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Chitosan, *Gymnema Sylvestre* and Ascorbic Acid as Complementary Medicine against Hypercholesterolemia in Rats.

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ABSTRACT

The present study was performed to evaluate the curative effects of chitosan and the combination of chitosan, *Gymnema sylvestre* and ascorbic acid (CGA) supplementation on oxidative stress and hypercholesterolemia induced in hypercholesterolemic rats. The plasma lipid levels, transaminases, lactate dehydrogenase activities, glucose, malondialdehyde and whole blood reduced glutathione, the activities of superoxide dismutase, glutathione peroxidase in erythrocytes and plasma glutathione reductase, glutathione-S-transferase and catalase were examined in hypercholesterolemic rats supplemented or not supplemented with chitosan or CGA. The results indicated that hypercholesterolemic rats fed basal or hypercholesterolemic diets revealed significantly higher mean plasma levels of total lipids, total cholesterol, triglycerides, low density and high density lipoprotein cholesterol, Atherogenic index, transaminases (ALT and AST), lactate dehydrogenase, malondialdehyde and glucose; in addition, significantly lower mean activities of enzymatic antioxidants and blood reduced glutathione were noted, compared to normal control. However, in hypercholesterolemic rats receiving the CGA supplement diet for two months; all these parameters were significantly better than those in hypercholesterolemic rats receiving basal or hypercholesterolemic diet. On the other hand, non significant improvement was observed by chitosan supplement diet. These results referred to possibility using CGA as curative natural supplement as hypocholesterolemic and antioxidative agents in hypercholesterolemic rats.

Keywords: Chitosan, *Gymnema sylvestre*, Ascorbic acid, Complementary medicine, Hypercholesterolemia, Oxidative stress.

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INTRODUCTION

Hyperlipidemia is a disorder of lipoprotein metabolism manifested as hypercholesterolemia, hypertriglyceridemia. Hyperlipidemia is a risk factor for atherosclerosis and pancreatitis, whereas atherosclerosis is a risk factor for coronary artery disease (CAD), myocardial infarction (MI) and hypertension [1]. Oxidative stress induced by reactive oxygen species (ROS) plays an important role in several diseases, including atherosclerosis and coronary heart disease. Hypercholesterolemia is reported to be associated with the oxidative stress that results from the increased production of ROS or impairment of the antioxidant system [2].

The growing mistrust of the general public on the pharmaceutical industry contributes to the decision of patients not to take prescription lipid-lowering drugs. As a result, patients seek alternative drugs or opt to rely on natural therapy in order to control their hypercholesterolemia [3].

Chitosan is a natural cationic polysaccharide consisting of [1-4]-2-amino-2-deoxy-D-glucopyranosyl units. It breaks down slowing to harmless products (amino sugars), which are completely absorbed by the human body [4]. The strong positive charge carried by chitosan makes it easy to bind negatively charged substrates, such as lipids and bile acids. Chitosan also interferes with the emulsification of neutral lipids by binding them with hydrophobic bonds [5]. Several studies have shown that chitosan has cholesterol-lowering properties both in animals and humans [6]. Other biological properties of chitosan, including biocompatibility, biodegradability, low toxicity, antitumoral and antiviral activity, make it suitable for use in biomedical and pharmaceutical formulations [7].

Gymnema sylvestre (Asclepiadaceae) a medicinal plant commonly known as Gurmar. In rat studied, the leaves of *G. sylvestre* improved serum cholesterol and triglyceride levels through influence of lipid metabolism [8]. *G. sylvestre* extract had hypolipidemic and antiatherosclerotic effect in albino rats fed on high fat diet [9]. Rachh et al. showed significant reduction in the levels of all lipids with increase in HDL-C in hypercholesterolemic rats group treated with *Gymnema* leaves as compared to high cholesterol diet control. The hypocholesterolemic activity of *G.sylvestre* may be due to the presence of flavonoids, tannins, saponins, acidic compounds found in photochemical screening [10]. Other biological properties of Gurmar, including antiviral, antimicrobial, anti-inflammatory, hypoglycemic, antioxidant, antiobesity agent [11].

Vitamin C or ascorbic acid is a well known, powerful and water-soluble antioxidant. It is endogenously present in the cellular cytosol. Vitamin C antioxidant activity is through donation of electron to the harmful free radicals generated during the oxidative stress process. It prevents oxidative injury in several organs via quenching the injurious free radicals and ROS produced in biological processes [12]. Ascorbic acid is well recognized to prevent various diseases including diabetes and heart disease [13, 14].

There are many studies on the protective effects of chitosan, *G. sylvestre* and ascorbic acid; however, no information on the curative effects of the combination of chitosan, *G. sylvestre* and ascorbic acid against hypercholesterolemia and oxidative stress has been performed until now. Therefore, the present study was designed to evaluate the curative effects of chitosan and the mixture of chitosan, *G. sylvestre* and ascorbic acid supplementation as complementary medicine on oxidative stress and hypercholesterolemia induced in rats by feeding hypercholesterolemic diet.

MATERIALS AND METHODS

Materials

High molecular weight (MW) chitosan and the mixture of high MW chitosan, *Gymnema sylvestre* and ascorbic acid (10:1:2) donated from Aldebeiky Pharma Co., Egypt.

Experimental animals

Thirty male Sprague-Dawley rats weighing 120 ±10 g were purchased from animal house of Helwan Station for Experimental Animals, Helwan, Egypt. They were raised in the animal house of Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt. The animals were housed in polyethylene

cages in groups of six rats per cage in a controlled environment (25±2 ºC, 50-60% relative humidity and 12-hour light-dark cycle) for two weeks for adaptation. During this period, the rats were fed on basal diet consisting corn starch 65%, casein 10%, corn oil 10%, salt mixture 4%, vitamins mixture 1% and cellulose 10%. The animals were fed *ad libitum* with a basal diet and water.

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Cairo.

Experimental design

To study the curative effect of chitosan and the mixture of chitosan, ascorbic acid and *G. sylvestre*, thirty rats were divided randomly in to equal five groups as follows: the first group, normal control group (NC), fed normal diet. Groups 2, 3, 4 and 5 fed hypercholesterolemia-induced diet which prepared as basal diet preparation, except that the 10% corn oil portion was replaced with 10% sheep perineal fat and it was supplemented with 1% cholesterol and 0.25% cholic acid for four weeks. Thereafter, group 2, high-cholesterol control group (HC) received hypercholesterolemia induced diet; group 3 (BD) received normal diet; group 4 (chitosan group) received normal diet supplemented with 3.6 g chitosan/kg diet. Group 5 (chitosan, *G. sylvestre* and ascorbic acid, CGA) received normal diet supplemented with 4.68 g mixture/kg diet.

During the experimental period (2 months), water and diets were available *ad libitum*. At the end of the experiment, all the animals were scarified by decapitation. Blood samples were collected in three heparinized tubes. The first one (0.1 ml blood) was used for the determination of reduced glutathione (GSH), the 2nd heparinized tube (0.5 ml blood) was used to extract the erythrocytes lysate to study antioxidants enzymes. The 3rd heparinized tube was centrifuged at 2500 rpm at 37 ºC for 15 min to separate the plasma.

Biochemical analysis

Lipid analysis

Plasma total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined according to Knight et al. [15], Fossati and Prencipe [16], Allain et al. [17], Levy [18] and Burstein [19], respectively. Atherogenic Index (AI) was calculated according to Lee and Niemann [20] using following equation:

$$\text{Atherogenic Index (AI)} = \frac{\text{Total cholesterd - HDL - C}}{\text{HDL - C}}$$

Determination of LDH, AST and ALT activities

Lactate dehydrogenase (LDH) activity in plasma was determined according to the method of Young [21]. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured colourimetricaly according to the method described by Reitman and Frankel [22].

Determination of glucose

Plasma glucose level was determined according to Trinder [23].

Determination of lipid peroxidation

Plasma lipid peroxidation was estimated by measuring the malondialdehyde (MDA) content [24].

Determination of non-enzymic antioxidant (GSH)

Reduced glutathione (GSH) in whole blood was determined by the method of Beutler et al. [25]. This method was based on the reaction of GSH with 5,5`dithiobis(2-nitrobenzoic acid) to give a yellow compound that absorbs at 412 nm.

Determination of enzymic antioxidant activities

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) in erythrocytes were assayed by the methods of Nishikim et al. [26] and Paglia and Valentine [27], respectively. Plasma glutathione reductase (GR), glutathione-S-transferase (GST) and catalase (CAT) activities were assayed by the methods of Goldberg and Spooner [28], Habig et al. [29] and Aebi [30], respectively.

Statistical analysis

Data were subjected to an analysis of variance, and the means were compared using the Least Significant Difference (LSD) test at the 0.05 levels, as recommended by Snedecor and Cochran [31].

RESULTS

The role of chitosan and mixture of chitosan, *G. sylvestre* and ascorbic acid to cure the hypercholesterolemia and oxidative stress induced in rats have been investigated in this study.

Hypercholesterolemic rats continued fed on hypercholesterolemia-induced diet (HC) and hypercholesterolemic rats received basal diet (BD) groups mark by significant ($P < 0.05$) increase in plasma total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and atherogenic index (AI) compared with normal control rats (NC). Supplementation with chitosan and mixture of chitosan, *G. sylvestre* and ascorbic acid (CGA) showed significant ($P < 0.05$) falls in total lipids, triglycerides, total cholesterol, LDL-C and AI compared with hypercholesterolemic group (HC) and hypercholesterolemic rats received basal diet group (BD) as shown in Table 1. On the other hand, no significant change in HDL-C level was observed. The best reduction in lipids profile was recorded by CGA group, the levels of total lipids, total cholesterol, triglycerides, LDL-C and AI were decreased by 2, 3.4, 1.3, 3.5, and 3.53-fold, respectively compared with HC group. Moreover, these levels were decreased by 0.75, 0.98, 0.82, 1.55 and 1.19-fold, respectively when compared with BD group. However, no significant change in lipids profile and AI was observed between NC, chitosan and CGA groups except HDL-C which significantly increased in CGA group compared with NC group. It must be mentioned that lipids profile and AI did not reached to the normal value (normal rats). Results showed that curative effect of CGA diet was more effective against hypercholesterolemia than chitosan only.

Table 1: Lipids profile of hypercholesterolemic rats fed chitosan and CGA supplemented diets

Groups	TL (mg/dl)	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	AI
NC	306.19±39.81^{c}	98.75±5.92^{c}	72.12±7.04^{d}	40.62±3.02^{b}	39.20±3.03^{c}	1.43
HC	1079.77±136.4^{a}	430.27±14.36^{a}	244.73±19.07^{a}	49.57 ± 2.94 ^{a}	268.83±15.04^{a}	7.68
BD	622.64±34.82^{b}	251.27±28.37^{b}	193.72± 9.80 ^{b}	53.31±2.51^{a}	152.54±10.47^{b}	3.71
Chitosan	414.53±28.83^{c}	137.73±15.86^{c}	121.38±13.22^{c}	46.81±1.92^{ab}	57.78±4.21^{c}	1.71
CGA	354.70±43.45^{c}	126.82±3.88^{c}	106.15±10.95^{cd}	51.15±3.04^{a}	59.79±6.98^{c}	1.69
LSD	195.22	45.84	39.01	8.69	25.18	

Values are expressed as means ± SE (n = 6).
Values with different superscript letters within the same column are significantly different ($P < 0.05$).

There were significant increases ($P < 0.05$) in the plasma ALT, AST and LDH activities of all hypercholesterolemic rat groups as compared to normal control rats (NC) as shown in Table 2. The activity of plasma ALT, AST and LDH significantly decreased ($P < 0.05$) in chitosan and CGA groups compared with hypercholesterolemic control (HC). On the other hand, CGA supplemented diet significantly decreased ($P < 0.05$) the activities of these enzymes by 31.3, 36.84 and 25.44%, respectively compared with BD group. Nevertheless, no significant different was observed between chitosan and BD groups. In the present study, it was observed that as a result of hypercholesterolemia, enzymes such as AST, ALT and LDH were released into blood. Their increase in the plasma activities of these enzymes was directly proportional to the degree of cellular damage. These values decreased by CGA and chitosan supplements.

Hypercholesterolemic rats fed hypercholesterolemic diet (HC) or normal diet (BD) showed significant ($P<0.05$) decrease and increase in blood reduced glutathione (GSH) content and plasma malondialdehyde

(MDA) level, respectively compared to normal control rats (Table 3). GSH significantly depleted (P<0.05) by 36.57% and 21.78% and MDA significantly increased (P<0.05) by 181.45% and 39.7% in HC and BD groups, respectively compared with NC group. The best results of GSH and MDA were recorded by hypercholesterolemic rats received CGA diet. GSH significantly increased (P<0.05) by 48.16% and 21.06% while, MDA significantly decreased (P<0.05) by 66.84% and 33.20% in CGA group compared with HC and BD groups, respectively. It must be noticed that all values of GSH and MDA in hypercholesterolemic rats fed on chitosan and GCA diets closed with that in normal rats.

Table 2: ALT, AST and LDH activities of hypercholesterolemic rats fed chitosan and CGA supplemented diets

Groups	ALT (U/L)	AST (U/L)	LDH (U/L)
NC	20.01 ± 1.04 ^{d}	30.25 ± 1.90 ^{d}	528.62 ± 18.23 ^{d}
HC	46.24 ± 2.26 ^{a}	94.78 ± 5.18 ^{a}	1445.49 ± 31.00 ^{a}
BD	40.73 ± 2.61 ^{b}	88.69 ± 3.28 ^{ab}	1315.64 ± 55.76 ^{b}
Chitosan	36.41 ± 0.76 ^{b}	78.9 ± 3.96 ^{b}	1204.70 ± 49.33 ^{b}
CGA	27.97 ± 0.77 ^{c}	56.02 ± 2.49 ^{c}	980.92 ± 40.63 ^{c}
LSD	5.08	10.71	120.31

Values are expressed as means ± SE (n = 6).
Values with different superscript letters within the same column are significantly different (*P* < 0.05).

Table 3: Effect of chitosan and CGA diets on GSH, MDA and glucose of hypercholesterolemic rats

Groups	GSH (mg/dl)	MDA (nmol/L)	Glucose (mg/dl)
NC	42.88 ± 3.23 ^{a}	26.52 ± 1.13 ^{c}	73.60 ± 2.23 ^{b}
HC	27.20 ± 2.37 ^{c}	74.64 ± 1.64 ^{a}	103.27 ± 3.67 ^{a}
BD	33.54 ± 2.65 ^{bc}	37.05 ± 4.48 ^{b}	81.73 ± 5.48 ^{b}
Chitosan	35.29 ± 2.40 ^{abc}	21.96 ± 3.63 ^{c}	84.85 ± 4.15 ^{b}
CGA	40.30 ± 1.24 ^{ab}	24.75 ± 2.55 ^{c}	76.20 ± 2.31 ^{b}
LSD	8.02	8.54	11.98

Values are expressed as means ± SE (n = 6).
Values with different superscript letters within the same column are significantly different (*P* < 0.05).

The decrease in the reduced glutathione and the increase in malondialdehyde levels of hypercholesterolemic group indicate that hypercholesterolemia damaged the integrity of the erythrocyte membrane. On the contrary, the observed increase in the amount of GSH and the decrease in MDA in the CGA group indicate that chitosan, *G. sylvestre* and ascorbic acid mixture effectively restore membrane integrity. On the other hand, Plasma glucose level of hypercholesterolemic rats (HC) was significantly (P<0.05) increased compared to normal control (NC). However, no significant changes were observed in other groups. Glucose level in CGA group reached to the normal state as similar as in normal rats (NC).

Table 4: Effect of chitosan and CGA diets on antioxidant enzyme Activities of hypercholesterolemic rats

Groups	SOD (U/ml)	GPx (U/L)	CAT (U/L)	GR (U/L)	GST (U/L)
NC	191.80± 5.03 ^{a}	337.42 ± 14.54^{a}	437.42 ± 19.58 ^{a}	40.19 ± 2.89^{a}	41.82 ± 2.08^{a}
HC	142.08± 3.88 ^{c}	301.45± 4.43^{b}	356.14 ± 13.17 ^{b}	29.14 ± 0.81^{c}	31.68 ± 2.73^{b}
BD	161.40± 5.18 ^{b}	312.00± 5.7 ^{b}	387.71 ±17.36^{ab}	30.14 ± 0.90^{bc}	31.06 ± 0.90^{b}
Chitosan	165.01± 5.00 ^{b}	322.75 ± 11.8 ^{ab}	415.73 ± 17.70 ^{a}	35.37 ± 1.73^{ab}	34.09 ± 0.92^{b}
CGA	170.55± 5.46 ^{b}	340.45 ± 15.22^{a}	402.09 ±13.56^{ab}	34.96 ± 1.62^{ab}	40.80 ± 1.52^{a}
LSD	17.91	23.94	50.77	5.01	6.16

Values are expressed as means ± SE (n = 6). Values with different superscript letters within the same column are significantly different (*P* < 0.05).

Table 4 shows the activities of antioxidant enzymes in erythrocytes and plasma. The erythrocytes SOD, GPx and plasma GR and GST were significantly (P<0.05) inhibited in HC and in BD groups compared with NC group. Also, significant inhibition in the activity of plasma CAT was observed in the HC group compared to the normal one. In general, Slight increase in the activity of antioxidant enzymes of chitosan and CGA groups when compared with BD group. The effect of CGA in this context was even better since the levels of antioxidant enzymes but they were still less than that in normal control group. CGA supplemented diet

significantly stimulated GPx and GST activities by 9.12% and 28.79%, respectively. As a result of that, the treatment with CGA supplemented diet improved the enzymic and non-enzymic (GSH) antioxidants.

DISCUSSION

The hypercholesterolemic-induced diet is regarded as an important factor in the development of cardiac diseases since it leads to the development of hyperlipidemia, atherosclerosis and abnormal lipid metabolism. The present finding are in agreement with those obtained by Osman et al. who reported that hypercholesterolemia state significantly increase blood lipids and release of some enzymes to plasma [32]. In the present study, the effects of chitosan and CGA on the alterations of lipids profile and oxidative stress in hypercholesterolemic rats were investigated. The results of Tao et al. studies confirmed the results of this study [5]. These results suggest that chitosan had anti-atherogenic, hypolipidemic and reduced oxidative stress via inhibition of reactive oxygen species and lipid peroxidation as well as increment of antioxidant enzymes. Hypercholesterolemic diet resulted in a significant increase in lipid parameters which has been shown to be a strong risk factor for coronary heart diseases in many populations [33]. Results of this study indicated that both chitosan and CGA supplements had a strong hypocholesterolemic effects in plasma of hypercholesterolemic rats. In addition, the atherogenic index markedly decreased in both groups fed diet supplemented with chitosan. Furthermore, the reduction of lipid levels in plasma may be because of the increased fecal lipid excretion. This result might suggest that chitosan can effectively curative the hypercholesterolemia, and the longer the time of treatment the better the hypocholesterolemic effect. Moreover, Zhang et al. reported that chitosan was efficacious in facilitating weight loss and reducing body fat in obese adults human [6]. Moon et al. suggest that chitosan account for the hypocholesterolemic effects by enhancement of cholesterol 7α-hydroxylase activity [34].

Oxidative stress induced by reactive oxygen species (ROS) plays an important role in the etiology of several diseases, including atherosclerosis and coronary heart disease [2]. ROS may react with variety of biomolecules including lipid, proteins and nucleic acids, leading to their oxidation. These may be increase oxidative stress by promoting the cellular consumption of glutathione and by inactivating selenium dependent glutathione peroxidase. Glutathione (GSH) serves as a substrate for the enzyme GPx, and it has been suggested that, through its activity, GSH protects plasma against oxidative damage [35]. However, various molecules including chitosan derivatives are able to scavenge the radicals at different levels [35]. The result of the present study related to results of Senevirathne et al. who reported that chitosan significantly increased catalase, glutathione peroxidase, and superoxide dismutase activities. Moreover, chitosan reduced the oxidative stress via inhibition of ROS and lipid peroxidation as well as increment of antioxidant enzymes [36].

On the other hand, supplementation with ascorbic acid produced a significant reduction of serum TG, TC, LDL-C levels, this may be due to ascorbic acid enhanced the conversion of cholesterol to bile acids simultaneous with increased fecal and liver bile acids through activation of cholesterol 7α-hydroxylase, the limiting step in cholesterol transformation to bile acids [37]. These facts confirmed the results of this study. Tsujikawa et al. showed that supplementary ascorbic acid augmented the reduction in the apparent fat digestibility and plasma TG level, of chitosan. The augmenting effect by ascorbic acid may be explained by a decrease in the viscosity of chitosan and the accompanying progress of mixing chitosan with oil [38].

Hypercholesterolemic diet was reported in several studies to cause hepatotoxicity and fatty liver [12, 39]. Similarly in the current study, HC and BD groups significantly induced elevation in plasma ALT and AST as well as LDH. Supplementation of chitosan and CGA significantly reduced hepatotoxicity and liver injury. CGA diet containing vitamin-C showing more effect as it decreased the elevated levels of ALT, AST and LDH with a higher extent than chitosan only.

The results of MDA and GSH levels in hypercholesterolemic rats are related to the results of Kapoor et al. [1]. They indicated that, the decrease in free radicals decreasing lipid peroxidation and MDA level. Supplementation of ascorbic acid significantly increased blood GSH compared to hyperlipidemic group, ascorbic acid restored MDA and GSH levels as well as erythrocytes GPx activity, due their free radical scavenging, metal chelating and radical chain reaction-breaking properties [37].

Thakur et al. found that *Gymnema* significantly reduced TG, TC, and LDL [40]. The hypolipidemic activity of *Gymnema* may be due to the presence of saponins, flavonoids, phenols, tannins and triterpenoids. Flavonoids inhibit HMG-CoA reductase by a dual mechanism for lowering of the blood cholesterol level.

Moreover, saponins inhibit intestinal absorption of dietary fat by inhibiting pancreatic lipase activity. These results prove the hypolipidemic activity of *G. sylvestre* [41].

Kanetkar et al. reviewed possible mechanisms by *G. sylvestre* exert its hypoglycemic effects: 1) it increases secretion of insulin, 2) it promotes regeneration of islet cells, 3) it increases utilization of glucose: it is shown to increase the activities of enzymes responsible for utilization of glucose by insulin dependent pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and 4) it causes inhibition of glucose absorption from intestine [42]. These mechanisms confirmed the hypoglycemic effect of CGA supplement group. Kumar et al. reported that antioxidant enzymes (SOD, CAT, GPx, GR and GST) GSH levels were significantly increased by water extract of *G. sylvestre* treated group as compared to the high fat diet fed group [43]. Further, *G. sylvestre* offers cardiac protection by decreasing cardiac caspase-3 levels, Na^+/K^+ ATPase activity, DNA laddering, oxidative stress, and maintaining normal architecture of myocardium. Saneja et al. reviewed that *G. sylvestre* have inhibitory effects of DPPH radicals and LDL oxidation [11].

These studies confirmed the results of the present study. In fact combination of chitosan, *G. sylvestre* and ascorbic acid may improve the factors causing coronary heart diseases (CHD), cardiovascular diseases (CVD) and oxidative stress nearby normal values more than chitosan only, so this mixture may have curative effect against atherosclerosis.

It could be summarized that both chitosan and CGA supplements improved and recovered the lipids profile compared with BD group. However the curative effects of the CGA supplement was better than chitosan supplement. CGA supplement significantly decreased plasma transaminases and LDH activities but they were still more than that in NC group, while no change in the activity of these enzymes was observed by chitosan supplement compared with BD group. Also, it can be show that CGA supplement recovered MDA and GSH levels and the activity of GPx and GST as those in NC group. Moreover, CGA supplement improves antioxidant enzymes (SOD, CAT and GR) better than chitosan only as compared to BD group but they still less than that in normal control group.

Chitosan and CGA are natural, normal, healthy and appropriate to recover oxidative agents, enzymic and non-enzymic antioxidants and hypercholesterolemic factors. This stand in stark contrast to the use of lipid lowering agents i.e. statins that have adverse effects like liver and muscle toxicity. Other risk factors are hepatic dysfunction, renal insufficiency, hypothyroidism, advanced age and serious infection [44].

CONCLUSIONS

The present results clearly refer to possibility using CGA as curative natural supplement as hypocholesterolemic and antioxidative agents, but may need further studies using higher concentrations of the CGA mixture and/or increasing experimental period to restore and normalize all biochemical parameters.

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