Celery (*Apium Graveolens*) Ethanolic Extract Ameliorates Experimental Atherosclerosis in Rats

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ABSTRACT

Atherosclerosis could be a common sickness which is seriously harmful to human health. Natural merchandise is a vital provenance of curative candidates for atherosclerosis. In this study, it was tended to evaluate the consequences of Celery (Apium graveolens) ethanolic extract (CE) on experimental atherosclerosis in rats, and explore the underlying mechanisms. Atherosclerosis was induced by high cholesterol diet (HCD) for twelve weeks. The atherosclerotic rats after that were treated with celery ethanolic extract at 200 mg/kg/d or simvastatin at 5mg/kg/d for twelve weeks. The current results clarified that CE or simvastatin decreased the serum scales of cholesterol, triglyceride and low-density lipoprotein accompanied with an improvement in serum level of high-density lipoprotein comparing to the control atherosclerotic rats. Additionally, the treatment of atherosclerotic rats with either CE or simvastatin resulted in a considerable depression in serum activities of C-reactive protein (CRP), Creatine Kinase (CK) and Lactate dehydrogenase (LDH) versus the control atherosclerotic rats. As well, significant suppression of serum Troponin-1 and Endothelin-1 levels was observed as an outcome of remediation with CE or simvastatin. Moreover, the curing of atherosclerotic rats with either CE or simvastatin resulted in significant inhibition in Cardiac transforming growth factor-ß (TGF-ß) and vascular endothelial growth factor (VEGF) versus the control atherosclerotic rats. Otherwise, noticeable depletion in serum ALT and AST activities was listed as a result of the remediation with either CE or simvastatin. The existent study has given a novel side to the perception of the role of ethanolic extract of celery against atherosclerosis. Consequently, the present results reinforced the advice recommending the consumption of celery to modulate atherosclerosis.

Key Words: *Atherosclerosis, Celery, Simvastatin, Rats, Cholesterol, Troponin-1, Endothelin-1, TGF-ß, VEGF.*

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INTRODUCTION

It has been realized that hypercholesterolemia is a risk agent for cardiovascular diseases (CVD), for example, atherosclerosis and myocardial infarction, which have been a widespread reason of mortality and morbidity [1].

Atherosclerosis (AS) has been supposed to be a critical risk agent for cardiovascular diseases (CVD) and has been considered as a principle wellspring of death around the globe [2].

AS is a medical state of the cholesterol, it demonstrates the solidifying and narrowing the arteries as the result of assembling and manufacturing of the fatty materials [3]. It for the most part happens because of the cardiovascular diseases, acute coronary diseases, strokes and heart attacks. While, the instrument of activity and occurrence of the AS still have remained not legitimately clarified, a couple of analysts asserted that the few risk factors, for example, hypotension, diabetes and dyslipidemia participated in the initiation of AS. Among the diverse risk reasons, dyslipidemia have had an imperative part in the pathogenesis of the AS, during the dyslipidemia, the patients regularly affirmed the high concentration of low-density protein, which can be oxidized through vessels' cells and changed into the oxidized LDL. Oxidized LDL level instigated the pathological changes as inflammation, oxidative stress, and endothelium damage [4].

For the dealing of the AS, statins have been commonly utilized because of their magnificent efficacy in limiting the LDL value in the serum, and repressing the vascular risk, but the continuous utilization of statins prompted more side effects on rhabdomyolysis, myopathy, liver

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injury, muscle toxicity and intense renal failure [5, 6].

Medicinal plant inferred metabolites have been known for various biological actions [7, 8]. Therefore, the plant flavonoids having antiartherosclerotic impact picked up the greater prevalence, and furthermore demonstrated to reduce the risk of the CVD including AS in a considerable number of clinical and fundamental status [9, 10].

Among the therapeutic plants in traditional medication can be pointed to the celery with different therapeutic advantages. Celery (*Apium graveolens*) is a two-year herbal with aromatic and divided stems fitting to the family of Apiaceae [11]. This plant is regional to the Mediterranean zone, and is developed in different parts of the universe [12]. Leaves and stems of celery comprise phenols, furanocoumarin, psoralen, bergapten, and xanthotoxin that their quantity is changing from 12 to 50mg /kg [13].

Water of leaves and roots of celery is efficient on biochemical parameters, for example, glutathione, catalase activity, glutathione peroxidase, xanthine oxidase, and peroxidase and lipid peroxidation in liver homogenate and blood hemolyzed [14].

Celery guaranties the anticoagulant action of blood plasma as well as the stoppage of the cardiovascular diseases [15]. Celery root prompts the increment in calcium besides the reduction in potassium within the heart tissues[16].

Sodium and potassium in celery aid the body's fluid to be managed, and they promote urine expulsion, which is a vital evidence for the withdrawal of the excess fluid of the body [17]. The experimental investigations have stated the antifungal possessions [18] and anti-inflammatory effects of the celery plant [19].

In the contemporary years, a lot of investigations have been centered around the deterrence and curing of obesity by the biological possessions of phenolic compounds. Phenolic compounds and flavonoids have pharmacological highlights as anti-oxidants, anti-mutagenic, anti-thrombosis, anti-inflammatory, anti-cancer, and hyperlipidemia. These compounds typically have a wide distribution in plants and make up a part of the human food [20]. Flavonoids cannot be formed in the human body and they are consumed by the body through the daily diet. An investigation proposed that flavonoids demonstrated biological indispensable roles, including clearing the active oxygen species [21]. Celery is rich in antioxidant compositions as flavonoids (such apiin and apigenin), vitamins E besides C [22].

In neoteric years, because of the amplified mortality due to the heart diseases, as well as, the vascular difficulties of the synthetic drugs, the investigation of the medicinal plants and their effects on lowering blood lipids has been considered essential. Therefore, the objective of this study was to inspect the effects and underlying mechanisms of Celery ethanolic extract on the experimental atherosclerotic rats, which would provide a basis for using Celery as medicine in the prohibition and treatment of atherosclerosis.

MATERIALS AND METHODS

Chemicals and drugs

Cholesterol was obtained from Sigma Chemical Co., USA. Simvastatin was obtained from Commercial Market, Cairo, Egypt. It was manufactured by MSD B.V Co., UAE. All the other reagents, solvents, and chemicals used for analysis met the quality criteria in agreement with the International Standards.

Plant preparation and extraction procedures

Air-dried aerial parts of Celery plant (*Apium graveolens*) were obtained from a regional market in Cairo, Egypt, and the plant was recognized by a botanist of the herbarium at the Botany Department, Faculty of Science, Cairo University, Giza, Egypt.

Dried aerial parts of Celery plant (2 kg) were pulverized into fine powder using a stainless-steel blender, and passed across a mesh opening of 35 mm sieve.

Then, the powder was extracted by cold percolation with 95% ethanol (3 × 4 L) till the exhaustion. Afterwards, the ethanol extract was concentrated under the reduced pressure to give 250 g of brown residue. The residue was preserved in a refrigerator till used in the biological assay.

Biological assay

Animals and treatments

Animal procedures were performed in accordance with the declaration of Helsinki and the guidelines for the care and use of the experimental animals established by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH) protocol.

A total of 50 adult female albino rats of Wistar strain weighing 130±10g at 90 days of age were registered in the current study. The animals were gained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were housed throughout the experiment (10 rats/cage) in polypropylene cages under specific pathogen-free conditions with controlled illumination (twelve hrs light/twelve hrs dark cycle), relative humidity (30-50%), and temperature (18-22°C). Animals were nourished with standard laboratory rat diet and water provided *ad libitum*. Animals were permissible to acclimatize to their environment for 2 weeks before the beginning of the experiment.

After the acclimatization period, 10 rats were nourished with standard laboratory rat diet containing 26.5% protein, 3.8% fat, 40% carbohydrate, and 4.5% crude fiber in 100 g of chow during 24 weeks of the investigational period, and attended as normal control group (Con gr). Another 10 rats were nourished as normal control group for twelve weeks,

then nourished with typical laboratory rat diet with simultaneous administration of Celery ethanolic extract by the intragastric gavage tube in a dosage of 200 mg/kg b.wt. according to Kooti et al. [23] for 12 weeks and attended as Celery extract group (CE gr). The other 30 rats were nourished with high-cholesterol diet (HCD) including 19.93% protein, 15% cholesterol, 57.50% carbohydrate, and 2.81% dietary fiber in 100 g of chow (modified method of Soliman et al. [24]) for twelve weeks. In distilled water, the dietary ingredients were homogenized and dehydrated in an incubator at 60°C for 24 hrs, and cut into small equal-sized pieces (pellets). HCD was given fresh each day as dry pellets; therefore, there was no spillage [25]. These rats were further assigned into three groups: Atherosclerotic group (AS gr) in which the rats were nourished with HCD for twelve weeks, then nourished with standard laboratory rat diet for other 12 weeks; Celery extract-treated group (AS+CE gr) in which the rats were nourished with HCD for 12 weeks, then nourished with standard laboratory rat diet with simultaneous administration of Celery ethanolic extract by intragastric gavage tube in a dosage of 200 mg/kg b.wt. according to Kooti et al. [23] for 12 weeks; and simvastatin-treated group (AS+Sim gr) in which the rats were nourished with HCD for twelve weeks, then nourished with typical laboratory rat diet with simultaneous administration of antihypercholesterolemic drug (simvastatin) by intragastric gavage tube in a daily dosage of 5 mg/kg b.wt. according to Mbikay, [26] for twelve weeks.

Sample collection

Orbital blood samples were got from the retro-orbital venous plexus using microcapillaries. The blood samples were collected in dry, clean centrifuge tubes, permissible to clot to get serum. Serum samples were detached by the centrifugation at 1800 ×g for 10 minutes at 4°C. Aliquots of serum samples were frozen and kept at -20°C pending for further analysis. Following the blood collections, the animals were sacrificed by cervical dislocation, and a midline abdominal incision was performed, and the whole heart of each animal was rapidly dissected out, carefully washed with ice-cold isotonic saline, blotted dry and thereafter weighed. After that, each heart was directly homogenized to give 10% (w/v) homogenate in ice-cold medium having phosphate buffer (pH: 7.4). The homogenate was centrifuged at 1800 ×g for 10 minutes at 4°C. The supernatant (10%) was separated and stored at -20°C pending for further analysis.

Biochemical determinations

Serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) levels were assessed by colorimetric method utilizing Reactivos GPL kits (Barcelona, Espana) according to Meiattini [27], Buccolo et al. [28] and Naito [29] methods respectively. Serum low density lipoprotein (LDL) level was assessed by a colorimetric technique using Centronic (Gmbh) kit (Wartenberg, Germany) according to Wieland and Seidel [30]. Serum Lactate dehydrogenase (LDH) was assessed by a colorimetric technique using kits obtained from Bio Systems Co. (Egypt) according to the methods of Young [31]. Serum C-reactive protein (CRP) was determined by CRP-HS II LT (Latex Turbidimetric Immunoassay) kit purchased from Wako Chemicals GmbH, according to Whicher [32]. Serum Creatine Kinase (CK) was determined by a kit bought from Diagnosticum Zrt., according to Mathieu et al. [33]. Serum Tropinin-1 and Endothelin-1 were assessed by enzyme linked immunosorbent assay (ELISA) method using Gscience kits obtained from Glory Science Co., Ltd, USA, according to the manufacturer's instructions. Cardiac transforming growth factor-ß (TGF-ß) and vascular endothelial growth factor (VEGF) were assessed by enzyme linked immunosorbent assay (ELISA) method by means of Gscience kits obtained from Glory Science Co., Ltd, USA, according to the manufacturer's instructions. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity was measured by the colorimetric technique using Salucea kit (The Netherlands) according to the way described by Young [34].

Statistical analysis

In the existing study, all the results were expressed as mean±standard error of the mean. Statistical Package for the Social Sciences program, version 14.0 was used to compare the significant difference between every two groups. The difference was considered statistically significant when p=0.05. Percentage difference representing the percent of the variation with respect to the corresponding control group was calculated according to the following formula:

% Difference = (Treated value − control value)/control value) × 100

RESULTS

Data in Table 1 illustrated the effect of ethanolic extract of Celery administration on the lipid profile of atherosclerotic rats. A significant increase ($p<0.05$) in serum cholesterol, triglycerides, and LDL levels (135.39%, 56.04%, and 77.94% individually) was detected in AS group versus the control group. Meanwhile, serum HDL level recorded the marked reduction ($p<0.05$) in AS group (-54.13%) versus the control group. Otherwise, AS group was remedied with ethanolic extract of Celery or simvastatin which exhibited a significant lessening ($p<0.05$) in serum cholesterol (-34.38% and -42.52% separately), triglycerides (-20.19% and -28.18% individually) and LDL (-24.11% and -28.24% individually) levels versus AS group. In contrast, a considerable increment in serum HDL level was demonstrated ($p<0.05$) in AS group which received Celery

ethanolic extract (58.48%) or simvastatin (75.16%) versus AS group. It has been worth mentioning that AS group in which Celery ethanolic extract was administered, showed a marked increment ($p<0.05$) in serum cholesterol level and a marked reduction ($p<0.05$) in serum HDL level versus AS group in which simvastatin was administered. However, serum LDL and triglycerides levels noted no significant change ($p>0.05$) in AS group which received Celery ethanolic extract as versus to AS group which received simvastatin (Table 1).

Table 1. Effectiveness of Celery ethanolic extract on lipid profile in atherosclerotic rats (Means±SE, n=10).

groups / parameters	Con	CE	AS	AS+CE	AS+Sim
Cholesterol (mg/dL)	61.65±2.15	65.12±3.25	145.12±5.61[a] a(135.39%)	95.23±2.33[bc] b(-34.38%) c(14.17%)	83.41±3.22[b] b(-42.52%)
Triglycerides (mg/dL)	59.35±1.92	61.51±2.31	92.61±1.52[a] a(56.04%)	73.91±1.33[b] b(-20.19%)	66.51±1.38[b] b(-28.18%)
HDL (mg/dL)	44.32±1.21	46.52±1.65	20.33±1.11[a] a(-54.13%)	32.22±1.55[bc] b(58.48%) c(-10.52%)	35.61±1.92[b] b(75.16%)
LDL (mg/dL)	23.12±1.23	25.23±1.71	41.14±1.08[a] a(77.94%)	31.22±1.22[b] b(-24.11%)	29.52±1.61[b] b(-28.24%)

a: Significant change at P< 0.05 when it compared to the normal control group; b: Significant change at P< 0.05 contra AS group; c: Significant change at P< 0.05 against to the Simvastatin treated group.

The activities of CRP, CK and LDH in serum of control and investigational groups have been represented in Table 2. The effectiveness of CRP, CK and LDH were significantly augmented ($p<0.05$) in AS group (107.16%, 123.59% and 51.16% respectively) contrary to the control group. Treatment with Celery ethanolic extract or simvastatin showed a drastic reduction in CRP (-30.53% and -42.86% respectively), CK (-33.57% and -45.45% respectively) and LDH (-21.52% and -27.17% respectively) activities contrary to AS group. Interestingly, no considerable alteration was detected (p?0.05) in serum LDH value between Celery ethanolic extract and simvastatin-treated AS groups. However, serum activities of CRP and CK confirmed considerable elevation ($p<0.05$) in Celery ethanolic extract-treated AS group contrary to simvastatin-treated AS group (Table 2).

Table 2. Influence of Celery ethanolic extract on serum activities of CRP, CK and LDH in atherosclerotic rats (Means±SE, n=10).

groups / parameters	Con	CE	AS	AS+CE	AS+Sim
CRP (IU/L)	3.21±0.08	3.30±0.09	6.65±0.28[a] a(107.16%)	4.62±0.25[bc] b(-30.53%) c(21.57%)	3.80±0.15[b] b(-42.86%)
CK (IU/L)	10.51±0.26	11.52±0.15	23.50±1.61[a] a(123.59%)	15.61±0.92[bc] b(-33.57%) c(21.76%)	12.82±1.02[b] b(-45.45%)
LDH (IU/L)	175.51±7.56	181.11±9.32	265.31±10.58[a] a(51.16%)	208.21±6.58[b] b(-21.52%)	193.21±8.61[b] b(-27.17%)

a: Significant change at P< 0.05 when it compared to the normal control group; b: Significant change at P< 0.05 contra AS group; c: Significant change at P< 0.05 against to the Simvastatin treated group.

The results in Table 3 represented the influence of dealing with Celery ethanolic extract on serum levels of Troponin-1 and Endothelin-1 in atherosclerotic rats. The AS group showed significant altitude ($P<0.05$) in serum Troponin-1 (92.81%) and Endothelin-1 (96.06%) levels relative to the control group (Table 3). The treatment of the AS group with Celery ethanolic extract elicited a significant reduction ($P<0.05$) in serum Troponin-1 (-31.00%) and Endothelin-1 (-22.28%) levels contrary to the untreated AS group (Table 3). It was noteworthy that there were no significant changes (P?0.05) in serum Troponin-1 and Endothelin-1 levels between AS group remedied with Celery ethanolic extract and the AS group remedied with Simvastatin (Table 3).

Table 3. Effect of Celery ethanolic extract on serum levels of Troponin-1 and Endothelin-1 in atherosclerotic rats (Means±SE, n=10).

groups / parameters	Con	CE	AS	AS+CE	AS+Sim
Troponin-1 (ng/ml)	14.32±1.22	16.31±2.13	27.61±2.12[a] a(92.81%)	19.05±2.04[b] b(-31.00%)	17.16±1.64[b] b(-35.82%)

Endothelin-1 (ng/ml)	23.12±1.02	25.61±1.08	45.33±2.35[a] a(96.06%)	35.23±1.65[b] b(-22.28%)	31.22±1.66[b] b(-31.13%)

a: Significant change at P< 0.05 when it compared to the normal control group; b: Significant change at P< 0.05 contra AS group.

The findings represented in Table 4, demonstrated the influence of Celery ethanolic extract treatment on the cardiac growth factors content of atherosclerotic rats. Where cardiac TGF-ß and VEGF contents displayed a significant altitude (p<0.05) in AS group (152.22% and 90.96%, respectively) versus the control group. However, Celery ethanolic extract or simvastatin-treated groups showed significant attenuation (p<0.05) in cardiac TGF-ß (-38.64% and -54.38%, respectively) and VEGF (-34.09% and -43.64%, respectively) versus AS group (Table 4). Of note, Celery ethanolic extract-treated AS group indicated a significant upregulation (p<0.05) in cardiac TGF-ß and VEGF contents contrary to simvastatin-treated AS group (Table 4).

Table 4. Influence of Celery ethanolic extract on cardiac levels of TGF-ß and VEGF in atherosclerotic rats (Means±SE, n=10).

groups / parameters	Con	CE	AS	AS+CE	AS+Sim
TGF-β (Pg/ mg protein)	126.21±4.22	138.61±5.11	318.33±6.76[a] a(152.22%)	195.32±6.22[bc] b(-38.64%) c(34.49%)	145.22±5.31[b] b(-54.38%)
VEGF (Pg/ mg protein)	242.62±4.56	251.28±3.32	463.31±8.14[a] a(90.96%)	305.33±5.81[bc] b(-34.09%) c(16.95%)	261.08±6.11[b] b(-43.64%)

a: Significant change at P< 0.05 when it compared to the normal control group; b: Significant change at P< 0.05 contra AS group; c: Significant change at P< 0.05 against to the Simvastatin treated group.

The results in Table 5 demonstrated liver functions recorded in atherosclerotic rats after Celery ethanolic extract administration. Where serum ALT (127.23%) and AST (93.39%) effectiveness were significantly amplified (p<0.05) in atherosclerotic rats versus the control group. Otherwise, AS groups treated with Celery ethanolic extract or simvastatin delivered marked reduction (p<0.05) in serum ALT (-42.64% and -48.45%, respectively), AST (-28.36% and -34.05%, respectively) activities contrary to AS group. Interestingly, no considerable alteration was detected (p?0.05) in serum ALT and AST effectiveness between Celery ethanolic extract and simvastatin-treated AS groups (Table 5).

Table 5. Effect of Celery ethanolic extract on serum activities of ALT and AST in atherosclerotic rats (Means±SE, n=10).

groups / parameters	Con	CE	AS	AS+CE	AS+Sim
ALT (U/L)	125.25±5.56	131.32±4.32	284.61±6.58[a] a(127.23%)	163.26±4.56[b] b(-42.64%)	146.72±3.64[b] b(-48.45%)
AST (U/L)	98.56±5.81	105.11±6.22	190.61±5.66[a] a(93.39%)	136.56±5.32[b] b(-28.36%)	125.71±6.35[b] b(-34.05%)

a: Significant change at P< 0.05 when it compared to the normal control group; b: Significant change at P< 0.05 contra AS group.

DISCUSSION

This contemporary study presented encouraging findings of Celery ethanolic extract to be hopeful candidate for mitigating Atherosclerosis. It has been commonly well-thought-out that hyperlipidaemia, inflammation, and oxidative stress would lead to the initiation and progress of AS [35].

The contemporary study detected that there was a noticeable hyperlipidemia in AS group feeding on a high cholesterol diet (HCD) versus the control group. The obtained data were congruent with Son et al. [36], who detected high cholesterol as well as TG level in HCD-fed rats. Additionally, Fruchart et al. [37] have established that adipose tissue lipid was generally derived from circulating TG, especially throughout HCD feeding. Furthermore, Novelli et al. [25] have been documented the serum LDL level amplified in both obese rats as well as HCD-fed rats. Moreover, the depletion in HDL has been declared by Raveh et al. [38], which was in agreement with the present study's results, due to depletion of the adverse cholesterol transmit from blood to the liver. The oxidative stress produced by HCD administration caused the augmentation of the reactive oxygen species (ROS) production. The overabundant of ROS encouraged the cellular spoilage through the oxidation of critical cellular components as

membrane lipids, proteins, and DNA [39]. In addition, AS often takes place in the huge and medium-sized artery. The manifestation of lipid aggregation (cholesterol, glucolipid etc.) in vessel wall intima was the risk agent in the manifestation and development of AS. Cholesterol mainly came from plasma lipoprotein. Plasma lipoprotein could rise the cholesterol internal flow and deposition within the arterial walls, like LDL that could promote the AS appearance. Moreover, plasma lipoprotein could hearten the cholesterol outward transmit from blood vessels, like HDL which had reserve effects on AS [40].

Most international and national lipid dealing guidelines have considered LDL the primary target of hypolipidemic remedy. Ethanolic extract of Celery or simvastatin, used in the existing study could diminish serum cholesterol, triglycerides and LDL levels while there was an augmentation of serum HDL level in AS group. These findings specified that Celery ethanolic extract has had helpful effects on lipid profile through cholesterol dropping effectiveness. These results were harmonious with Grundy et al. [41] and Kooti et al. [23]. Tsi and Tan [42] studied the characteristics of anti-hyperlipidemia of the celery in hypercholesterolaemic (RICO) rats. Eventually, in the experiment, a considerable decrease was observed in the concentration of serum total cholesterol and triglyceride values in the treatment group. Celery has been among the vegetations which are affluent in flavonoids as apigenin and apiin. As well, this plant contains vitamins E besides C, which have potent antioxidant possessions [22]. Cheng et al. [43] presented that celery seed extract has a part in the scavenge of free radicals due to the antioxidant property.

Simvastatin medication mended the lipid profile via depressing serum total cholesterol, triglycerides and LDL cholesterol concentrations and increasing serum HDL level as contrary to the AS group. Simvastatin has been considered as one of HMG-CoA reductase inhibitors; a class of lipid dropping drugs. The findings were in stratification with previous studies of Matikainen et al. [44] and Yao et al. [45]. Also, Delbosc et al. [46] stated that Simvastatin ameliorated the lipid profile and rose HDL serum level through an enhancement of lipid dysfunction of obese rats and delayed the development of the obesity complications.

Measurement of CRP, CK and LDH effectiveness in serum has been considered to be diagnostic markers for certain cardiovascular diseases, and they have been sensitive markers of myocardial infarction [47, 48].

The contemporary study demonstrated an increment in the serum activities of CRP, CK and LDH in AS group contrary to the normal control group. The possible mechanism underlying the increased CRP, CK and LDH efficacies has been related to the inflammation indicated in atherosclerosis. The indication from epidemiological investigations have shown that atherosclerosis was depicted by a complex pathology that contains several processes including hyperlipidemia [49], oxidative stress [50, 51], inflammation [52] and platelet aggregation [53]. Capria et al. [54] stated that tumor necrosis factor-a (TNF-a) has been an important factor in atherosclerosis and vascular function in several ways. It has been central in vascular inflammation, implicated in oxidative stress besides apoptosis, and has thrombogenic potentials. Moreover, CRP acted in atherosclerosis through increasing the scales of reactive oxygen species (ROS) and nuclear factor kappa-B (NFkB). NFkB in turn encouraged the levels of chemokines and cytokines [55]. Thus, the inflammation may have a direct influence on CRP, CK and LDH efficacies in atherosclerosis.

The treatment of AS group with celery ethanolic extract produced considerable amelioration in serum CRP, CK and LDH efficacies in contrast with the untreated AS group. It has been stated that the action mechanisms of Flavones could be clarified with the repression of different enzymes as prostaglandin synthase, lipoxygenase and cyclooxygenase, implicated in the inflammatory development and tumorigenesis, and with the inducement of detoxifying enzymes like glutathione S-transferase [56-59]. Atta and Alkofahi [60] stated that crude ethanol extracts of *Apium graveolents* (celery) showed anti-inflammatory efficacy in rats in vivo on carrageenan induced paw oedema. Thus, celery ethanolic extract may have a suppressive influence on the efficacy of heart enzymes due to its anti-inflammatory properties.

The present study demonstrated significant depletion in the efficacies of heart enzymes CRP, CK and LDH in the AS group cured with simvastatin versus AS group. Statins, beside their primary lipid-lowering influence, have been related with numerous pleiotropic actions, like the improvement of endothelial function [61], improved NO bioavailability [62], anti-oxidant [63] and anti-inflammatory influences [64]. Simvastatin possesses anti-inflammatory capacity as it could restrain the production of oxLDL-induced mRNA besides the production of tumor necrosis factor-a (TNF-a) and monocyte chemoattractant protein-1 [65]. In addition, simvastatin applied anti-inflammatory action in adipose tissue via repression of endoplasmic reticulum stress. The suppression of endoplasmic reticulum exertion in adipocytes represented an alternative mechanism of the pleiotropic achievement of statins [66]. Subsequently, simvastatin could suppress the inflammation induced by atherosclerosis, consequently, it could decrease the efficacies of seral CRP, CK and LDH.

The data of the existing study exhibited a considerable elevation in troponin-1 besides endothelin-1 serum levels in AS group in contrast with the normal control group. The production of cardiac troponins into the circulation happens with cardiomyocyte damage. Cardiac troponin has

been a favorite marker for identifying severe myocardial infarction and has been used as a key investigative tool for decision making in patients presenting with chest pain [67]. Traditionally, it was thought that the liberation of cardiac troponin has been equivalent to myocardial necrosis. In vitro trails have established caspase-3 activation consequences in cleavage of cardiac troponin and subsequent release [68]. Consequently, the increment in cardiac troponin levels noticed in patients with coronary artery disease may be the outcome of the activation of caspase-3 within cardiac myocytes and the resulting cleavage and liberation of cardiac troponin, but they did not necessarily associate with myocardial cell death [68]. Moreover, research studies have shown that coronary atherosclerosis has been responsible for two-thirds of coronary syndrome [69]. Therefore, the outcomes of the study of Laufer et al. [70] suggested that cardiac troponin has the possibility to become a serum biomarker that would advance the identification of patients at risk for promoting cardiac proceedings.

Atherosclerosis which occurred because of the obesity which was encouraged by the high cholesterol diet was contributed to the imbalance between the increased calorie intake, moreover, the decreased physical activity was unique for developing global health issues and was linked with the stimulated endothelin system in humans with or without hypertension [71]. The levels of the endothelium-derived peptide endothelin-1 (ET-1) were increased in obese subjects, and ET-1 interceded vascular tone was elevated. Blood vessels of obese rats included an increase in the liberation of ET-1 gene as well as ETA receptor protein, but the influence of the elevated body weight because of vasoconstrictor peptide was not identical among murine aqueduct arteries [72]. The increased ET-1 could be due to NO release caused by the activation of endothelial ETB receptors. Furthermore, the increased plasma ET-1 concentrations in human obesity might perhaps depend on fasting insulin concentrations, abnormal peptide clearance, or both [73].

The administration of ethanolic extract of celery showed significant retardation in troponin-1 and endothelin-1 serum levels comparing with AS group. The improvement in the troponin-1 serum level by the administration of celery ethanolic extract might be referred to its active ingredients of n-butylphthalide, Vitamin C besides vitamin E which have shown numerous pharmacological activities including the antioxidant efficacy so, they could sweep free radicals, inhibit the secretion of cytokines, and suppress the cell death [22, 74]. The capability to regulator ROS was thus critical in cardiovascular diseases, because myocardial injury occurred when the "oxidant–antioxidant" balance was troubled in favor of the excess oxidative stress [39]. A growing body of evidence suggested that ROS-scavengers effectively protect myocardials against apoptotic cell death. The inhibitory effect of celery ethanolic extract hydrolysed phenolic fractions on the apoptotic cell death indicated by the inhibition of caspase-3 activity might be resulted from the relieve of the oxidative stress and limited inflammation [56, 75].

It has been originated that in hypertensive obese subjects possessed improved vascular efficacy to endogenous ET-1. Recently, a correlation between ET-1 gene besides blood pressure levels was reported in obese Japanese individuals [75]. N-butylphthalide (NBP) was the most important active constituents in celery [76]. Some researchers have described antihypertensive effectiveness of some differentherbs, like, Solanaceae, in which NBP was one of the foremost fractions [77]. Moreover, celery seed was used by Moghadam et al. [78] for the curing the rats suffering from hypertension. Thus, the reduction in serum ET-1 level due to the treatment with celery ethanolic extract might be referred to the antihypertensive action of celery ethanolic extract.

The treatment of AS group with simvastatin produced a considerable reduction in serum troponin-1 as well as endothelin-1 levels when contrasted with those in the AS group. Statins had straight anti-inflammatory influences on adipocyte. Wu et al. [65] specified that Simvastatin curing significantly repressed the oxidized-LDL. Otherwise, it has been stated that the oxidized-LDL induced mRNA appearance, and the secretion of TNF-α and monocyte chemoattractant protein-1 (MCP-1) was also noticeably decreased by Simvastatin curing. By this mechanism, Simvastatin could diminish troponin-1 level in the serum of simvastatin treated group.

In addition, statins have been revealed to recover endothelial function [61, 79] and reduce the arterial stiffness [80, 81] in many patient groups. Wallace et al. [82] suggested that aortic stiffening and endothelial dysfunction caused by the acute inflammatory trauma might be adjustable to pharmacological involvement with statins. Thus, simvastatin could attenuate the level of endothelin-1 in simvastatin treated group comparing to AS group.

The present study registered a considerable excess in the level of growth factor of VEGF in AS group compared with the control group. VEGF has been a powerful growth factor for endothelial cells and an inducer of angiogenesis, it has been serious for endothelial integrity and thus for vascular function [83]. Furthermore, VEGF may enhance the pathophysiologic mechanism of plaque development and plaque undermining [83]. The previous research on angiogenesis in tumors demonstrated that as a tumor grew, hypoxia became a key agent for angiogenesis initiation [84]. The same phenomenon happened in the rising atherosclerotic plaque. Most neovessels in the plaque originated from the branches of vasa vasorum [85]. As the

intima of an atherosclerotic vessel thickened, a consequence of a rising plaque, oxygen diffusion from the lumen became more difficult. The development of the intima to a fateful thickness (about 100 mm) could induce a low oxygen tension and the activation of proangiogenic substances, of which hypoxia inducible factor (HIF)-1 was the most important agent. HIF was produced in hypoxic conditions in almost all tissues physiologically as well as pathologically. The HIF-1 transcription agent consisted of HIF-1b and HIF-1a, the latter of which was responsive to hypoxia [86]. Under physiological (normoxic) situations, HIF-1a was modified (hydroxylated) by prolyl hydroxylases. This procedure was oxygen-dependent and made use of such constituents as vitamin C and iron. After HIF-1a was hydroxylated, it was further degraded by proteases. Under hypoxic conditions, however, the prolyl hydroxylases converted to inactive, and HIF-1a could form a dimer with HIF-1b and invigorated the transcription of different genes, such as nitrous-oxidesynthase and VEGF [84, 87].

The investigational studies have revealed that the repression of the VEGF-system reduced atherosclerosis. In mouse models, it was established that the repression of VEGF receptors led to the reduction of atherosclerotic lesions, which was independent of hypercholesterolemia [88] or even angiogenesis [89]. The current results showed significant suppression in cardiac VEGF level in celery ethanolic extract-treated AS group versus AS group. This result was in resemblance with a previous report of Tülay et al. [90]. They confirmed that VEGF value was decreased by curing with *Apium graveolens* extract. Moreover, Tülay et al. [90] stated that this finding could be referred to the antiproliferative influence exerted by an ethanolic extract of *Apium graveolens.*

The current data showed that there was a considerable decline in cardiac VEGF level of simvastatin-treated AS rats. Simvastatin has been mentioned to reduce VEGF expression and angiogenesis at the high concentrations in a paradigm of hypercholesterolemia [91] but encourage angiogenesis in ischemic models at lower doses [92, 93].

Moreover, the present data registered a considerable increment in the cardiac TGF-ß level in the As group contra normal control group. The increased evidence proposed that atherosclerosis was an inflammatory illness promoted by hypercholesterolemia. The influence of adaptive immunity has been controversial, however [94]. Robertson et al. [94] hypothesized that proatherogenic T cells were managed by immunoregulatory cytokines. Among them, TGF- ß has been implicit in atherosclerosis, but its mechanism of action was still unclear. The data in the existing study were in line with the preceding clinical observations that both activated plasma TGF-ß, and plasma TGF-ß receptors were significantly amplified in patients with coronary artery disease and that the increased plasma TGF-ß receptor levels in these patients were positively correlated to plasma cholesterol [95]. Surprisingly, an inverse correlation was also stated between LDL and TGF-ß levels in human being [96]. The discrepancy between these results might reflect the alterations in TGF-ß levels in diverse stages of hyperlipidemia and atherosclerosis. It has also been recommended that TGF-ß could reduce lipid accumulation in the artery and indeed tamoxifen, which elevated TGF-ß levels, and inhibited lipid lesion formation [96].

Otherwise, the curing of AS group with celery ethanolic extract registered a significant depletion in the cardiac TGF-ß level in AS group. This could be due to the influence of the celery extract with its active ingredients n-butylphthalide and luteolin which could encourage the expression of TGF-ß [97]. In addition, this inhibition influenced luteolin has been thought to be associated to its suppression of H2O2-induced JNK phosphorylation, proportionate with that luteolin suppressed the adipocyte dependent activation of macrophages through the repressed JNK phosphorylation. Taken together, the antioxidant properties of luteolin emerged through the inhibition of JNK phosphorylation and TGFß1 induction [98].

The treatment of AS group with simvastatin caused a significant decrease in the cardiac level of TGF-ß. The mechanisms causing the protective influence of statins have not been fully clarified. The balance between nitric oxide (NO) and oxidative stress played a critical role in keeping cardiovascular homeostasis [99]. It has been detected that TGF-ß has been upregulated by oxidative stress, and increased glomerular pressure, and was inhibited by NO [100]. Hence, increasing NO and the reduction of oxidative stress by statins might participate at least in portion, the decrease of TGF-ß.

The significant augmentation in serum AST and ALT activities represented a clinical indicator for liver tissue injury caused by toxicants or disease situations [101]. In AS group, the efficacy of liver enzymes AST besides ALT in serum was considerably elevated relatively to those in the control group. The quantity of the released cellular enzymes registered in the blood indicated the change in plasma membrane integrity and/or hepatocytes permeability. Obesity has been recognized to yield oxidative stress and promote the releasing of ROS, which caused the overproduction of peroxidized lipid molecule in liver tissue as shown by the enhanced malondialdehyde content in the liver. The overproduction of lipid peroxides caused destabilization in cellular lipid substances inducing oxidative damage, especially of membrane structures. This led to the seepage of liver enzymes to the circulation [39, 102].

Celery ethanolic extract-treated AS group showed the marked betterment in liver functions as it was evident from

the significantly blunted activity of liver enzymes in serum relative to AS counterparts. The therapeutic influences of the most medicinal plants were attributed to their antioxidant possessions which in turn, could be ascribed to their antioxidant phytochemicals. The hepatoprotective influences of celery extract could be mainly due to its antioxidant and free radical scavenging possessions of its active constituents which have been exemplified in numerous studies [103]. Thus, this influence of celery extract asserted the capability of its active ingredients to preserve the architectural integrity of hepatocytes and in turn, restricted the leakage of liver enzymes into circulation. This indicated the membrane-stabilizing property of celery extract.

The administration of simvastatin in AS rats markedly decreased serum efficacy of liver enzymes in AS rats. Abbas and Sakr [104] described that the protective influence of simvastatin on the liver was recognized by the significant reduction of the oxidative stress and the alleviation of liver functions. These results were in harmony with the stated results of Cui et al. [105] who illustrated the downregulation of liver enzymes in the serum of HFD-fed rats cured with simvastatin.

CONCLUSION

In conclusion, the existing study provided the experimental evidences for the anti-atherosclerotic activity of celery (*Apium graveolens*). This effect was documented by the improvement of dyslipidemia, the amelioration of the cardiac enzyme's activities, troponin-1, endothelin-1 and the growth factors levels. The anti- atherosclerotic influence of celery (*Apium graveolens*) might be attributed to its hypolipidemic, antioxidant and anti-inflammatory capacity.

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