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Impact effect of lycopene and tomato-based products network on cardio-protective biomarkers *in vivo*

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ABSTRACT:

Background: Dietary intake plays an important role as nutritional supplements are known to provide potential health benefits in cardiovascular disease. Recent studies suggest that the dietary intake of tomatoes and tomato products containing lycopene are associated with a decreased risk of cardiovascular disease. In order to substantiate these facts, the present study was undertaken to investigate the effectiveness of lycopene from tomato products on the potential effects of oxidative stress and atherosclerosis *in vivo*, focusing on early atherosclerotic events.

Methods: Thirty male albino rats were assigned randomly into 5 groups; group C was the negative control group fed a basal diet, group H was the positive control fed a high-fat diet (HFD), group TS (HFD) was fed a 8% lyophilized tomato paste, group TW (HFD) was fed a 24% lyophilized raw tomato, and group L (HFD) was fed 0.1% mg pure lycopene. The level of serum; total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-$_c$), and low-density lipoprotein cholesterol (LDL-$_c$) was measured after 8 weeks of experimental treatment. Malondialdehyde (MDH) plasma levels were measured in heart tissue homogenate. Furthermore, pathologic changes of the heart and aorta were also assessed.

Results: We found that TC, TG, LDL-$_c$ and MDH, levels were significantly increased in group H ($P < 0.05$) compared to the negative control group. Administration of TS, TW and L demonstrated significant changes in these parameters ($P < 0.05$). The TW group (fed 24% of lyophilized raw tomato components) had more positive effects than the TS & L groups. Furthermore, morphologic changes of heart and aorta revealed that TW and TS had a similar preventive effect against the development of atherosclerosis.

Conclusion: Our study indicated that raw tomatoes have a higher potential effect when compared to tomato paste or lycopene alone. This potential effect includes the ability to attenuate and/or reverse oxidative stress and other atherosclerosis related parameters induced by the consumption of a high-fat diet.

Key words: Cardioprotective, Lipid profile, Lycopene, Oxidative stress, Tomatoes products

BACKGROUND:

Coronary heart disease (CHD) is the most common type of all heart diseases. In fact, it is the number one cause of death for both men and women. Current strategies of public health focus on reducing the risk of coronary heart disease by lowering serum cholesterol and improving lifestyles through theuse of medications or medical procedures. These various forms of treatment can help prevent and/or cure CHD, thereby reducing the risk of other related health problems [1].

Preceding studies have established that decreased cholesterol is associated with a reduced risk of CHD. In addition, recent findings have drawn several more parallels between triacylglycerol and low density lipoproteins (LDL) as significant lipid risk factors for CHD, while also highlighting cholesterol levels [2].

Diet plays an important role in lowering these circulating lipid levels and offers long-term efficacy that's comparable with most effective drug treatments that are currently being used. One dietary regimen that may be beneficial for improving lipid profile involves a supplementation of the diet with dietary antioxidants, such as lycopene, which is naturally present in tomatoes and tomato products [3].

Epidemiologic studies have shown that tomato and tomato product intake is associated with a reduced risk of degenerative diseases [4]. Moreover, a high intake of tomato juice prevents low density lipoprotein (LDL) oxidation and thiobarbituric reactive species (TBARS) formation in healthy men [5, 6].

Numerous studies have shown that lycopene may be beneficial in the fight against diseases such as cancer and coronary heart disease, as well as other chronic conditions. Further investigations strengthened the hypothesis that lycopene could be a fundamental factor for the preventive effects of tomatoes and tomato products [8]. These studies have used epidemiological, biochemical and bioavailability methodologies in order to confirm its protective role. However, the underlying mechanisms of lycopene and extent of its efficacy still remains unclear [7].

Recently, it was found that lycopene is present in most human tissues, but it is not accumulated uniformly. There is a preferential accumulation of lycopene, particularly in the adrenals and testes. The confirmed ability to increase lycopene levels in these tissues is one prerequisite for using it as a dietary supplement to improve health. In fact, it has recently been reported that supplementation of tomato lycopene oleoresin in volunteers undergoing elective surgery produced a significant increase of carotenoids in the plasma, skin, and adipose tissues [4]. Still however, little is known about the metabolism or degradation of lycopene in mammals. A number of oxygenated metabolites have been found in plasma and tissues [6]. On the other hand, other investigations have shown a higher *in vivo* effect with the whole tomato compared to the lycopene alone [9]. Most of the experiments comparing lycopene, tomatoes and tomato

products have been made with the assumption that phytochemicals other than lycopene might also be involved in the preventive health effects of raw tomatoes [10]. Evidence from recent studies especially implies the crucial role of both whole tomato and lycopene intake with the incidence of CHD [11].

This study was thus designed to determine the effects of lycopene from various tomato sources on oxidative stress levels and other risk-factors of atherosclerosis. Therefore the role of, lyophilized raw tomato, lyophilized tomato paste and pure lycopene was evaluated in male albino rats, focusing on early atherosclerotic events induced by a high-fat diet *in vivo*.

MATERIALS AND METHODS:

Lyophilized tomato paste and lyophilized raw tomato Preparation: Fifty kg of raw tomatoes were obtained from the market and used in this study (Almadena Almonura, Kingdom of Saudi Arabia). Four kg of tomato paste was prepared by first grinding fifteen kg of raw tomatoes and then heating until the tomato paste concentrate reached 39% Brix. From there, the tomatoe paste was frozen with the rest of the raw tomatoes using the Freeze-Drying Model Ez550Q FTS (Germany). This yielded 1.5 kg of freeze-dried raw tomato powder (TW) and 0.7 kg of freeze-dried tomato paste powder (TS). Both ofwhich were stored at 4°C in a dark environment [12].

High performance liquid chromatography: High performance liquid chromatography (HPLC) was used to separate and identify the carotenoid compounds in the freeze-dried raw tomato (TW) and the freeze-dried tomato paste (TS). The carotenoid samples of TW and TS were then extracted by using methanol, trichloromethane and deionized water. Finally, these extracted samples were mixed gently and centrifuged at 400g for 10 minutes. The lower layer (organic phase) was aspirated, transferred into glass tubes and then evaporated under nitrogen. A second extraction was made using tetrahydrofuran, dichloromethane and deionized water. This was also centrifuged at 400g for 10 minute. The resulting second bottom layer was pooled and evaporated for dryness under nitrogen. The extract was then dissolved in 200µl of acetonitrile/dichloromethane (50/50 v/v) and injected into a HPLC apparatus. Carotenoids were separated out using two columns in a series (Nucleosil C18, 150 x 4.6 mm, 3 µm followed by Vydac C18, 250 x 4.6 mm (France)). The mobile phase involved an acetonitrile, dichloromethane, methanol (containing 50 mM ammonium acetate), and water (70/10/15/5, v/v/v/v) mixture. The flow rate was isocratic (2 ml/min). Carotenoids were detected at 450 nm, and identified by the comparison of their retention time as well as spectral analysis against those in the pure mixture of lutein, lycopene, and beta-carotene [6]. Quantification was performed using the Waters Millenium 32 Software (version 3.05.01). All solvents used were of HPLC grade from the Sigma Chemical Company(USA).

Animal model: Thirty male albino rats, weighing between 120-125 grams, were used in this study. They were bred in the animal housing section of Taibah University. These rats were maintained according to the recommendations of the local and national ethics committee. Prior to the experiment, they were fed a standard diet for one week in order to promote adaptation. This was also in accordance with the NIH guide for the care and use of laboratory animals [13].

Experimental design: The animals were distributed into two main groups (Table 1). The first group was the negative control (C) (n=6), fed a basal diet [14] and the second group (n=24) was the positive control, fed a high-fat diet (basal diet + 5% tallow + 1% cholesterol+ 0.02% bile salt). The second group was then divided into 4 subgroups (n=6). Subgroup 1 in the positive control (H) was fed a high-fat diet only, group 2 (TS) was fed a high-fat diet plus 8% of the tomato paste powder, group 3 (TW) was fed a high-fat diet plus 24% of the raw tomatoes powder, and group 4 (L) was fed a high-fat diet plus 0.1% lycopene. Groups TS, TW and L were all supplemented as to have the same quantity of lycopene (100mg per kg of diet) (Table 2).

Table 1: Diet composition (%)

	Control Negative (C)	**High- fat diet**			
		Control Positive (H)	**(TS)**	**(TW)**	**(L)**
Casein	12	12	12	12	12
Corn oil	10	10	10	10	10
Mineral mixture	4	4	4	4	4
Vitamin mixture	1	1	1	1	1
Wheat bran	4	4	4	4	4
Tallow	0	5	5	5	5
Cholesterol	0	1	1	1	1
bile salt	0	0.02	0.02	0.02	0.02
Lyophilized tomato paste	0	0	8	0	0
Lyophilized raw tomato	0	0	0	24	0
Lycopene	0	0	0	0	0.1
Wheat starch	up to 100				

Lyophilized raw tomato group (TW), lyophilized tomato paste group (TS), 0.1% lycopene (L) groups all contain 100 mg of lycopene per kg of diet.

Table 2: Carotenoids content in TW and TS used for the supplementation (%)

Carotenoids content of freeze-dried samples		
Carotenoids	Lyophilized tomato paste (TS)	Lyophilized raw tomato (TW)
beta-carotene	0.63	0.29
Lycopene	1.31	0.42
Lutein	0.29	0.056

Chemicals: Pure lycopene was obtained as a bright red powder from the Sigma Chemical Company(USA). Cholesterol powder and bile salts were obtained from the Elgamhoria Company for Medical Preparations, Chemicals and Medical Equipments (Cairo, Egypt).

Biological evaluation: During the experimental period (8 weeks), the diet consumed was recorded every day. The body weight gain (BWG) and food efficiency ratio (FER) were determined according to Chapman *et al* [15].

Biochemical analysis: At the end of experiment period, animals were sacrificed after a 12 hr period of fasting. Blood samples were collected in clean dry tubes at room temperature and centrifuged at 3000 rpm for 10 minutes. The clear supernatant serum were aspirated and stored at -20˚C, until it was used for biochemical parameters [16].

Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were determined using an enzymatic colorimetric method [17-21]. Very low density lipoprotein cholesterol (VLDL-c) was determined according to that of Lee and Nieman [21] as follows VLDL-c =TC - LDL-c – HDL-c. The atherogenic index (AI) was calculated as HDL-c /TC% and LDL-c / HDL-c [22].

Sample preparation: The hearts and lungs of the rats were excised and weighed. The heart of each rat was divided into two parts. The first part was used for determining the susceptibility of the heart to peroxidation through the estimation of malondialdehyde (MDA) in the heart homogenate [23]. The second part of the remaining hearts, the aorta, and the lungs were rapidly washed in cold saline (9 g/l NaCl) and then stored in formalin solution (10%) for 24 hours. Washing was done with tap water and then serial dilutions of alcohol (methyl, ethyl and absolute ethyl alcohols) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C. in a hot air oven for 24 hours. Paraffin beeswax tissue blocks were prepared by sectioning off at every 4 microns by a slide microtome. The resulting tissue sections were then collected onto glass slides, deparaffinized and stained by hematoxylin and eosin [24] for histopathological examination by a light microscope (80x, 160x magnification).

Statistical Analysis: Statistical Analysis was performed by using the SPSS 10.1.7 program package (SPSS Inc., USA). The data are presented in terms of means ± SD, the differences between the groups were determined by one way ANOVA, and significance was defined at a 0.05 level of confidence.

RESULTS:

Determination of the carotenoid content in TS and TW by HPLC: Three compounds including beta-carotene, lycopene and lutein were identified in the TS & TW samples. The lowest value of lycopene (0.42g/ 100g) was recorded for the freeze-dried raw tomato sample. The highest value of lycopene (1.31g/100g) was recorded for the freeze-dried tomato paste sample (Table 2).

The effect of tomato products and lycopene on body weight, food intake and feed efficiency rate: There was no significant difference between the mean values of IBW (initial body weight) of all the experimental groups. After 8 weeks of treatment, the mean value of FBW (final body weight) for all the groups had increased. The mean value of FBW in group H (192±4.9g) was significantly higher when compared to the corresponding mean values of group C, group TS, group TW and group L (139± 13.31, 131.67±5.61, 131.17±15.25 and 132.83±11.89 g,

respectively) . Concerning FBW and IBW, the mean values of BWG (body weight gain) for group TS, TW and L had no significant differences when compared with group C. Furthermore, the mean values of FER% (food efficiency rate) for groups TS and L also indicated no significant differences when compared to group C (Table 3).

Table 3: Body weight gain, food intake and feed efficiency rate after 8-weeks of feeding

	IBW(g)	**FBW**(g)	**BWG**(g)	**FI**(g)	**FER** (%)
(C)	123.17 ±4.29 [a]	139.00 ±13.31 [a]	19.17 ±17.13 [a]	681.33 ±129.73 [a]	0.03 ±0.032 [a]
(H)	124.67 ±3.44 [a]	192.00 ±4.90 [b]	83.83 ±6.77 [b]	1092.00 ±98.59 [b]	0.075 ±0.009 [b]
(TS)	118.50 ±2.07 [a]	131.67 ±5.61 [a]	15.83 ±5.98 [a]	952.00 ±132.52 [c]	0.016 ±0.005 [ca]
(TW)	121.33 ±14.50 [a]	131.17 ±15.25 [a]	12.00 ±6.99 [a]	1054.66 ±42.15 [bc]	0.011 ±0.006 [c]
(L)	122.67 ±11.13 [a]	132.83 ±11.89 [a]	12.50 ±5.58 [a]	746.6 6±76.51 [a]	0.017 ±0.009 [ca]

C: normal diet; **H**: high-fat diet; **TS**: high-fat diet plus freeze-dried tomato paste; **TW**: high-fat diet plus freeze-dried fresh tomato; L, high-fat diet plus lycopene; **IBW:** initial body weight; **FBW**: final body weight; **BWG:** body weight gain; **FI**: food intake; **FER**: feed efficiency rate. Results are expressed as means ± SD for *n*=6 animals per group. **[a,b,c]**Mean values within a column not sharing a common superscript letter were significantly different ($p < 0.05$).

Effect of tomato paste, fresh tomato and lycopene on the relative weight of the heart and lung: The mean values of heart weight for group H (0.83±0.25g) was significantly higher than the corresponding mean values of group C, group TS, group TW and group L (0.46±0.05, 0.45±0.10, 0.38±0.07 and 0.57±0.05 g), respectively ($p < 0.05$). The mean values of heart weight for group TS, group TW and L group showed no significant difference when compared with group C. The mean values of lung weight for group H, group TS and group TW (0.73±0.05, 0.76±0.20 and 0.855±0.12 g), respectively, again had no significant differences when compared with the corresponding mean value for group C (0.78±0.14 g). At the same time, the mean value of lung weight for group L (0.93±0.08 g) was significantly higher than the corresponding mean values for group C ($p < 0.05$)(Table 4).

Table 4: Effect of tomato paste, fresh tomato and lycopene on relative weight of heart and lung

	Heart weight(g)	**Lung weight** (g)
Normal diet (C)	0.46±0.051[ab]	0.78±0.14[ab]
High-fat diet (H)	0.83±0.25[c]	0.73±0.05[b]
High-fat diet plus freeze-dried tomato paste (TS)	0.45±0.10[ab]	0.76±0.20[b]
High-fat diet plus freeze-dried fresh tomato (TW)	0.38±0.07[a]	0.855±0.12[ab]
High-fat diet plus lycopene (L)	0.57±0.05[b]	0.93±0.08[a]

Results are expressed as means ± SD for *n*=6 animals per group. [a,b,c]Mean values within a column not sharing a common superscript letter were significantly different ($p < 0.05$).

Lipid profile: There were comparative changes among the groups in the serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c), and high density lipoprotein cholesterol (HDL-c). Group TW had the lower concentrations of TC, TG, LDL-c and VLDL-c when compared with group H, group TS and group L. The mean values in group H (97.50±4.00, 189.16±16.55, 36.17±1.04 and 37.83±3.31 mg/dl, respectively) had significantly increased when compared with control group C (84.66±1.21, 146.66±10.20, 16.67±0.75 and 29.33±2.04 mg/dl, respectively) ($p < 0.05$).In group TW, the mean value of TC, TG, LDL-c and VLDL-c (78.67±1.86, 106.00±11.85, 19.00±1.09 and 21.20±2.37 mg/dl, respectively) were significantly lower than those of group H ($p < 0.05$). In parallel with the suppression effect of freeze-dried tomato powder, there were a significant increase in the mean values of HDL-c for group TS, group TW, and group L (35.83±3.06, 38.00±1.89, and 37.83±2.31mg/dl, respectively) when compared with the group H (23.50±1.41 mg/dl) ($p < 0.05$). However, there was no significant difference in the mean values of HDL-c in group TW when compared with control group C (38.66±2.16 mg/dl). The lycopene group (L) showed an apparently lesser effect. (Table 5).

Table 5: Effect of tomato paste, fresh tomato and lycopene on serum lipid profile after 8 weeks

	TG (mg/dl)	**TC** (mg/dl)	**LDL-$_C$** (mg/dl)	**VLDL-$_C$** (mg/dl)	**HDL** (mg/dl)
	M ±SD	M ±SD	M ±SD	M ±SD	M ±SD
(C)	146.66±10.20 [a]	84.66±1.21 [a]	16.67±0.75 [a]	29.33±2.04 [a]	38.66±2.16 [a]
(H)	189.16±16.55 [b]	97.50±4.00 [b]	36.17±1.04 [b]	37.83±3.31 [b]	23.50±1.41 [b]
(TS)	112.50±22.41 [c]	80.12±2.89 [c]	20.33±2.80 [c]	22.50±4.48 [c]	35.83±3.06[c]
(TW)	106.00±11.85 [c]	78.67±1.86 [c]	19.00±1.09 [c]	21.20±2.37 [c]	38.00±1.89[ca]
(L)	120.00±5.47 [c]	80.83±1.72 [c]	20.92±1.85 [c]	24.00±1.09 [c]	37.83±2.31 [ca]

C: normal diet; **H**: high-fat diet; **TS**: high-fat diet plus freeze-dried tomato paste; **TW**: high-fat diet plus freeze-dried fresh tomato; **L**: high-fat diet plus lycopene; **TC**: total cholesterol; **TG**: triglyceride; **LDL-c**: low density lipoprotein cholesterol; **VLDL-c**: very low density lipoprotein cholesterol; **HDL-c**: high density lipoprotein cholesterol. Results are expressed as means ± SD for *n*=6 animals per group. [a,b,c]Mean values within a column not sharing a common superscript letter were significantly different ($p < 0.05$).

Biomarker of oxidative stress*:* The mean value of MDH for group H (18.75±1.44 nmol / mg tissue protein) had significantly increased when compared to control group C (4.47±0.61 nmol/mg tissue protein)($p < 0.05$). Whereas the mean values of MDH in groups TW (4.53±1.13 nmol/mg tissue protein), TS (9.11±0.50 nmol/mg tissue protein), and L (5.15±0.59 nmol/mg

tissue protein) were significantly lower than group H ($p < 0.05$).The L group showed a lesser but still significant decrease when compared to group H (Table 6).

Table 6: Effect of tomato paste, fresh tomato and lycopene on oxidative stress parameter

	MDH (nmol / mg tissue protein)
	M±SD
Normal diet (C)	4.47±0.61[a]
High-fat diet (H)	18.75±1.44 [b]
High-fat diet plus freeze-dried tomato paste (TS)	9.11±0.50 [c]
High-fat diet plus freeze-dried fresh tomato (TW)	4.53±1.13 [a]
High-fat diet plus lycopene (L)	5.15±0.59 [a]

Results are expressed as mean ±SD for *n*=6 animals per group. [a,b,c] Mean values within a column not sharing a common superscript letter were significantly different ($p < 0.05$).

Atherogenic indices: Table 7 shows the comparative change between the groups in total (HDL-c/TC %) and as a ratio (LDL-c/HDL-c) for atherogenic indices. The mean value of HDL-c/TC % for group H (24.09±0.90) was significantly lower than that of the control group C (45.66±2.25) ($p < 0.05$). However, the mean value of LDL-c/HDL-c ratio for group H (1.54±0.14) was significantly higher than that of control group C (0.43±0.03) ($p < 0.05$). At the same time, the mean value of HDL-c/TC % for the TW group (4.53±1.13) showed a significant increase in comparison to group H ($p < 0.05$). Demonstrating a protective effect, group TW showed a significant decrease when the LDL-c/HDL-c ratio was compared to group H ($p < 0.05$).

Table 7: Effect of tomato paste, fresh tomato and lycopene on atherogenic indices

	HDL/TC %	**LDL/ HDL**
	M±SD	M±SD
Normal diet (C)	45.66±2.25 [a]	0.43±0.03[a]
High-fat diet (H)	24.09±0.90 [b]	1.54±0.14[b]
High-fat diet plus freeze-dried tomato paste (TS)	45.56±3.94 [a]	0.56±0.06[c]
High-fat diet plus freeze-dried fresh tomato (TW)	47.43±1.86 [a]	0.50±0.05[ca]
High-fat diet plus lycopene (L)	46.78±2.28 [a]	0.55±0.04[c]

HDL-c/TC %: high density lipoprotein cholesterol/ total cholesterol%; **LDL-c/HDL-c**: low density lipoprotein cholesterol/ high density lipoprotein cholesterol. Results are expressed as means ±SD for *n*=6 animals per group. Different superscript letter(s) in each column indicate significant differences between groups ($p >0.05$). Results are expressed as means ±SD for *n*=6 animals per group. [a,b,c]Mean values within a column not sharing a common superscript letter were significantly different ($p < 0.05$).

Histopathological findings

Morphologic changes in rat hearts: Representative heart sections stained with hematoxylin and eosin from each group are shown in Figure 1. The hearts of the control rats in group C showed no histopathological alteration and normal histological structures of the myocardium (**my**) were observed. In the heart sections of the rats in group H, we observed fat (**f**) deposition in the pericardium along with inflammatory cell infiltration (**m**).Furthermore, in section **H2,** the underlying myocardium had focal inflammatory cell infiltration (**m**) in between the myocardial bundles. While the hearts of the rats in group TS was showing the same focal inflammatory cells infiltration in the myocardium (**m**) underneath the pericardium. The examined hearts of the rats in group TW indicated less inflammatory cell infiltration in the myocardium (**m**) underneath the pericardium. The examined sections of the hearts of rats from group L were illustrated congestion in the myocardial blood vessels (**bv**). The morphological features of the hearts in the rats of group TW were almost identical to that of the control rats (group C). At the same time, the changes observed in the rats of group TW were quite similar to that of what was seen in group TS.

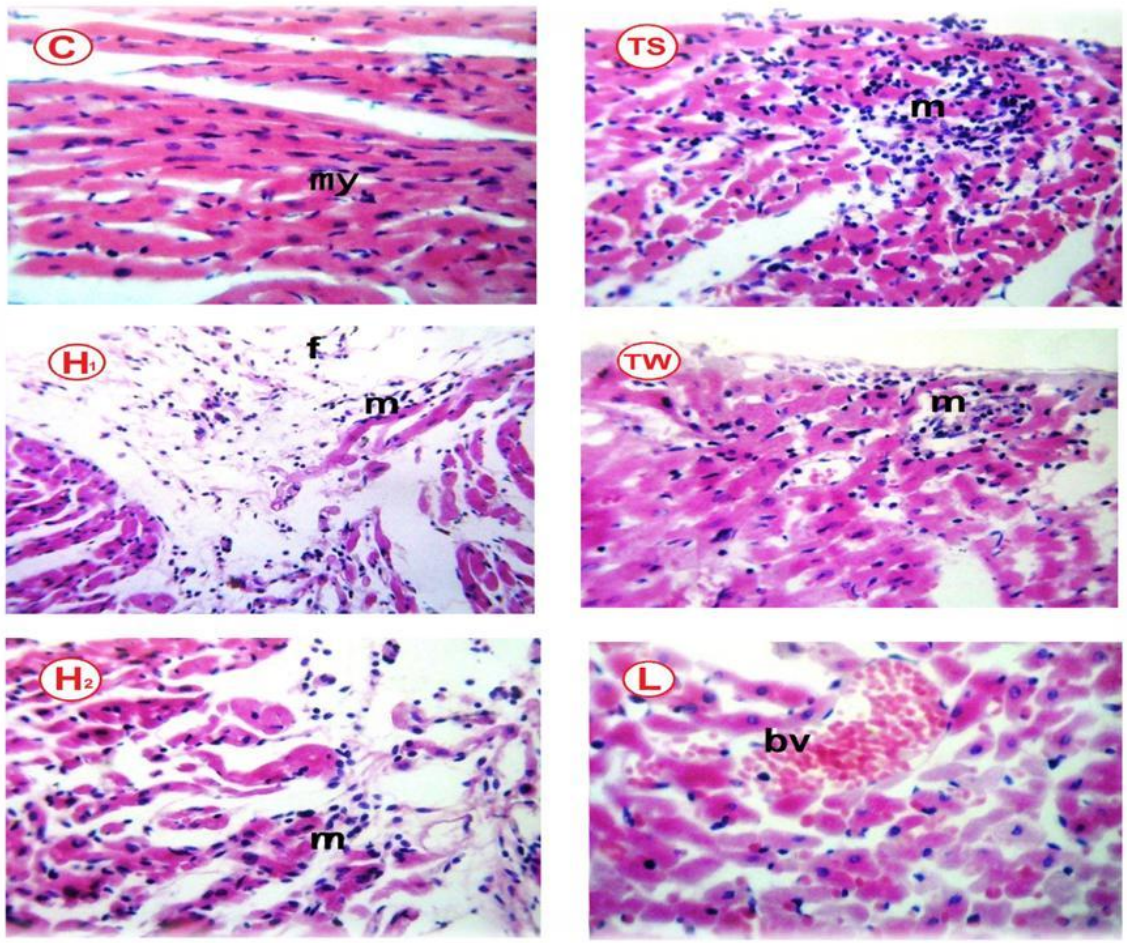

Fig. 1. The effect of ingestion of TS, TW and L on the morphologic changes of the rat heart stained with hematoxylin and eosin using a light microscope (magnification 80×). (C): Rat fed the standard diet (control); (H1,H2): rat fed the high-fat diet; (TS): rat fed the high-fat diet plus 8% lyophilized tomato paste ; (TW): rat fed the high-fat diet plus 24% lyophilized raw tomato; (L): rat fed the high-fat diet plus 0.1% lycopene.

Morphologic changes of rat aorta: Representative aortic sections from each group, stained with hematoxylin and eosin, are shown in Figure 2. The aortic walls in the control rats of group C are seen as smooth and intact, the structures of intima (**i**), medial (**m**), and adventitial layers (**a**) aree clearly distinguishable with no pathologic changes. The aortic walls of the rats in group H, show vacuolization in the tunica media (**m**). In effect this caused a disorganized structure of the superficial tunica media. The aorta of the rat in group TW showed a normal histopathological structure of the intima (**i**), media (**m**) and adventitia (**a**). Examined aortas of rats in group TS show an intact histopathological structure of the intima (**i**), media (**m**) and adventitia (**a**). On the other hand, some of examined sections in the rat aortas of group L showed an intact intima (**i**) with vacuolization in tunica media (**m**). Furthermore, aorta rats of group TW expressed morphologic features similar to that of the control rats in group C. At the same time, the changes seen in the TW rats groups were similar to those in group TS.

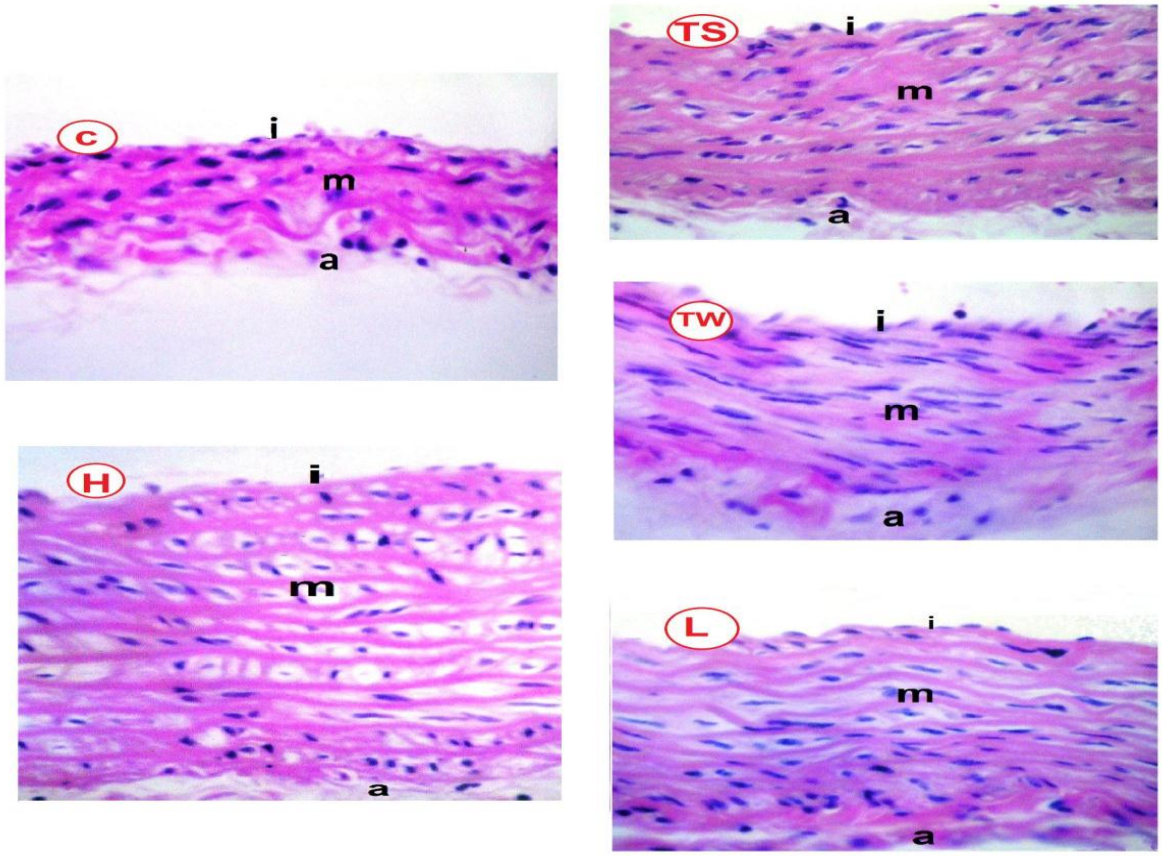

Figure 2. Effect of ingestion of TS, TW and L on the morphologic changes of the rat aorta stained by hematoxylin and eosin using a light microscope (magnification 160×). (C) Rat fed the standard diet (control); (H) rat fed the high-fat diet; (TS) rat fed the high-fat diet plus 8% lyophilized tomato paste; (TW) rat fed the high-fat diet plus 24% lyophilized raw tomato; (L) rat fed the high-fat diet plus 0.1% lycopene.

DISCUSSION:

Lycopene is the pigment that gives certain fruits their red coloring. This hydrocarbon carotenoid is also believed to have strong antioxidant powers, which some studies show can reduce the risk of cardiovascular disease. Besides cardiovascular diseases, recent evidence also points to carotenoids as an effective means for receiving antioxidants which can inhibit the development of diseases such as cancer, cataracts, and macular degeneration. An intake of β-carotene has been inversely linked to incidence of lung cancer. Similarly, and lycopene and tomato-based products have also been inversely correlated with prostate cancer [25, 26].

However, lycopene, being the strongest single oxygen quencher, as well as a potent antioxidant compared to many other carotenoids, has rarely been tested in studies for its unique role in cardiovascular disease prevention [27]. In the past, studies reported an inverse relationship between incidence for degenerative diseases and the consumption of fruits and vegetables. This is in contrast to singling out one single ingredient of these foods. As a result there is only scarce scientific knowledge on the interactions between different food components regarding their protective potential. Likewise, many other important points regarding the bioavailability of lycopene, as well as the molecular mechanisms behind its protective effects, have not yet been completely investigated [28].

In this study, we sought to gain more insight into the effects and mechanism of action of lycopene derived from tomato products. We analyzed weight gain after 8 weeks of exposure to pure lycopene, raw tomato and tomato paste in conjunction with a high-fat diet, and found that there were non-significant differences between group C and group TW. Furthermore, food intake of group TW was significantly higher than group C. These results indicated that the consumption of raw tomatoes may have helped to maintaine normal body weight by decreasing intestinal fat absorption. This in turn, may have led to a decrease in the risk for cardiovascular disease that was seen in these rats. These results have been mentioned and published by Lee *et al* [29].

Having been identified as an independent risk factor for coronary heart disease, plasma lipoprotein (LDL-c) plays a crucial role in inducing atherosclerosis [30]. At the same time, HDL-c levels are inversely related to coronary heart disease [31]. The results of our experiment showed that group TW was the superior in lowering the serum levels of TC, LDL-c and VLDL-c and TG. This data agrees with the other findings which suggest that healthy human subjects who ingested lycopene in the form of tomato juice or tomato paste for a week had a significantly lower level of LDL than controls [3]. Interestingly, TW was shown to have the lower levels of these lipids and the higher levels of HDL-c.This may be due to the lycopene compounds inhibiting the activity of an essential enzyme involved in cholesterol synthesis (macrophage 3-hydroxy-3-methyl glutaryl coenzyme A reductase) [32]. Based on these observations, the dietary supplementation of lycopene, may in fact act as a moderate hypocholesterolemic agent. This agrees with findings of Fuhrman *et al* [33].

Moreover, we found that TW inhibited the increase of serum MDA which was induced by the high-fat diet. In comparison, TS and L presented a lesser effect in this respect. Thus, the consumption of raw tomato proved helpful in facilitating the clearance of free radicals and blocking the oxidative modification of LDL while simultaneously counteracting inflammatory reactions [34]. These findings provide a theoretical rationale for the use of raw tomato as a preventive treatment for atherosclerosis. The higher HDL-c/TC % ratio reflects the lower risk of

CHD, as does the lower LDL-c/HDL-c. This conclusion agrees with the findings of Aviram and Fuhrman [31].

The results of our experiment on rats that were fed a high-fat diet showed that TW had the higher HDL-c /TC % ratio and lower LDL-c/HDL-c rate when compared with all other treatment groups. These changes were significant in comparison to group H. Thus, it was assessed that our findings were in accordance with other similar studies which have reported the dietary intake of tomatoes and tomato products as being associated with a decreased risk of chronic diseases such as cardiovascular disease (Rao and Agarwal) [35].

Furthermore, results suggest a higher potential in TW than TS and L alone in protecting against oxidative stress. Observation indicates that both tomato juice and lycopene reduced the extent of lipid peroxidation. However it is tomato juice not lycopene that possesses cardio-protective ability [36]. Moreover, yellow tomatoes which do not contain lycopene, have a higher potential than pure lycopene to attenuate and/or to reverse oxidative stress-related parameters. The findings of this study are in accordance with this claim by Gitenay *et al* [6]. Explanations for these findings may be considered under two interpretations. The first of which is that the phytochemicals and micronutrients (other than lycopene) of a raw tomato, can act synergistically. The second interpretation is that metabolism of the tomato compounds can lead to many bioactive molecules, called lycopenoids that are identified with inducing such beneficial health effects in vivo and in vitro [6, 37].

Unfortunately, there were several limitations with the current study. Firstly, it was quite a narrow scope of research in that we were not able to analyze tomato products other than raw tomatoes and tomato paste. Other notable tomato products such as ketchup, or cooking sauce would have been valuable to include in our research. On that note, we also did not get to analyze other sources of lycopene such as watermelon, pink grapefruit, apricots, etc. Our study only proved that raw tomatoes are in fact beneficial in alleviating oxidative stress and more so than tomato paste or pure lycopene. In addition, the choice of rats as a model for carotenoid absorption was also another weak point since rats are considered poor absorbers of carotenoids [37, 38]. Furthermore, to determine if our findings are applicable to humans, future studies must evaluate the effects of more reasonably ingestible amounts of raw tomatoes for human subjects.

CONCLUSION:

Our study established that although all three were successful in reducing the extent of lipid peroxidation, freeze-dried raw tomato had a higher protective effect than freeze-dried tomato paste and lycopene alone. . Consequently it was confirmed that tomato feeding alleviates an experimentally induced oxidative stress more than lycopene alone. Thus, because of the positive effects of freeze-dried raw tomatoes on the tested parameters, an increase in the consumption of raw tomatoes in the overall diet is highly recommended with predictive future applications to human clinical trials.

Authors' contribution: Elnashar NN designed the study in addition to collecting and analyzing the data. All the authors listed contributed to the interpretation of the data and reviewed the manuscript. None of the authors had conflicting interests.

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