma*(2013)**reported that onions and fenugreek supplemented can decrease the body weight gain than that of control group but the difference was not significant.

Parveen*et al* .(2013)found that results were comparable with orlistat, a anti - obesity drug, and standard provide clear evidence that the AqE-TFG treatment offered significant protection against MSG-induced dyslipidemia and oxidative stress.

Vats*et al*.(2004) found that administration of all the three plant extracts exerted a favorable effect on body weight and blood glucose.

Gisele *et al*.(2011) found that NAC improved high- sucrose diet-induced obesity and its effects on glucose tolerance, lipid profile, in vivo LDL-oxidant defences.

Table (1)*:* Feed intake (F1) g, body weight gain (BWG%) and feed efficiency ratio (FER) of control and obese rats treated withonions ,fenugreek. and mixture from them .

parameters / Grops	Feed intake g/day	BWG %	FER
Negative group	18.72 ±7.35	40.85±3.15	2.182±0.081
Positive group	16.86 ±2.38	95.62±1.38	5.671±0.39
Fenugreek 5%	16.08 ±1.75	53.83±1.13**	3.347±0.014
Fenugreek 10%	16.71 ±1.61	42.5±2.04**	2.543±0.026
Fenugreek 15%	15.65 ±1.54	25.72±1.62***	1.643±0.091
Onions 5%	16.91 ±3.23	68.81±3.28*	4.069±0.251
Onions 10%	16.42 ±3.19	58.59±2.16*	3.568±0.136
Onions 15%	15.82 ±3.52	35.76±1.54***	2.260±0.101
Mixture2.5:2.5%	16.18 ±2.16	46.69±1.46**	2.885±0.026
Mixture 5:5%	16.32 ±1.81	40.95±1.16**	2.509±0.031
Mixture7.5:7.5%	16.38±1.19	15.45±1.31***	.0943±0.061

Mixture : (onions and fenugreek).

*Differences are significant at P≤0.05 **Differences are highly significant at P≤0.01 ***Differences are highly significant at P≤0.001.

1 Feed efficiency ratio

Table (2) illustrate the festing serum lipids and the effect of feeding with different concentrations (5%, 10% and 15%)foronions, fenugreek and mixture from them on serum lipids of obese rats.

It could be observed that total cholesterol, TG and LDL decreased as a result of adding onions,fenugreek and mixture from them in the diet. There were very highly significant difference in TC and TG of mixture from onions and fenugreek 15% group as compared to positive group. As for HDL, the.mean value of negative control was higher than that of positive control. While the mean values of groups fed on diets with 15% mixture for onions and fenugreek and 15% onions were more significantly higher than that of positive control.

Concerning LDL, the mean value of negative control was lower than that of positive control. The mean values of all the groups were significantly lower than that of positive control.

The results agreed with **Kim *et al*. (2013)** and **Atta *et al*. (2012)** they showed that,onions and fenugreek decreased total cholesterol,LDL cholesterol and triglycerides .

Parveen *and Uma*(2013) found that AqE-TFG (Aqueous extract of Trigoellafoenumgarecum) produced significant reduction in serum total cholesterol (TC), triglycerides (TGs), lactate dehydrogenase (LDH) and elevationin serum high density lipoprotein cholesterol (HDL-C), hepaticand cardiac antioxidant enzymes

Kim *et al*.(2013) found that the total cholesterol level, low-density lipoprotein cholesterol level and atherogenic index significantly decreased ($P \leq 0.05$).

Table (2): Mean values of lipids parameters of control and obese rates treated fed on onions,fenugreek and mixture from them.

Serum lipids	Total cholesterol	Triglycerides	HDL-cholesterol	LDL-cholesterol	VLDL-cholesterol
Negative group	168.13±5.06	70.85±3.27	54.91±1.17	99.05±2.32	14.17±0.73
Positive group	196.87±2.31	93.73±2.15	43.48±0.92	134.65±4.15	18.74±0.56
Fenugreek 5%	188.33±3.19	88.61±1.37	47.16±0.76*	123.45±4.50	17.72±0.89
Fenugreek 10%	180.02±2.57*	82.45±1.16*	50.53±2.31*	113.0±2.46*	16.49±0.92
Fenugreek 15%	178.15±1.88**	77.55±1.33**	51.07±1.19**	111.57±1.88*	15.51±0.64*
Onions 5%	186.19±1.51	85.47±1.11	50.38±1.38*	118.72±1.93*	17.09±0.79
Onions 10%	178.46±1.67**	79.75±2.47**	51.01±2.15**	111.50±0.65*	15.95±0.25*
Onions 15%	175.77±6.13**	76.81±3.81**	53.46±3.08***	106.95±2.29**	15.36±0.67**
Mixture2.5:2.5%	180.48±2.31	80.17±2.97*	50.78±1.27**	113.67±2.28	16.03±.044
Mixture 5:5%	177.67±5.43**	75.31±1.81**	50.29±3.11**	111.69±4.67*	15.06±0.27**
Mixture7.5:7.5%	173.23±3.11**	73.28±1.05***	53.87±1.32***	104.71±1.63**	14.65±0.66***

Mixture : (onions and fenugreek).

*Differences are significant at $P \leq 0.05$ **Differences are highly significant at $P \leq 0.01$ ***Differences are highly significant at $P \leq 0.001$.

Table (3) showsserum ALT, AST and serum glucose of control positive and different groups of obese rats fed on different concentration (5%, 10% and 15%) from onions, fenugreek and mixture from them. It's clear that ALT and AST for control positive was higher than control negative.

It could be observed the improvement of ALT and AST was highest for mixture

Fenugreek 5 % from onions and fenugreek 15% diet (6.20 and 16.11) while was lowest for diet (7.11 and 19.76) difference were high significant ($P \leq 0.001$) when compared with control positive group.

This result in table (3) agreed with those reported by **Parveen and Uma(2013)** aspartate amino transferase (AST), alanine amino transferase (ALT), hepatic and cardiac lipid peroxides (MDA) levels.

Mete *et al* . (2013) indicated that pretreatment with ACE (*Allium cepa*) protects against DOX- (Doxorubicin) induced hepatotoxicity due to the antioxidant properties of ACE. Further studies on efficacy of antioxidant treatment by ACE in DOX-mediated toxicity and underlying mechanisms would provide a better explanation.

Table (3): Mean values of liver functionparameters of control and obese rates treated fed on onions, fenugreek and mixture from them.

parameters / Grops	ALT u/l	AST u/l
Negative group	6.17±0.13	16.25±0.091
Positive group	7.71±0.36	20.67±0.53
Fenugreek 5%	7.11±0.011	19.76±.075
Fenugreek 10%	6.53±0.049*	19.33±1.10
Fenugreek 15%	6.37±0.021**	17.63±.073
Onions 5%	6.65±0.033	18.46±0.90
Onions 10%	6.43±0.018*	17.15±0.54*
Onions 15%	6.31±0.011**	16.32±0.18**
Mixture 2.5:2.5%	6.38±0.218**	17.17±0.26*
Mixture 5:5%	6.33±0.027**	16.56±0.88**
Mixture 7.5:7.5%	6.20±0.015***	16.11±0.37**

Mixture : (onions and fenugreek).

*Differences are significant at P≤0.05 **Differences are highly significant at P≤0.01 ***Differences are highly significant at P≤0.001.

Table (4) showsserum glucose of control positive and different groups of obese rats fed on different concentration (5%, 10% and 15%) of onions, fenugreek and mixture from them It's clear that serum glucose of control positive was higher than control negative.

It could be observedthe improvement of serum glucose was highest for 15% diet mixture of onions and fenugreek (79.63mg/dl) while it was lowest for fenugreek

5% diet (81.75mg/dl) ,the difference was high significant ($P \leq 0.01$) when comparedwith control positive group.

This result agreed with those reported by **Tripathiand Chandra (2009)** That extractsin diabetic rats has remarkably improved the elevated levels of lipid fasting blood glucose . A significant decreasein peroxidation ($p \leq 0.001$) significant.

Imad*et al*.(2010) found that in assessment of hypoglycaemic activity of *Allium cepa* in type 1 and type 2 diabeticpatients, ingestion of crude*Allium cepa* (100 g) caused a considerable reduction in fasting blood glucoselevels by about 89
mg/dl in relation to insulin (145 mg/dl) in type 1 diabetic patients and it reduced fasting blood glucose levels by 40 mg/dl.

Table (4): Mean values of serum glucose parameters of control and obese rates treated fed on onions, fenugreek and mixture from them.

parameters / Grops	Serum Glucose mg/dl
Negative group	75.17±3.38
Positive group	94.22±6.18
Fenugreek 5%	89.93±1.19
Fenugreek 10%	86.32±2.65
Fenugreek 15%	84.97±1.11
Onions 5%	85.36±2.06
Onions 10%	84.22±1.87
Onions 15%	81.75±2.34*
Mixture 2.5:2.5%	83.27±3.63
Mixture 5:5%	82.81±5.16*
Mixture 7.5:7.5%	79.63±4.82**

Mixture : (onions and fenugreek).
*Differences are significant at P≤0.05 **Differences are highly significant at P≤0.01 ***Differences are highly significant at P≤0.001. 1 Feed efficiency ratio

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