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Ameliorative role of a combination of chitosan, calcium, vitamins A and E against high fat diets-induced adverse effects in rats

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Abstract

Background: Chitosan is a promising natural source for controlling obesity. Obese people tend to use supplementary nutrients to compensate for their deficiencies in their diets. The present study aimed to investigate the combined effect of chitosan, calcium, and vitamins A and E supplements against the adverse effects of high-fat diet consumption in rats.

Methods: Twenty-five male albino rats were assigned into five equal groups. Control rats were fed standard basal diet (SBD). Another group was fed high-fat diet (HFD) without supplements. The other three groups were fed HFD containing 0, 400, and 800 mg of chitosan per kilogram diet, in presence of calcium and vitamins A and E supplements, daily for 10 weeks.

Results: As compared to controls, rats fed HFD without supplements showed significant elevations in body weight gain, feed consumption, relative weights of the heart and liver, serum levels of total cholesterol, triglycerides, low-density lipoprotein cholesterols, very low-lipoprotein triglycerides, urea, creatinine, and malondialdehyde, as well as serum activities of alanine and aspartate aminotransferases, alkaline phosphatase, and creatine kinase. Moreover, significant declines in the relative kidney weight, serum levels of high-density lipoprotein cholesterols, total proteins, albumin, globulin, calcium, vitamins A and E, erythrocyte glutathione content, and superoxide dismutase activity were recorded. Histopathological alterations were observed in rats fed HFD with or without supplements. On contrary, rats fed HFD containing chitosan and supplements showed remarkable improvement in all the studied parameters towards control values, in dose-dependent manner.

Conclusion: Chitosan, calcium, and vitamins A and E attenuated the adverse effects caused by HFD intake in rats.

Keywords: Biochemical variables, Biological parameters, Calcium, Fat-soluble vitamins, High-fat diet, Chitosan, Histopathology, Rats

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Background

Obesity is considered one of the biggest challenges threatening public health. Obesity is mainly produced by an imbalance between the input of calories and the energy expenditure (Delamater, Pulgaron, & Daigre, 2013). Such imbalance results in an abnormal accumulation of fats in non-adipose tissues such as the muscles, liver, kidney, and heart (Bahreini et al., 2013 and Snel et al., 2012). High-fat diet (HFD) contributes largely to obesity prevalence (Myles, 2014). The quality of life is adversely affected by obesity as being related to the incidence of several disorders such as hypertension, diabetes, osteoarthritis, and cardiovascular diseases (Boden, 2011). Therefore, more attention of scientific community was initiated to solve this serious problem. Several medications were approved for obesity controlling. However, most of these drugs have adverse side effects, expensive, and take long time to produce their effects (Mhurchu, Dunshea-Mooij, Bennett, & Rodgers, 2005).

Chitosan is a natural polysaccharide that is produced from chitin by N-deacetylation (Anraku et al., 2018). Globally, chitin is considered one of the most plentiful natural water-insoluble polymers (Muxika, Etxabide, Uranga, Guerrero, & De La Caba, 2017). Exoskeletons of invertebrates especially crustaceans represent the chief sources of chitin (Kumirska et al., 2010). Chitosan has cationic nature that facilitates its penetration through cellular membranes (Cheung, Ng, Wong, & Chan, 2015). Recently, chitosan was involved in gene delivery due to its biocompatibility (Choi, Nam, & Nah, 2016). Chitosan also has the ability to accelerate wound healing (Patrulea, Ostafe, Borchard, & Jordan, 2015). Moreover, chitosan is effectively used in management of body weight via scavenging lipids for further excretion (Walsh, Sweeney, Bahar, & O'Doherty, 2013). Lately, chitosan was reported to exert antioxidant properties both in vivo and in vitro (Anraku et al., 2011).

Calcium (Ca^{2+}) is primarily component in bone formation (Bonjour et al., 2014). In addition, Ca^{2+} also mediates muscle contraction, nerve signaling, vasodilation, and hormone secretion (Nobre et al., 2011). Vitamin A represents a vital micronutrient that is mainly found in carrot, mangos, liver, and eggs (Eastep & Chen, 2015). Vitamin A and retinoids are responsible for regulation of growth, reproduction, and epithelial tissue maintenance and repair in mammals (Eastep & Chen, 2015). Vitamin E is a general term that describes a group of fat-soluble vitamins (tocopherols and tocotrienols) with remarkable antioxidant properties (Niki & Traber, 2012). Vitamin E represents a major component of the endogenous antioxidant system that is obtained mainly from vegetable oils (Rizvi et al., 2014). The high lipophilicity of vitamin E allows its storage in adipose tissue and consequently, enhances its bioavailability (Zingg, 2007).

Obese people usually tend to use mineral and vitamin supplements to compensate for their deficiency in the unbalanced diet. To our knowledge, studies on the interaction between chitosan and other supplements are still rare (Rodrigues, Silva, & Lacerda, 2012). Accordingly, the present work was designed to study the potential combined effects of chitosan, calcium, and vitamins A and E supplements against the adverse effects induced by HFD consumption in rats.

Methods

Experimental diet

Two types of diets were applied in the present study. The first type was the standard basal diet (SBD) that was prepared according to the method described by Reeves, Nielsen, and Fahey Jr (1993). The second diet was the high-fat diet (HFD) that was prepared by modification of SBD by increasing the amount of lipids while decreasing the carbohydrate content (Chien, Ku, Chang, Yang, & Chen, 2016). The chemical composition of both diets is presented in Table 1.

Supplements

Chitosan of high molecular weight (310,000–375,000 Da), vitamin A (retinyl palmitate), and vitamin E (DL-α-tocopherol acetate) were obtained from Sigma-Aldrich chemical company, USA. Calcium (Ca^{2+}) was purchased as in the form of calcium carbonate from El Nasr pharmaceutical chemical company, Egypt. The amounts of vitamin A, calcium and vitamin E were supplemented according to Mahassni and Al-Shaikh (2013), Nobre et al. (2011) and Shen, Tang, Huang, and Cai (2010), respectively.

Animals

Adult healthy male Sprague Dawley rats, weighing 100 ± 10 g, were purchased from VACERA vivarium, Helwan,

Table 1 The percentage of chemical constituents of the standard basal diet (SBD) and high-fat diet (HFD)

Constituent	SBD	HFD
Cornstarch (%)	56.07	26.07
Casein (%)	14.00	14.00
Sucrose (%)	10.00	10.00
Corn oil (%)	10.00	10.00
Cellulose (%)	5.00	5.00
Minerals (%)	3.50	3.50
Vitamins (%)	1.00	1.00
Methionine (%)	0.18	0.18
Choline chloride (%)	0.25	0.25
Tert-butyl hydroquinone (%)	0.00	0.00
Lard (%)	0.00	30.00

*Significantly differed ($P < 0.05$), as compared to the SBD

Egypt. The rats were housed in an animal house of the Nutrition National Institute (NNI), Cairo, Egypt and maintained on standard diet and tap water for 1 week, for acclimatization, before starting the experiments.

Study design

Twenty-five male rats were divided into five groups (5 rats/group) as shown in Table 2.

Sampling

At the end of the experiment, rats were fasted for 12 h then were sacrificed under anesthesia using sodium pentobarbital. Blood samples were collected from the vagal vein and divided into two parts. The first part was collected in dry test tubes and allowed to stand, then were centrifuged at 3000 rpm for 15 min to separate the serum. The clear serum was stored in the deep freezer at − 20 °C for subsequent biochemical analysis. The second part was collected on EDTA and then centrifuged at 1000×*g* for 10 min at 40 °C. The resultant plasma and white buffy layer were discarded. The remaining erythrocytes were lysed in ice-cold HPLC grade water and then centrifuged at 10000×*g* for 15 min at 4 °C. The supernatant erythrocyte lysate was collected and stored in a deep freezer at − 80 °C. Dissection was executed immediately after scarifying to extract the heart, kidney, and liver. The extracted organs were minced in physiological saline (0.9%) and then dried using filter papers and weighed immediately.

Biological parameters

The amount of food consumed by each group was determined weekly throughout the experiments and then divided by the number of animals per group to estimate the average of food consumption. The starting and final weights of all the experimental rats were measured to calculate the body weight gain (BWG), after receiving diets. The relative weights of the heart, kidney, and liver of all the experimental groups were estimated at the end of experiments.

Table 2 Summarized experimental design of the study

Conditions	Experimental groups (*N* = 25 rats)				
	I	II	III	IV	V
Diet type	SBD	HFD			
Chitosan content (mg/kg diet)	0	0	0	400	800
Calcium content (g/kg diet)	–	–	10	10	10
Vitamin A content (mg/kg diet)	–	–	11	11	11
Vitamin E content (mg/kg diet)	–	–	350	350	350
Frequency	Daily				
Duration	For 10 weeks				
Sampling	At the end of experimental period				

SBD Standard basal diet; *HFD* high fat diet

Biochemical analysis

Determination of serum lipid profile

The levels of serum total cholesterol (TC), triglycerides (TG), and high- and low-density lipoprotein cholesterols (HDL-C & LDL-C), were analyzed using Analyticon Biotechnologies kit, Germany, according to the techniques designated by Allain, Poon, Chan, Richmond, and Fu (1974), Nauck et al. (1997), Sullivan, Kruijswijk, West, Kohlmeier, and Katan (1985), and Wieland and Seidel (1983), respectively. The levels of very low-lipoprotein triglycerides (VLDL-TG) were computed according to the Friedewald formula: VLDL-TG = TG/5.

Determination of serum protein profile

The levels of total proteins (TP) and albumin were estimated according to the pamphlet of ELiTech kit, France, according to the methods described by Bartholomew and Delaney (1964) and Doumas (1975) respectively. The levels of globulin and albumin/globulin ratio were calculated as described by Cui et al. (2015).

Determination of serum aminotransferases activity

The activities of serum alanine and aspartate aminotransferases (ALAT and ASAT) were determined according to the method stated by Schumann et al. (2002a, 2002b), using kits obtained from Stanbio Laboratory company, USA.

Determination of serum alkaline phosphatase activity

Serum alkaline phosphatase (ALP) was determined according to the manual instruction of the kit from Biosystem, Spain. The method was described by Tietz, Rinker, and Shaw (1983).

Determination of serum creatine kinase activity

The activity of creatine kinase (CK) was analyzed as described by Burtis, Ashwood, and Bruns (2012) using Spectrum kits (Cairo, Egypt).

Determination of serum creatinine and urea levels

Serum creatinine level was determined according to the manual instruction of the kit from BioMed, Egypt (Toora & Rajagopal, 2002). Serum urea was determined according to the manual instruction of the kit from Diamond, Egypt (Tabacco, Meiattini, Moda, & Tarli, 1979).

Determination of serum total calcium levels

The serum levels of the calcium determination test was performed according to Kessler and Wolfman (1964), using Spectrum kits (Cairo, Egypt).

Determination of serum vitamins A and E concentrations

The levels of vitamins A and E were determined in the serum of rats by aid of high-performance liquid chromatography (HPLC). The method of determination was according to Bieri, Tolliver, and Catignani (1979).

Determination of lipid peroxidation biomarker and endogenous antioxidants

Serum levels of malondialdehyde (MDA) were determined as lipid peroxidation (LPO) biomarker according to the method described by Uchiyama and Mihara (1978). The level of glutathione (GSH) and activity of superoxide dismutase (SOD) were detected in the erythrocyte as stated by Beutler (1963) and Winterbourn, Hawkins, Brian, and Carrell (1975), respectively. The serum levels of MDA as well as erythrocyte GSH content and SOD activity were estimated using Bio-diagnostic Kits (Giza, Egypt).

Histological examination

Small parts of the heart, kidney, and liver were fixed in 10% formalin for histological examination. The fixed tissues were processed for preparation of paraffin sections with thickness of 5 μm. These sections were then stained with hematoxylin and eosin, as described by Suvarna, Layton, and Bancroft (2018).

Statistical analysis

The present data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) version 22. Kolmogorov-Smirnov tests revealed that data was normally distributed, i.e., variables were parametric (Razali & Wah, 2011). Post hoc Duncan's test was used to show similarities in all the studied parameters among the experimental groups. Pearson's correlation coefficient was applied to correlate the studied parameters to the amount of chitosan in diet.

Results

Effect on biological parameters

The BWG, feed consumption, and relative weights of the heart, kidney, and liver of all the experimental rats, after 10 weeks of receiving the appropriate diets, were displayed in Table 3. In rats of groups II, III, and IV, BWG and feed consumption were significantly higher than in group I. Rats of group IV showed remarkable reductions in BWG and feed intake, as compared to groups II and III, but were markedly higher than in group V. In rats of groups III, IV, and V, the chitosan amount was negatively correlated with the BWG and feed consumption. Rats of groups II and III exhibited a marked decline in the relative kidney weight, but remarkable elevations in relative weights of the heart and liver, in comparison to the other groups. Relative liver weight of group IV was noticeably higher than in group I.

Table 3 Body weight gain (BWG), feed consumption, and relative weights of the heart, kidney, and liver of all the experimental groups, at the end of the experiments

Variables	Experimental groups				
	I	II	III	IV	V
BWG (g/10 weeks)	24.40 ± 0.98^{a}	36.80 ± 2.15^{c}	37.60 ± 0.51^{c}	31.40 ± 1.54^{b}	25.80 ± 0.58^{a}
	–	(+ 50.8%)[#]	(+ 2.2%)[¶]	(– 14.7%)[¶]	(– 29.9%)[¶]
				$r^{d} = -0.92$, $P < 0.001$	
Feed consumption (g/day)	9.43 ± 0.35^{a}	12.56 ± 0.50^{c}	11.20 ± 0.37^{c}	10.88 ± 0.32^{b}	9.27 ± 0.23^{a}
	–	(+ 33.2%)[#]	(– 10.8%)[¶]	(– 13.3%)[¶]	(– 26.2%)[¶]
				$r^{d} = -0.75$, $P < 0.01$	
Relative heart weight (g/100 g BW)	0.29 ± 0.01^{a}	0.43 ± 0.01^{b}	0.41 ± 0.01^{b}	0.27 ± 0.01^{a}	0.26 ± 0.01^{a}
	–	(+ 48.3%)[#]	(– 4.7%)[¶]	(– 37.2%)[¶]	(– 39.5%)[¶]
				$r^{d} = -0.80$, $P < 0.001$	
Relative kidney weight (g/100 g BW)	0.82 ± 0.02^{b}	0.67 ± 0.03^{a}	0.66 ± 0.01^{a}	0.80 ± 0.06^{b}	0.80 ± 0.02^{b}
	–	(– 18.3%)[#]	(– 1.5%)[¶]	(+ 19.4%)[¶]	(+ 19.4%)[¶]
				$r^{d} = +0.55$, $P < 0.05$	
Relative liver weight (g/100 g BW)	2.49 ± 0.11^{a}	3.20 ± 0.06^{c}	3.16 ± 0.08^{c}	2.88 ± 0.08^{b}	2.65 ± 0.04^{ab}
	–	(+ 28.5%)[#]	(– 1.3%)[¶]	(– 10.0%)[¶]	(– 17.2%)[¶]
				$r^{d} = -0.83$, $P < 0.001$	

Data is displayed as mean ± standard error of mean. $P < 0.05$, $P < 0.01$, and $P < 0.001$ represent significant correlations. In the same raw, values marked with similar superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are statistically different ($P < 0.05$)

r^{d}: correlation coefficient between the studied variables and the administered doses of chitosan, I: control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400 mg chitosan and V: rats fed HFD + supplements + 800 mg chitosan

[#]Percent of change in comparison to the group I

[¶]Percent of change in comparison to the group II

Effect on lipid profile in serum

In Table 4, the levels of TC, TG, HDL-C, LDL-C, and VLDL-TG in the serum of all the experimental groups were recorded. Rats of groups II and III exhibited marked elevations in serum levels of TC, TG, LDL-C, and VLDL-TG, whereas a remarkable decline in HDL-C levels, as compared to group I. In comparison to groups II and III, rats of groups IV and V showed significant reductions in the serum levels of TC, TG, LDL-C, and VLDL-TG, whereas a remarkable elevation in HDL-C levels towards the control values.

Effect on protein profile in serum

The concentrations of TP, albumin, globulin, and albumin/globulin ratio of all groups were clarified in Table 5. In rats of groups II and III, serum levels of most protein variables were markedly lower than in groups I, IV, and V. By increasing the amount of chitosan in HFD containing supplements, serum levels of all protein variables were significantly elevated towards the normal values of controls.

Effect on activities of aminotransferases, alkaline phosphatase, and creatine kinase, as well as levels of creatinine and urea in serum

Table 6 displayed the serum activities of ALAT, ASAT, ALP, and CK, as well as the serum levels of creatinine and urea of all rats. Rats of groups II, III, IV, and V exhibited marked elevations in serum activities of ALAT, ASAT, and ALP, in comparison to group I. The serum activities of ALAT, ASAT, and ALP of group IV were significantly higher than in group V, whereas remarkably lower than in groups II and III. Among all the experimental groups, serum activity of CK and serum levels of creatinine and urea were similar except for marked elevations in groups II and III.

Effect on serum levels of calcium, and vitamins A and E

The serum levels of calcium and vitamins A and E of all groups were shown in Table 7. In rats of group II, serum levels of calcium, and vitamins A and E were significantly depleted, as compared to the other groups. Group III showed marked elevations in serum levels of calcium and vitamins A and E, in comparison to the rest of groups. In rats of group IV, serum levels of calcium and vitamins A and E were significantly greater than in group V. Rats of groups IV and V revealed remarkable elevations in vitamins A and E levels in serum, as compared to groups I and II.

Effect on serum levels of malondialdehyde and erythrocyte glutathione content and superoxide dismutase activity

The serum MDA level as well as erythrocyte GSH content and SOD activity of all groups were shown in

Table 4 The levels of total cholesterol (TC), triglycerides (TG), high- and low-density lipoprotein cholesterols (HDL-C & LDL-C) and very low-density lipoprotein triglyceride (VLDL-TG) in serum of all the experimental groups, at the end of the experiments

Variables	Experimental groups				
	I	II	III	IV	V
TC levels (mg/dL)	92.20 ± 1.24^{a}	165.80 ± 2.78^{d}	160.60 ± 2.78^{d}	124.00 ± 0.89^{c}	111.60 ± 0.81^{b}
	–	(+ 79.8%)[#]	(– 3.1%)[¶]	(– 25.2%)[¶]	(– 32.7%)[¶]
				$r^{d} = -0.94$, $P < 0.001$	
TG levels (mg/dL)	75.20 ± 0.49^{a}	175.40 ± 4.23^{d}	173.60 ± 4.01^{d}	99.80 ± 1.07^{c}	88.20 ± 2.15^{b}
	–	(+ 133.2%)[#]	(– 1.0%)[¶]	(– 43.1%)[¶]	(– 49.9%)[¶]
				$r^{d} = -0.91$, $P < 0.001$	
HDL-C levels (mg/dL)	44.80 ± 1.39^{b}	23.60 ± 1.47^{a}	24.60 ± 1.50^{a}	46.20 ± 1.81^{b}	46.00 ± 1.87^{b}
	–	(– 47.3%)[#]	(+ 4.2%)[¶]	(+ 95.8%)[¶]	(+ 94.9%)[¶]
				$r^{d} = +0.81$, $P < 0.001$	
LDL-C levels (mg/dL)	30.40 ± 2.26^{a}	107.04 ± 2.53^{d}	104.06 ± 1.72^{d}	57.36 ± 2.18^{c}	48.32 ± 0.75^{b}
	–	(+ 252%)[#]	(– 2.3%)[¶]	(– 46.4%)[¶]	(– 54.9%)[¶]
				$r^{d} = -0.92$, $P < 0.001$	
VLDL-TG levels (mg/dL)	15.00 ± 0.06^{a}	35.08 ± 1.71^{c}	34.40 ± 1.21^{c}	20.06 ± 0.04^{b}	17.44 ± 0.41^{a}
	–	(+ 133%)[#]	(– 1.9%)[¶]	(– 42.8%)[¶]	(– 50.3%)[¶]
				$r^{d} = -0.91$, $P < 0.001$	

Data is displayed as mean ± standard error of mean. $P < 0.001$ represents significant correlation. In the same raw, values marked with similar superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are statistically different ($P < 0.05$)

r^{d}: correlation coefficient between the studied variables and the administered doses of chitosan (0, 400, and 800 mg), I: control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400 mg chitosan and V: rats fed HFD + supplements + 800 mg chitosan

[#]Percent of change in comparison to the group I

[¶]Percent of change in comparison to the group II

Table 5 The levels of total proteins (TP), albumin, globulin, and albumin/globulin ratio in the serum of all the experimental groups, at the end of the experiments

Variables	Experimental groups				
	I	II	III	IV	V
TP levels (g/dL)	6.30 ± 0.21^{d}	3.08 ± 0.12^{a}	3.04 ± 0.10^{a}	4.04 ± 0.07^{b}	4.48 ± 0.02^{c}
	–	(− 51.1%)[#]	(− 1.3%)[¶]	(+ 31.2%)[¶]	(+ 45.5%)[¶]
				$r^{d} = +0.95$, $P < 0.001$	
Albumin levels (g/dL)	4.18 ± 0.19^{d}	1.94 ± 0.11^{a}	1.96 ± 0.09^{a}	2.76 ± 0.14^{b}	3.26 ± 0.09^{c}
	–	(− 53.6%)[#]	(+ 1.0%)[¶]	(+ 42.3%)[¶]	(+ 68.0%)[¶]
				$r^{d} = +0.92$, $P < 0.001$	
Globulin levels (g/dL)	2.12 ± 0.04^{d}	1.14 ± 0.04^{a}	1.21 ± 0.07^{a}	1.28 ± 0.04^{b}	1.54 ± 0.02^{c}
	–	(− 46.2%)[#]	(+ 6.1%)[¶]	(+ 12.3%)[¶]	(+ 35.1%)[¶]
				$r^{d} = +0.78$, $P < 0.01$	
Albumin/globulin ratio (%)	1.97 ± 0.09^{ab}	1.70 ± 0.10^{a}	1.73 ± 0.09^{a}	2.16 ± 0.12^{b}	2.12 ± 0.07^{b}
	–	(− 13.7%)[#]	(+ 1.8%)[¶]	(+ 27.1%)[¶]	(+ 24.7%)[¶]
				$r^{d} = +0.59$, $P < 0.05$	

Data is displayed as mean ± standard error of mean. $P < 0.05$, $P < 0.01$, and $P < 0.001$ represent significant correlations. In the same raw, values marked with similar superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)
r^{d}: correlation coefficient between the studied variables and the administered doses of chitosan (0, 400, and 800 mg), I: control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400 mg chitosan and V: rats fed HFD + supplements + 800 mg chitosan
[#]Percent of change in comparison to the group I
[¶]Percent of change in comparison to the group II

Table 6 The activities of alanine and aspartate aminotransferases (ASAT and ALAT), alkaline phosphatase (ALP), and creatine kinase (CK) as well as levels of creatinine and urea in the serum of all the experimental groups, at the end of the experiments

Variables	Experimental groups				
	I	II	III	IV	V
ALAT activity (U/L)	27.60 ± 0.51^{a}	63.80 ± 1.43^{d}	61.40 ± 1.88^{d}	54.80 ± 1.39^{c}	44.00 ± 1.41^{b}
	–	(+ 131%)[#]	(− 3.8%)[¶]	(− 14.1%)[¶]	(− 31.0%)[¶]
				$r^{d} = -0.90$, $P < 0.001$	
ASAT activity (U/L)	84.00 ± 2.23^{a}	195.60 ± 4.37^{d}	189.80 ± 5.29^{d}	134.40 ± 4.84^{c}	120.20 ± 1.74^{b}
	–	(+ 133%)[#]	(− 3.0%)[¶]	(− 31.3%)[¶]	(− 38.5%)[¶]
				$r^{d} = -0.91$, $P < 0.001$	
ALP activity (U/L)	117.60 ± 2.16^{a}	286.60 ± 18.10^{d}	278.00 ± 12.41^{d}	179.60 ± 2.93^{c}	153.80 ± 2.91^{b}
	–	(+ 144%)[#]	(− 3.0%)[¶]	(− 37.3%)[¶]	(− 46.3%)[¶]
				$r^{d} = -0.91$, $P < 0.001$	
CK activity (U/L)	922.80 ± 11.97^{a}	1153.20 ± 13.28^{b}	1106.00 ± 32.19^{b}	935.20 ± 41.94^{a}	913.60 ± 40.63^{a}
	–	(+ 16.2%)[#]	(− 4.1%)[¶]	(− 18.9%)[¶]	(− 20.8%)[¶]
				$r^{d} = -0.68$, $P < 0.01$	
Creatinine level (mg/dL)	0.66 ± 0.04^{a}	0.96 ± 0.05^{b}	0.89 ± 0.03^{b}	0.66 ± 0.05^{a}	0.64 ± 0.02^{a}
	–	(+ 45.5%)[#]	(− 7.1%)[¶]	(− 31.3%)[¶]	(− 33.3%)[¶]
				$r^{d} = -0.75$, $P < 0.001$	
Urea level (mg/dL)	33.40 ± 2.16^{a}	58.40 ± 1.50^{b}	54.80 ± 1.39^{b}	37.20 ± 1.62^{a}	37.40 ± 0.87^{a}
	–	(+ 74.9%)[#]	(− 6.2%)[¶]	(− 36.3%)[¶]	(− 36.0%)[¶]
				$r^{d} = -0.81$, $P < 0.001$	

Data is displayed as mean ± standard error of mean. $P < 0.01$ and $P < 0.001$ represent significant correlations. In the same raw, values marked with similar superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)
r^{d}: correlation coefficient between the studied variables and the administered doses of chitosan (0, 400, and 800 mg), I: control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400 mg chitosan and V: rats fed HFD + supplements + 800 mg chitosan
[#]Percent of change in comparison to the group I
[¶]Percent of change in comparison to the group II

Table 7 The serum levels of calcium, vitamins A and E, and malondialdehyde (MDA) as well as erythrocyte content of glutathione (GSH) and activity of Superoxide dismutase (SOD) of all the experimental groups, at the end of the experiments

Variables	Experimental groups				
	I	II	III	IV	V
Calcium levels (mg/dL)	10.24 ± 0.16^{bc}	8.58 ± 0.15^{a}	12.38 ± 0.14^{d}	10.58 ± 0.19^{c}	9.92 ± 0.09^{b}
	–	(− 16.2%)[#]	(+ 44.3%)[¶]	(+ 23.3%)[¶]	(+ 15.6%)[¶]
				$r^{d} = -0.93$, $P < 0.001$	
Vitamin A levels (μg/dL)	35.93 ± 1.20^{b}	24.62 ± 0.91^{a}	65.22 ± 3.81^{e}	52.78 ± 1.75^{d}	39.08 ± 0.87^{c}
	–	(− 31.5%)[#]	(+ 165%)[¶]	(+ 114%)[¶]	(+ 58.7%)[¶]
				$r^{d} = -0.91$, $P < 0.001$	
Vitamin E levels (μg/dL)	18.03 ± 0.32^{b}	13.31 ± 0.71^{a}	62.78 ± 0.78^{e}	49.74 ± 0.55^{d}	46.20 ± 0.49^{c}
	–	(− 26.2%)[#]	(+ 372%)[¶]	(+ 274%)[¶]	(+ 247%)[¶]
				$r^{d} = -0.94$, $P < 0.001$	
MDA levels (nmol/mL)	0.36 ± 0.02^{a}	1.39 ± 0.18^{c}	1.34 ± 0.15^{c}	0.73 ± 0.02^{b}	0.38 ± 0.02^{a}
	–	(+ 286%)[#]	(− 3.6%)[¶]	(− 47.5%)[¶]	(− 72.7%)[¶]
				$r^{d} = -0.91$, $P < 0.001$	
GSH content (mg/dL)	27.37 ± 0.66^{b}	15.16 ± 1.04^{a}	15.40 ± 1.12^{a}	25.89 ± 1.71^{b}	27.28 ± 0.83^{b}
	–	(− 44.9%)[#]	(+ 1.6%)[¶]	(+ 70.8%)[¶]	(+ 79.9%)[¶]
				$r^{d} = +0.83$, $P < 0.001$	
SOD activity (U/L)	363.20 ± 5.70^{b}	264.43 ± 7.87^{a}	266.40 ± 8.18^{a}	346.63 ± 24.50^{b}	342.81 ± 9.38^{b}
	–	(− 27.2%)[#]	(+ 0.7%)[¶]	(+ 31.1%)[¶]	(+ 29.6%)[¶]
				$r^{d} = +0.64$, $P < 0.05$	

Data is displayed as mean ± standard error of mean. $P < 0.05$ and $P < 0.001$ represent significant correlations. In the same raw, values marked with similar superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are statistically different ($P < 0.05$)
r^{d}: correlation coefficient between the studied variables and the administered doses of chitosan, I: control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400 mg chitosan and V: rats fed HFD + supplements + 800 mg chitosan
[#]Percent of change in comparison to the group I
[¶]Percent of change in comparison to the group II

Table 7. In rats of groups II and III, significant elevations in MDA levels in serum were reported in association with marked depletions in GSH content and SOD activity, as compared to groups I, IV, and V. Rats of groups IV and V showed a marked decline in MDA levels in serum, with increasing chitosan content in HFD. In addition, the GSH levels and SOD activity of groups IV and V were returned to the normal levels of group I.

Histological investigations

In the present study, no obvious lesions were observed in the heart and renal tissues of all groups except for myocardial blood vessel congestion (Fig. 1) and epithelial lining necrosis of renal tubules (Fig. 2) of groups II and III. In Fig. 3, the hepatic tissues of groups II, III, and IV exhibited fat accumulation (steatosis) in hepatocytes. In the liver tissue of group V, no marked lesions were detected except for slight vacuolation.

Discussion

In the present study, HFD was applied for two reasons. The first one was to enhance the absorption of fat soluble vitamins A and E. Secondly, this was an attempt to induce obesity in rats. As expected, in the current work, rats of groups II and III showed a significant elevation in their BWG, as compared to group I. This can be linked to the reported increase in feed consumption rates by these groups. It is noteworthy that fatty diets are characterized by high palatability that enhances their uptake (Melhorn et al., 2010). In addition, HFD can increase size and number of adipose cells (Melhorn et al., 2010), leading to increased tissue weight, as confirmed by the recorded elevations in the relative weights of the liver and heart of groups II and III, in the present data. Such fluctuations in relative organ weights reflect an induction of adverse reactions (Sayed, Ali, & Mohamed, 2018). In the present study, histological examination of the hepatic tissue revealed abnormal fat deposition of groups II and III. Rats fed HFD without supplements showed significant reduction in the levels of serum calcium and vitamins A and E. The reduced calcium levels in serum may be linked to the documented obesity-associated vitamin D deficiency that subsequently can alter calcium absorption from the intestine

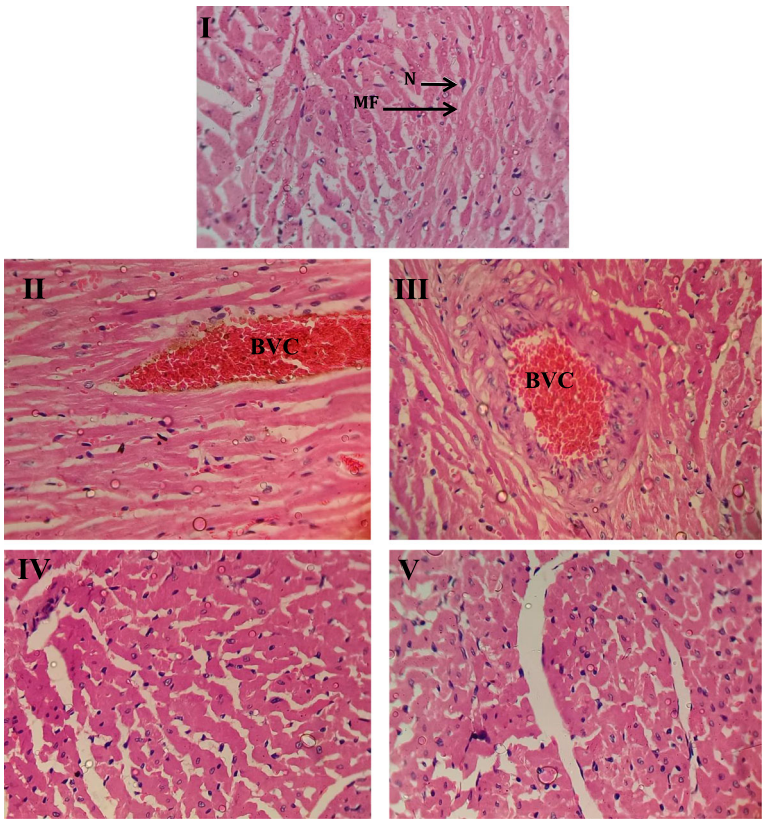

Fig. 1 Histological examination of the heart tissue of all experimental rats, at the end of experiments. I: Control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400mg chitosan and V: rats fed HFD + supplements + 800mg chitosan. BVC: blood vessel congestion, MF: myocardial fiber and N: nucleus. Haematoxylin & Eosin, X200

(Eastep & Chen, 2015 and Frommelt et al., 2014). However, the reported decrease in serum levels of vitamins A and E may be explained by continuous displacement of fat soluble vitamins from the blood to the adipose tissue in presence of high amount of dietary fats (Sohet et al., 2009). The observed depletion in serum levels of calcium can modulate calcium-sensing receptor causing suppression of certain enzymes required for lipolysis in adipose tissue (He et al., 2011), leading to an enhanced fat deposition. Abnormal fat accumulation can induce secretion of tumor necrosis factor-alpha (TNF-α) (Fonseca-Alaniz, Takada, Alonso-Vale, & Lima, 2007). TNF-α cytokine can boost lipid synthesis in the liver and reactive oxygen species (ROS) production leading to induction of lipid peroxidation (LPO) (Marseglia et al., 2015). This was ensured by the reported elevations in MDA serum levels and reduced levels of GSH and activity of SOD. These findings were accompanied by significant increases of ASAT, ALAT, and ALP activities of groups II and III reflecting that hepatic functions of those rats were harmfully affected. Similarly, Haggag, Elsanhoty, and Ramadan (2014) reported that high dietary fats and oils can expressively surge LPO and can increase tissue vulnerability to injury by ROS. The reported elevation in relative heart weight of groups II and III was associated with remarkable elevations in CK activity which reflects myocardial injury (Mythili & Malathi, 2015). This was manifested by the observed congestion of the blood vessels of the heart of these groups. In the present study, rats of group III showed remarkable elevations in the serum levels of calcium and vitamins A and E. It was documented that an excess of vitamin A can cause oxidative damage through protein thiol oxidation in tissues of rats (Cha, Yu, & Seo, 2016). This was confirmed by the increased levels of MDA in rats of group III. This could be due to the increased absorption of fat-soluble vitamins associated with high-fat diet intake. The reported decline in relative kidney weight of rats of groups II and III can be attributed to the observed necrosis in renal tubules. These findings can explain the increased levels of urea and creatinine indicating occurrence of renal dysfunction. Consequently, serum protein profile of rats of groups II and III were depleted due to leakage of albumin and amino acids from the damaged renal tubules.

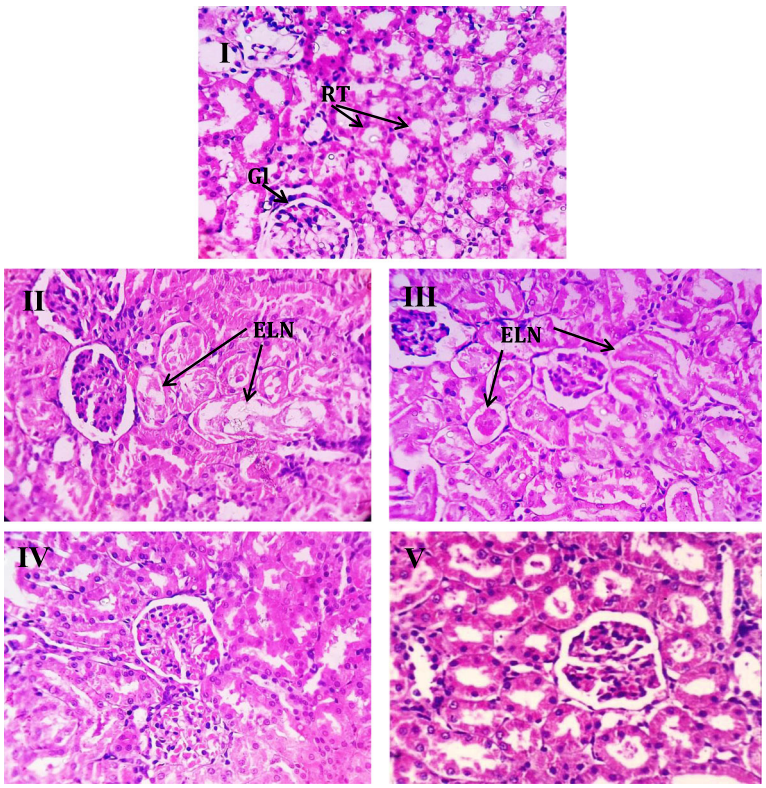

Fig. 2 Histological examination of the renal tissue of all experimental rats, at the end of experiments. I: Control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400mg chitosan and V: rats fed HFD + supplements + 800mg chitosan. ELN: Epithelial lining necrosis of renal tubules, Gl: glomerulus, and RT: renal tubules. Haematoxylin & Eosin, X200

On the other hand, rats fed HFD containing supplements in addition to chitosan showed significant reductions in the average BWG and feed intake, as compared to those of groups II and III, in a dose-dependent manner. This can be linked to the reported reduction in most lipid variables after application of chitosan to the HFD, in the present study. As by increasing the chitosan content of HFD, in presence of calcium and vitamin supplements, significant reductions were recorded in the levels of all lipid variables in serum, except for marked elevations in HDL-C. This can be attributed to the ability of chitosan to bind lipids in the intestine impeding their absorption (Anraku et al., 2014). Moreover, the increased levels of HDL-C can enhance lipid transport to the liver for further elimination with bile acids (Abdel--Ghaffar, Ali, & Soliman, 2018). Thus, chitosan in association with calcium and vitamins A and E can render the occurrence of hyperlipidemia associated obesity. In the present data, serum levels of TP, albumin, and globulin of groups IV and V were significantly elevated, as compared to groups II and III, towards the control. This can be linked to the recorded normal levels of creatinine and urea in serum of groups IV and V, reflecting the return normal renal functions and consequently, the stoppage of protein leakage from the renal tubules. In addition, chitosan can protect proteins from denaturation and oxidation (Kerch, 2015). Furthermore, this may be explained on basis of reduced oxidative stress, as manifested by the recorded reduction in the MDA levels as well as the enhanced levels of GSH and activity of SOD. This also can explain the observed marked inhibitions in the serum activities of ASAT, ALAT, ALP, and CK, with increasing the dose of chitosan in HFD. These findings could be ascribed to the antioxidant activities of chitosan (Wang et al., 2018) and vitamin E (Rizvi et al., 2014) that improved protection of liver and heart against the adverse effects of fat accumulation. This can be also attributed to the above mentioned ability of chitosan to eliminate fats. This was manifested by the reduced histopathological alterations in all the studied organs of rats fed HFD containing supplements and chitosan, as compared to the groups II and III, in the present study.

Conclusion

In rats fed HFD without supplements, significant alterations were recorded in all the studied biological, biochemical parameters, and histological examinations. By inclusion of supplements with HFD without chitosan,

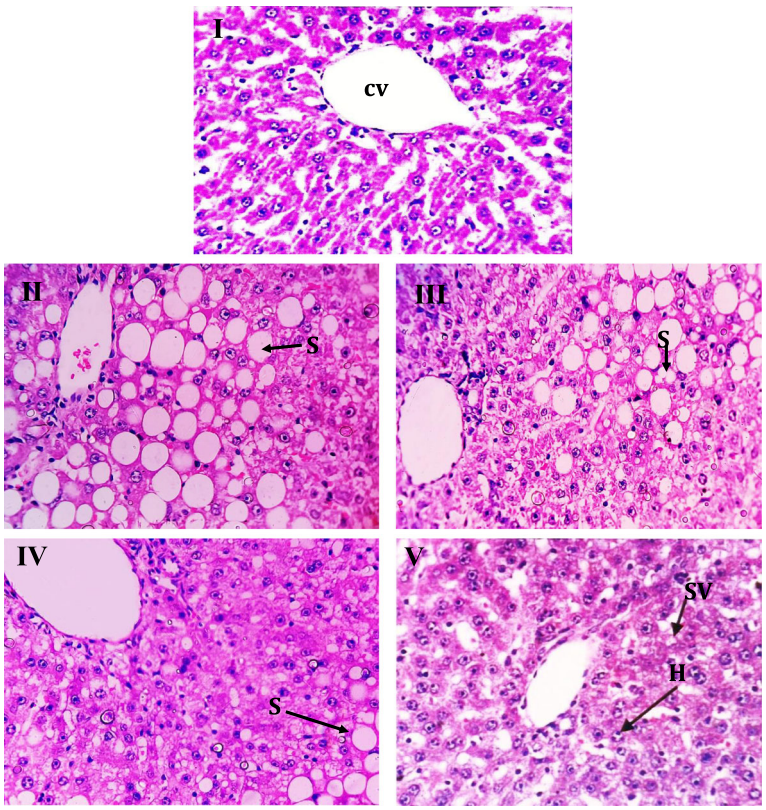

Fig. 3 Histological examination of the hepatic tissue of all experimental rats, at the end of experiments. I: Control rats, II: rats fed HFD without supplements, III: rats fed HFD + supplements, IV: rats fed HFD + supplements + 400mg chitosan and V: rats fed HFD + supplements + 800mg chitosan. CV: central vein, H: hepatocyte, S: steatosis (fat accumulation), and SV: slight vacuolation. Haematoxylin & Eosin, X 200

marked disturbances in most of the studied variables, but remarkable elevations in serum levels of calcium and vitamins A and E were observed. However, by increasing chitosan amount in HFD containing supplements, substantial alleviations of all the studied parameters were reported, in dose-dependent manner.

Abbreviations

ALAT: Alanine aminotransferase; ALP: Alkaline phosphatase; ASAT: Aspartate aminotransferase; BVC: Blood vessel congestion; BWG: Body weight gain; CV: Central vein; ELN: Epithelial lining necrosis; Gl: Glomerulus; GSH: Glutathione; H: Hepatocytes; HDL-C: High-density lipoprotein cholesterol; HFD: High-fat diet; LDL-C: Low-density lipoprotein cholesterol; LPO: Lipid peroxidation; MDA: Malondialdehyde; MF: Myocardial fiber; N: Nucleus; RT: Renal tubules; S: Steatosis; SBD: Standard basal diet; SOD: Superoxide dismutase; SV: Slight vacuolation; TC: Total cholesterol; TG: Triglycerides; TP: Total proteins; VLDL-TG: Very low-density lipoprotein triglycerides

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Availability of data and materials

All data are available upon request.

Authors' contributions

AAA shared in study designing and carried out the statistical analysis of data and manuscript writing. AM and EAH are responsible for study designing and manuscript editing. AHA-R shared in study designing and carried out the biochemical analysis. HAHM is responsible for animal handling and experimental procedures. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocol of the present study was approved by the ethical committee of the Nutrition National Institute at December 2015. The animal handling was done according to the standard international guidelines.

Consent for publication

The data provided here is original.

Competing interests

The authors declare that they have no competing interests.

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