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Original Research Article

Effect of *Trigonella Foenum-Graecum L.* Seed Powder on Dyslipidemia and Oxidative Stress in High Calorie Diet-Induced Obese Rats

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ABSTRACT

Background: As obesity has reached the level of epidemic proportions according to the World Health Organization, with an approximate number of 1.4 billion worldwide overweight and 300 million persons clinically obese. Obesity is not limited to developed countries but it is spreading globally. Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Almost all researchers believe that prevention could be the key strategy for controlling obesity. World Health Organization estimates traditional medicines, mostly plant drugs cater to the health needs of nearly 80% of world population. The present study was planned to investigate the influence of *Trigonella foenum-graecum* seed powder (FSP) on dyslipidemia in experimental obesity, which was induced by the high-calorie diet.
Results: Animals were divided into four groups: a control group fed a regular diet and tap water with/without 2% FSP, and HCD groups that were fed a high-calorie diet with/without 2% FSP for 14 weeks, respectively. Treatment with FSP significantly suppressed the increments of body weight, liver weight, and epididymal fat weight. FSP improved serum aspartate amino transferase (AST), alanine amino transferase (ALT), and lactate dehydrogenase (LDH) levels. FSP elevated the antioxidant enzyme (glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT)) levels.
Conclusions: These findings demonstrated the preventive effect of *Trigonella foenum-graecum* seed powder on dyslipidemia, due to inhibition of impaired lipid digestion and absorption, in addition to improvement in lipid metabolism, increased antioxidant defense.

Keywords: *Trigonella foenum-graecum* seed powder; Fenugreek; Obesity; High calorie diet; Oxidative Stress; Dyslipidemia; Prevention.

INTRODUCTION

The growing prevalence of obesity in the population, historically considered a problem of high-income countries, is increasingly affecting developing countries. [(1)] A number of studies have gone beyond the mere estimation of the distribution of BMI in the population and prevalence of obesity, and analysed the association between obesity and a series of socio-economic and behavioural variables [(2-10)] Replicating consistent research results from high-income countries, these studies have produced evidence of an association between BMI and gender, alcohol use, tobacco, physical exercise, urban vs. rural living. [(11-14)]

Intercountry comparable overweight and obesity estimates from 2008 show that 53.5% of the adult population (>20 years old) in Ukraine was overweight and 21.3% were obese. The prevalence of overweight was lower among men (50.5%) than women (56.0%). The proportion of men and women that were obese was 15.9% and 25.7%, respectively. Adulthood obesity prevalence forecasts (2010-2030) predict that in 2020, 32% of men and 10% of women will be obese. By 2030, the model predicts that 49% of men and 6% of women will be obese. [15]

Obesity in youth adversely affects their psychological, musculoskeletal, cardiovascular and respiratory health [16,17] Furthermore, it is associated in the long-term with adult obesity, insulin resistance, type-2 diabetes, cancer, respiratory disease, osteoarthritis, hypertension, dyslipidemia, cardiovascular morbidity and premature mortality [18-21]

As obesity has reached the level of epidemic proportions according to the World Health Organization, with an approximate number of 1.4 billion worldwide overweight and 300 million persons clinically obese [22] During the last years, changes in lifestyle, changes in the food system (increased consumption of dietary fats), reduced physical activity seem to be the major drivers of the rise of the global epidemic of obesity. [23,24] Obesity is increasing in adults and children, and has been described by the WHO as a global epidemic with an estimated 500 million obese adults and 1.5 billion overweight or obese individuals worldwide [25,26]

Prevention strategies must be based on a better evidence-based knowledge of factors able to either increase or decrease obesity risk. Obesity is a multifactorial disease involving genetic, environmental and behavioral factors, the latter including nutritional factors that comprise diet, alcohol consumption, body fatness and physical activity.

Over the years, many medications have been used to manage obesity. Due to obscure etiology, the treatment of obesity is difficult. Further, the cause of concern is the non-availability of drugs for its treatment and the short-term efficacy and limiting side effects of the available drugs. [27] Currently, orlistat is the only Food and Drug Administration (FDA) approved drug for long term management of obesity but this drug has undesirable gastrointestinal side effect such as steatorrhea. [28] Further studies for obesity treatment will give us chance to manage weight problem more effectively.

Herbal supplements are being extensively used due to their effectiveness in managing many chronic disorders. They are cost-effective, and exert less to no toxic side-effects in comparison with many chemically synthesized drugs. [29] Out of many such medicinal plants, fenugreek [Trigonella foenum-graecum Linn (Fabaceae)] has recently attracted the attention of scientists from across the globe. Fenugreek is traditionally used in India, especially in the Ayurveda and Unani systems. [30,31] Fenugreek belongs to the family Fabaceae and is applied in many parts of the world for the treatment of diabetes. At maturity the pods contain hard brown seeds of fenugreek, which is known and utilized for its medicinal use. In the ancient Indian traditional system of medicine, Ayurveda, fenugreek has been suggested as an important medicine to treat a variety of digestive and mucosal conditions. [32,33] The presence of proteins and fiber in TFG seeds offers high nutritive value as it contains approximately 26% protein and 48% fiber and might exert a lipid lowering effect. Fenugreek has been shown to possess antioxidant activity in different experimental animal models. The plant has also been employed against diseases such as bronchitis, fever, sore throat, wound, swollen glands, skin irritation, diabetes and ulcers. Fenugreek is well known for its multiple pharmacological properties including antidiabetic, antioxidative, hypocholesterolemic, antineoplastic, anti-inflammatory, antiulcer-rogenic, antipyretic, immunomodulatory and antitumor [34-36] Active compounds of fenugreek included soluble fiber, saponins, trigonelle, diosgenin, and 4 hydroxyisoleucine. [37-45] Hypoglycemic activities have mainly been attributed to dietary fiber and saponin. [46-48]

There is greater need to study the pharmacological and toxicological effects of herbal products to examine their clinical efficacy and safety. Because, every drug has potential side effects. Is no exception and even herbal products. They are not completely safe in this regard.

In this regard, side effects of Trigonella foenum-graecum L. have been studied to spell out its potential side and therapeutic effects. Despite the significant number of scientific papers devoted to the study fenugreek, draws attention to the lack of definitive views on the mechanism of action fenugreek and therefore the principles of its application, which generally determines the relevance of research under this specific problem. Therefore, finding out the positive and negative effects of fenugreek need for evidence-based use as a drug for the treatment and prevention of various diseases.

It was shown obesity is one of the conditions that decrease antioxidant capacity. The present study was planned to investigate the influence of *Trigonella foenum-graecum* seed powder (FSP) on obesity induced by high calorie diet.

MATERIALS AND METHODS

Research was conducted according to with the standards of the Convention on Bioethics of the Council of Europe's **'**Europe Convention for the Protection of Vertebrate Animals**'** used for experimental and other scientific purposes**'** (1997), the general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001) and other international agreements and national legislation in this field. Animals were kept in a vivarium that was accredited in accordance with the 'standard rules on ordering, equipment and maintenance of experimental biological clinics (vivarium)'. The tools used to research were metrological control.

Animals and housing conditions

Studies conducted on 40 non-linear rats and divided to four groups of 10 animals each. The animals of each experimental group were individually housed in polypropylene cages in an environmentally controlled clean air room, with a temperature of 22±3$^{\mathbf{0}}$C, a 12 h light/12 h dark cycle and a relative humidity of 60±5%.

Animals and diet

Rats of group 1 (Control, C) were given water ad libitum and were fed by a standard food during 14 weeks of the experimental period. Food consumption was measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week.

The (HCD) group was fed by a high-caloric diet, which contained: standard nutrition (60%), lard (10%), eggs (10%), sucrose (9%), peanut (5%), dry milk (5%), vegetable oil (1%) and water ad libitum. [49] Food consumption was measured daily at the same time (09:00 to 10:00 h). The body weights were determined once a week.

Rats of group 3 (CFg) were fed by a standard nutriment supplemented with 2% fenugreek seed powder. Food consumption was measured daily at the same time (09:00 to 10:00 h). The body weights were determined once a week.

The (HCDFg) group was fed by, a high-caloric diet, which contained: standard nutrition (60%), lard (10%), eggs (10%), sucrose (9%), peanut (5%), dry milk (5%), vegetable oil (1%) and fenugreek seed powder (2%) during 14 weeks of the experimental period. Food consumption was measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week.

Body mass index (BMI) = body weight (g) / length^2 (cm^2) [50]

Seeds of Trigonella foenum graecum L. varieties Ovari 4 were provided by Professor of the University of West Hungary Sándor Makai (Institute of Crop

Sciences, Department of Medicinal and Aromatic Plants).

Analytical methods

Liver function markers as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and triglycerides (TGs), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) were estimated by biochemical analyzer Microlab 300 (Elitech, France) and commercial kits from Elitech diagnostic (France) according to the standard protocols provided by manufacturers. Very low density lipoprotein cholesterol (VLDL-C) level was estimated using Friedewald's equation: VLDL-C = TGs/5.

The activity of superoxide dismutase (SOD) and catalase (CAT) were determined according to previously described methods in [51,52] The activities of glutathione peroxidase (GPx) was estimated according to methods described by Vlasova. [53] The total proteins were measured using Bradford assay. [54]

Histopathological Analysis

Autopsy material (areas piece of liver) was fixed in a formaldehyde solution with 10% volume fraction of at least 24 hours, dehydrated in alcohols of increasing concentration series, enlighten in chloroform and embedded in paraffin. 7 µm mikron thick sections were stained with hematoxylin and eosin, azure -2- eosin according to AA Maksimov. It was carried Schick-reaction to determine the level of glycogen in hepatocytes and cardiomyocytes using morphometric software Paradise in conventional arbitrary units of optical density. Using a light microscope Leica ICC50 HD we examined histological specimens and conducted photomicrography.

Statistical analysis

Statistical analysis of data was carried out by the software package 'Statistica 8.0'. For the analysis of data distribution type, Shapiro-Wilks criterion was used. As the data were normally distributed, we used Levan criterion for evaluating the equality of variance and Student's t test for independent samples. We calculated mean values (M) and standard deviations (SD). Significant difference was considered at $p \leq 0.05$

RESULTS

Effect of TFG on Body Weight and Food Intake

The feeding of HCD for 14 weeks caused a significant ($p < 0.05$) increase in body weight gain and BMI of rats, in comparison with the control rats. Add FSP to HCD (HCDFg group) for 14 weeks significantly ($p < 0.05$) suppressed the increase in the body weight gain and BMI in comparison with the HCD rats. Despite variation in body weight gain and BMI, there was no significant difference in food intake between all groups except group of CFg. A statistically significant ($p < 0.05$) increase in food intake was observed in the CFg group than those in control group.

Table 1: Effect of *Trigonella foenum-graecum seed powder* on initial body weight, final body weight, body weight gain, accumulated food intake, and - body mass index in high calorie diet- (HCD-) induced obese rats.

Parameters	Groups			
	C	HCD	CFg	HCDFg
IBW (g)	167,333±17,332	179,9±18,076	153,2±17,718	172,866±13,319
FBW (g)	325,266±4,911	416,3±9,511*	297,666±16,021*	343,333±25,116#
BWG (g/day)	1,611±0,177	2,412±0,174*	1,474±0,115	1,739±0,146#
AFI (g)	2022,14±63,901	2097,06±52,539	2324,21±121,162*	1973,22±64,773
BMI (g/cm^2)	0,705±0,066	0,953±0,115*	0,793±0,052*	0,776±0,063#

IBW - initial body weight; FBW - final body weight; BWG - body weight gain; AFI - accumulated food intake; BMI - body mass index
Data are presented as the M ± SD for ten animals in each group. Values are statistically significant at p<0.05.
* p<0.05 compared to control rats; # p<0.05 compared to HCD rats.

The weight of liver was significantly ($p < 0.05$) higher in HCD group than those in control group (Figure 1). There was no significant difference in weight of liver in CFg and HCDFg groups compared to the control group.

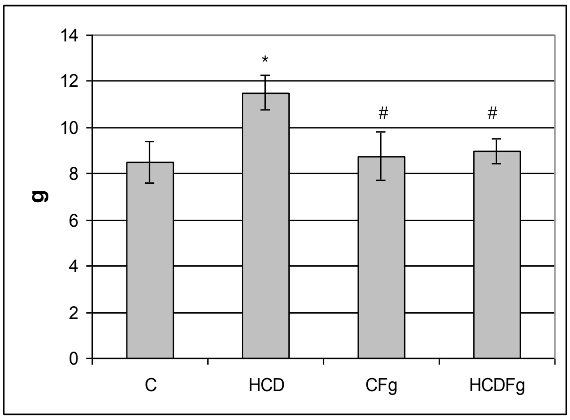
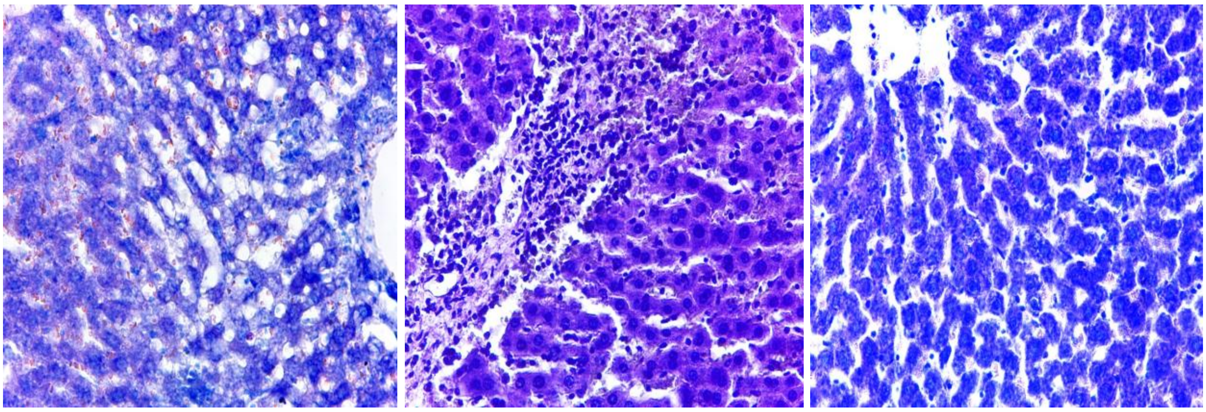


Figure 1: Effect of *Trigonella foenum-graecum L.* seed powder on liver weight in high calorie diet- (HCD-) induced obese rats. Data are presented as the M ± SD for ten animals in each group. Values are statistically significant at $p<0.05$.
* $p<0.05$ compared to control rats; # $p<0.05$ compared to HCD rats.

Hepatic Histopathology

Figure 2 represents liver histopathologies of experimental rats. The histopathological examination of HCD group showed signs of granular and vacuolar degeneration (Figure 2, a). Small foci of lymphocytic infiltration were located mainly in the stroma, in the course of the small arteries and veins increases the amount of tissue basophils. It was observed local periportal proliferation stroma. When high-calorie diet during treatment with fenugreek (FSP) in the liver revealed a moderately pronounced signs of granular dystrophy of hepatocytes, lymphocytic infiltration is almost not observed (Figure 2, c). The glycogen level in the cytoplasm of parenchymal cells in comparison with the above observation increased. At the same time, only some animals receiving a standard diet of vivarium with the addition fenugreek (Figure 2, b) were observed weakly expressed granular degeneration of the liver cells, sporadic congestion of lymphocytes.



a **b** **c**

Figure 2. Effect of *Trigonella foenum-graecum L.* seed powder on hepatic histopathological changes in high calorie diet- (HCD-) induced obese rats. **Group 1 (a)**: HCD control, showing fatty degeneration and greater hepatic lipid accumulation; **Group 2 (b):** C + TFG, showing mild congestion, no fatty changes, and less hepatic lipid accumulation; **Group 3 (c):** HCD + TFG, showing no fatty changes and considerably lower hepatic lipid accumulation.

Effect of FSP Serum Biochemistries

The lipid profiles are shown in Table 2. The HCD-induced obese rats exhibited a significant ($p < 0.05$) increase in the levels of TC, TGs, LDL-C, and VLDL-C and a significant ($p < 0.05$) decrease in HDL-C in comparison with control group. The levels of serums TC, TGs, LDL-C, and VLDL-C were significantly ($p < 0.05$) reduced, and the level of HDL-C was significantly ($p < 0.05$) increased in HCDFg group when compared to HCD group (Table 2).

The serum AST, ALT, and GGT activities were significantly ($p < 0.05$) increased in HCD group, when compared to the control group. A significant ($p < 0.05$) reduction in AST, ALT, and GGT activities was observed in HCDFg group in comparison to the HCD group (Table 3).

Determination of tissue biochemical indicators

Table 4 represents the oxidative stress marker in hepatic tissue. A marked decrease of antioxidant enzyme status (GPx, SOD, and CAT) was observed in the hepatic tissue of rats in HCD group when compared with control group. Add TFG to HCD (HCDFg group) for 14 weeks significantly ($p < 0.05$) raised the antioxidant enzyme (GPx, SOD, and CAT) activity.

Table 2: Effect of *Trigonella foenum graecum L.* seed powder on serum lipid levels in high calorie diet- (HCD-) induced obese rats.

Parameters	Groups			
	C	HCD	CFg	HCDFg
TC (mg/dL)	1,50±0,15	1,99±0,15*	1,59±0,13#	1,67±0,09#
TGs (mg/dL)	0,62±0,04	1,18±0,09*	0,66±0,11#	0,89±0,06*#
HDL-C (mg/dL)	0,742±0,065	0,575±0,041*	0,634±0,105	0,721±0,038#
LDL-C (mg/dL)	0,222±0,03	0,308±0,024*	0,246±0,027#	0,236±0,021#
VLDL-C (mg/dL)	0,124±0,008	0,236±0,018*	0,132±0,022#	0,178±0,01*#

Data are presented as the M ± SD for ten animals in each group. Values are statistically significant at p<0.05.,
* p<0.05 compared to control rats; # p<0.05 compared to HCD rats.

Table 3: Effect of *Trigonella foenum-graecum L.* seed powder on serum levels of liver enzyme markers in high calorie diet- (HCD-) induced obese rats.

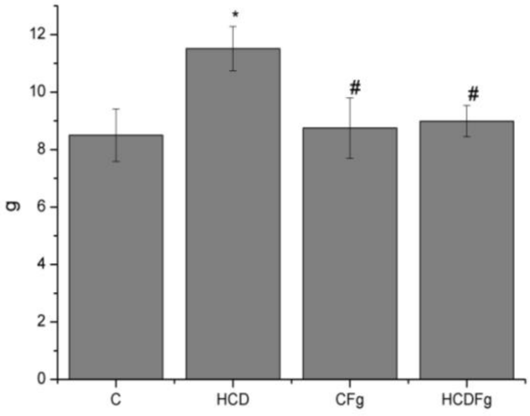
Parameters	Groups			
	C	HCD	CFg	HCDFg
ALT (U*L-1)	63,35±2,17	88,75±9,92*	65,38±9,87	71,93±3,59*#
AST (U*L-1)	250,6±25,1	310,9±22,9*	352,7±78,1	254,8±21,8#
GGT (U*L-1)	1,52±0,15	3,52±0,32*	2,1±0,56	1,88±0,4#

Data are presented as the M ± SD for ten animals in each group. Values are statistically significant at p<0.05.
* p<0.05 compared to control rats; # p<0.05 compared to HCD rats.

Table 4: Effect of *Trigonella foenum-graecum L.* seed powder on the activities of SOD, catalase and glutathione peroxidase in liver

Parameters	Groups			
	C	HCD	CFg	HCDFg
SOD (U/mg protein)	2,59±0,45	1,71±0,22*	1,77±0,37	1,90±0,20*
CAT(μmol H_2O_2/min*mg protein)	0,059±0,006	0,036±0,005*	0,057±0,005#	0,049±0,002*#
GPx (nmol GSH/min*mg protein)	11,33±2,87	3,00±1,83*	8,28±1,76#	4,99±1,12*

Data are presented as the M ± SD for ten animals in each group. Values are statistically significant at p<0.05.
* p<0.05 compared to control rats; # p<0.05 compared to HCD rats.



Graph 1

DISCUSSION

The present study was conducted to compare the various experimental groups (C, HCD, HCDFg, and CFg). It was established different levels of dyslipidemia, hepatosteatosis and oxidative stress. [55,56] Body mass index (BMI) is widely used to measure body fat. Published data have shown that BMI levels correlate with body fat and with future health risks. The prevention with *Trigonella foenumgraecum L.* seed powder for 14 weeks suppressed the increase in body weight and weight of liver induced by a HCD. [57] In our study, a significant reduction in body weight gain and BMI with FSP prevention indicates that FSP suppresses the HCD-mediated increase in body weight gain.

In the present study it was found dyslipidemia and oxidative stress in the HCD rats. Our study showed an increase total cholesterol levels and hypertriglyceridemia in rats with HCD-induced obesity (HCD-group). Our results agree with the data of Lavie and Milani, who showed that obesity adversely affects plasma lipids, especially by increasing TC, LDL-C, VLDL-C, TGs and decreasing the level of HDL-cholesterol. [58]

This is a high risk dysmetabolic situation. Metabolic disturbance is the main cause of dyslipidemia. The HCD might lead to an increase in the synthesis of phospholipids and cholesterol esters in rats. [59] Hyson et al indicated that the blood level of LDL-cholesterol and its oxidation are related to cardiovascular risk and the LDL-cholesterol level of blood is an index of health. [60] Add FSP in high caloric diet (HCDFg-group) resulted in significant reduction in levels of TC, TGs, VLDL-C and elevation in HDL-C which is similar to the findings of other researchers. Because fenugreek contains fiber, which have effect of dietary fiber on lipoprotein cholesterol is due to its association with absorption and transport of lipids. Also, according reports, Fenugreek seeds also lower serum triglycerides, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). These effects may be due to sapogenins, which increase biliary cholesterol excretion, in turn leading to lowered serum cholesterol levels. The lipid-lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone.

Either, the hepatic lipid-lowering effect of fenugreek seeds can be attributed to its role in modulating the activity of several glucose and lipid metabolism enzymes or to its ability to enhance biliary cholesterol excretion [61,62] (Raju J, 2001).

It is well known that hyperlipidemia decreases the strength of the antioxidative defense system. [63] Thus, the present study hypothesizes that the possible explanation for improvement in dyslipidemia following administration of FSP may be due to reduction in oxidative stress in HCD-induced obese rats.

As per earlier reports, the ALT enzyme is a sensitive marker of liver damage and AST levels are predictive of damage to the liver and other organs with high metabolic activity (brain, heart, and lungs). [64] Hence any necrosis or membrane damage to the liver and heart leads to leakage of these enzymes into the blood circulation. [65,66] The results of our study proved that obese rats were more exposed to hepatotoxicity as evidenced by increased levels of serum AST, GGT and ALT. Administration (application) of FSP significantly reduced the elevated GGT, AST, and ALT levels, which could be attributed to the protective effect on hepatic tissue.

Trigonella foenum graecum L. seeds (flavonoids and polyphenols) have antioxidative properties; reduce oxidative stress, what have been found in our experimental model of obesity. In the present study, HCD-induced obesity in rats showed decreased activities of GPx, SOD, and CAT enzymes. Our results are consistent with published data. [67,68] Therefore, it may be concluded that HCD causes the induction of oxidative stress in the hepatic tissue and may lead to the consequences like fatty liver disease. Administration of TFG for a period of 14 weeks resulted in significant elevation in antioxidant enzymes GPx, SOD, and CAT.

CONCLUSION

In conclusion, Trigonella foenum graecum L. seems to have an important role in preventing the development of HCD-induced dyslipidemia and oxidative stress. The antioxidant benefits of fenugreek related to its polyphenols and saponins, because of this it can be used for the treatment of liver. The protective effect of Trigonella foenum graecum L. also may be related to its free radical scavenging and membrane stabilizing properties, and may be helpful in protection from the metabolic disorders.

Thus, our experimental results allow to recommend the use of Trigonella foenum graecum L. seeds for the prevention of obesity.

Abbreviations

HCD: high-carbohydrate diet;
TFG: Trigonella foenum-graecum;
FSP: *Trigonella foenum-graecum* seed powder;
ALT: alanine aminotransferase;
AST: aspartate aminotransferase;
GGT: gamma-glutamyl transpeptidase;
TGs: triglycerides; TC: total cholesterol;
HCL-C: high density lipoprotein cholesterol;
LDL-C: low density lipoprotein cholesterol;
VLDL-C: very low density lipoprotein cholesterol;
SOD: superoxide dismutase;

CAT: catalase;
GPX: glutathione peroxidase;
BMI: body mass index.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GII, KVV, KLI, LDO performed experiments and statistical analysis of obtained data and prepared the article. KVV, KTD, YIV, PTD performed experiments and analysis of the study, did the literature review in part of the discussion, formulated prospects and performed the final article drafting. OLI did the organization, literature review and analysis of the study. All authors read and approved the final manuscript.

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