The Biochemical Role of Pomegranate Peel Extract in Tissues Antioxidants of High Sucrose Stressed Rabbits

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A B S T R A C T

Pomegranate (Pomg) fruit contains many phenolic compounds with high antioxidant activity, which may have useful health properties including flavonoids anthocyanins, flavonoids and hydrolysable tannins (punicalagin). The pomegranate peel includes tannins, gallagic acid ellagic acid and its esters of glucose and flavonoids. Oxidative stress is an imbalance between free radical production and the antioxidant defense system which could lead to lipid, protein and/or DNA alterations in this respect, the possibility exists that sucrose feeding facilitates oxidative damage. For the present study, the strong antioxidant activity of POMG on some antioxidant parameters in liver and heart of male rabbits exposed to high sucrose diet (HSD) induced oxidative stress, were investigated. Eighty (80) male rabbits at age of 4 weeks, weighting 500- 600 g. were used in this study. The rabbits were divided into four equal groups. **1)** Control Normal group: kept on basal ration. **2)** Stressed group (HSD): reared on the 25% sucrose concentration ration, daily for 4 months. **3)** Pomegranate peel extract (PPE) group: reared on 25% pomegranate peel powder ration only, daily for 4 months. **4)** Sucrose + PPE: kept on equal amounts of 25% sucrose and 25% PPE rations, daily for 4 months. Liver and heart samples were collected from all animal groups two times, after 2 and 4 months of experiment period. Levels of reduced glutathione (GSH) and activities of Catalase (CAT) and Glutathione-S-Transferase (GST) were determined in liver and heart tissues. Extent of oxidative stress was also assessed by liver and heart lipid peroxide L-Malondialdehyde (MDA) and Hydrogen peroxide H_2O_2. The obtained results revealed that, HSD supplementation exhibited a significant increase in: 1) Liver and heart MDA and H_2O_2 concentrations. PPE supplementation to stressed rabbits exhibited a significant decrease in the previously mentioned parameters. On contrast, HSD supplementation exhibited a significant decrease in liver and heart GSH, CAT and GST. Supplementation of PPE to stressed rabbits exhibited a significant increase in all mentioned parameters. From the obtained results, it could be concluded that, the potential of POMG as natural antioxidant act as a powerful agent against the harmful effects of sucrose induced oxidative stress.

Keywords: Pomegranate, Sucrose, Lipid peroxide, Oxidative stress.

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1.INTRODUCTION:

Pomegranate is a drought tolerant and long lived plant which grows popularly in arid and semiarid zones in the Mediterranean countries such as Egypt Iran and India (Ercisli et al., 2011). The botanical Family of

pomegranate is Punicaceae Punica is the only gene with predominant species called p.granatum and it is classified as a berry (Newman, 2011).

Pomegranate juice and seed extracts have antioxidant capacity more than thus of red wine or green tea by 2-3 times (Sharrif and Hamed 2012). In elderly humans increasing of plasma antioxidant capacity and scavenging of free radicals and decreasing macrophage oxidative stress and lipid peroxidation in animals are claimed to pomegranate extracts (Rosenblat et al., 2006). Pomegranate leaf extract have antioxidant activity that may be due to the presence of Potent antioxidants in it that are called phytochemicals (flavonoids, tannins, glycosides) (Jurenka, 2008). It is known that the damaged β-cells are regenerated by flavonoids in alloxan-induced diabetic rats (Hakkim et al., 2007).

Hydrolysable tannins are attribuated to nearby 92% of pomegranate antioxidant activity. The main component of pomegranate husk is Punicalagin (Lee et al., 2008). Punicalagin is one of polyphenols of pomegranateare agents that have the ability for restraining ROS effect on the body (Seeram et al., 2005). It is reported that punicalagin have protective activities against inflammation, cancer and atherosclerosis (Lee et al., 2008).

Vos et al ., (2008) indicated that, over 10% of daily calories in sucrose come from its 50% fructose, of which 75% (in adults) and 82% (in children) is attributed to added sweeteners rather than naturally occurring fructose. When D-fructose is joined with a molecule of glucose, is a component of the disaccharide sucrose, higher levels of fructose have been associated with several metabolic disorders in humans and in laboratory animals (Henry et al., 1991).

McDonald (1995) suggested that, the dietary sucrose, as opposed to complex carbohydrates, may have a differential effect on net oxidative stress and that, these differences are reflected in the accumulation of advanced glycation product. It appears that fructose moiety of the sucrose molecule plays a larger role than the glucose moiety. A high-fructose diet stimulates de novo lipogenesis; it increases the hepatic VLDL secretion and decreases the peripheral triglyceride clearance. Also it promotes oxidative damage and exert detrimental effects by reducing antioxidant defenses, and increasing generation of free radicals (Watson et al., 2003).

The oxidative stress linked damage of high fructose consumption in pro-oxidant conditions in rodents. The hypothesis that fructose itself, even in case of sufficient dietary antioxidant supply, could be pro-oxidant (Busserolles et al., 2002b). The hypothesis that the protein could be damaged by oxidative stress and/or glycation reactions has already been proposed , especially in vitro studies which have already shown the fructose capacity to glycate this enzyme (Yan and Harding, 1997).

Fructose has a stronger reducing capacity than glucose and its glycation reaction being easily and usually a long lasting phenomenon and further investigation is needed to ensure the validity of such a side effect of fructose. (Kaneto et al., 1996).

A diet rich in sucrose could alter cellular metabolism via increasing oxidative stress. Indeed, current evidence suggests that dietary sucrose, as opposed to other complex carbohydrates, may have a differential effect on net oxidative stress and that the difference is reflected in the accumulation of advanced glycation products (Maillard reaction), so this oxidative stress and the associated lipid peroxidation could be due to oxygen free

radical production and/or decreased protection from nonenzymatic or enzymatic antioxidants (Sies et al., 2005).

Moreover, Srividhya and Anuradha (2002) reported that increases in the levels of the thiobarbituric acid reactive substances (TBARS) and hydroperoxides were observed in the liver of fructose-fed rats which induce free radical formation by a number of mechanisms. It causes down regulation of the key enzymes of the hexose monophosphate pathway, namely glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase that generate a reduced environment in the form of NADPH and NADH, Impaired regeneration of NADPH could result in an increased oxidative state of the cell.

Aim of the work:

Investigate the biochemical effect of Pomegranate Peel Extract in tissues antioxidants of high sucrose stressed rabbits.

2. Materials and Methods:

2.1. Ration and additives:

Animals were fed on a basal ration throughout 14 days acclimatization period of the experiment in the form of pelleted concentrated ration shown in the following table (1):

1	Carbohydrates	58%	5	Minerals	1.49%
2	Protein	20.%	6	Calcium	0.98%
3	Lipid	3.4%	7	Phosphorus	0.53%
4	Cellulose	3.1%	8	Moisture	12%
Total 100 %					

2.2. Pomegranate peel:

After gathering fresh Pomegranate from fruit farm in Cairo-Alexandria desert road at the region of Borg El-Arab (Egypt), Fresh peel were carefully cleaned and washed by distilled water and dried, then were powdered and passed through mesh to increase their contact with powdered ration.

2.3. Chemical composition of Pomegranate peel: Table (2)

1	Moisture	13.7%	5	Fiber	11.22%
2	Protein	3.10%	6	Total phenolic	27.90%
3	Carbohydrates	39.0%	7	Ash	3.30%
4	Fat	1.73%			
Total 100 %					

Then the powder sent to ration manufacture to be pellet as 25 % of the ration ratio according to Maha (2017) as following: Table (3)

1	Pomegranate peel powder	25%	6	Minerals	1.4%
2	Carbohydrates	38%	7	Calcium	0.9%
3	Protein	15.5%	8	Phosphorus	0.5%
4	Lipid	3.4 %	9	Moisture	12%
5	Cellulose	3.1 %			
Total 100 %					

2.4. Feed additives:

Sucrose is powdered and mixed with ration in 25% concentration of the ration then pelleted according to (Tabbakh, 2014). Table (4)

1	Sucrose	25%	6	Minerals	1.4%
2	Carbohydrates	38%	7	Calcium	0.9%
3	Protein	15.5%	8	Phosphorus	0.5%
4	Lipid	3.4%	9	Moisture	12%
5	Cellulose	3.1%			
Total 100 %					

2.5. Experimental animals:

A total number of eighty (80) male rabbits at age of 4 weeks old after winning and weighting about 500-600 gm, were used in the experimental investigation of this study, and obtained from the Laboratory Animals Research Center, Fac. Vet. Med., Benha University, rabbits were housed in separated metal cages, exposed to good ventilation, humidity and to a 12-hr light-dark cycle, and provided with a constant supply of standard

pellet diet and fresh, clean drinking water ad libitum.

2.6. Experimental design:

Rabbits were allocated into four groups of 20 rabbits in each, placed in individual cages and classified as following: (Group 1): 20 rabbits, served as control normal group, kept on basal ration only for ; (Group 2): 20 rabbits, served as sucrose stressed group, reared on the 25% sucrose concentration ration for 4 months; (Group 3): 20 rabbits, reared on 25% PPE ration only; (Group 4): 20 rabbits, reared on equal amounts of 25% sucrose and PPE rations.

2.7. Sampling:

At end of the 2nd and 4th months of experiment periods, 10 rabbits were slaughtered from each group. They were eviscerated, the abdomen was opened and the liver and heart were rapidly excised gently, rinsed with ice-cold isotonic saline, cleared off blood, photographed and immediately transferred into ice-cold isotonic saline again, then blotted between 2 filter papers and quickly frozen in a deep freezer at (-20 °C) for subsequent biochemical analysis, and molecular investigation.

2.8. Determination of serum biochemical markers:

GSH (Beutler et al., 1963), Catalase (Aebi, 1984), GST (Habig and Jakoby, 1974), L-MDA (Ohkawa, et al., 1979), H_2O_2 (Aebi, 1984).

2.9. Statistical analysis

The results were expressed as mean (±S.E.) and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when $p < 0.05$.

3. RESULTS:

The obtained data in Tables (5,6) revealed that high sucrose ration exhibited a significant decrease in liver and heart GSH concentrations in addition to CAT and GST activities, after 2 and 4 months. On contrast, it exhibited a significant increase in liver L-MDA and H_2O_2 concentrations in sucrose stressed rabbits, after 2 and 4 months, when compared with control normal group.

Administration of pomegranate peel extract as a treatment to high sucrose stressed group, resulted in significant increases in liver and heart GSH concentrations in addition to CAT and GST activities, after 2 and 4 months. On contrast, it exhibited a significant decrease in liver L-MDA and H_2O_2 concentrations, after 2 and 4 months, in comparison with non treated sucrose stressed group.

The obtained data in Tables (5,6) revealed that, supplementation of pomegranate peel powder only to normal rabbits exhibited a significant increase in in liver and heart GSH concentration in addition to CAT and GST activities, after 2 and 4 months. On contrast, it exhibited a non-significant decrease in liver L-MDA and H_2O_2 concentrations, after 2 and 4 months, when compared with control normal group.

Table (5): The effect of pomegranate peel extract on antioxidant and lipid peroxide parameters in *LIVER* tissues of male rabbits exposed to sucrose-induced oxidative stress for 2 and 4 months.

Parameters	Animal groups / Durations	Control	Sucrose Stressed	Pomegranate only	Pomegranate + Sucrose
GSH mg/g. tissue	After 2 months	670.27 ± 47.44^{a}	538.42 ± 10.99^{b}	740.47 ± 23.85^{a}	759.15 ± 36.20^{a}
	After 4 months	636.79 ± 45.06^{a}	500.73 ± 10.22^{b}	689.73 ± 21.70^{a}	675.64 ± 32.21^{a}
Catalase U/g. tissue	After 2 months	293.30 ± 3.00^{a}	240.10 ± 2.60^{c}	266.85 ±2.80^{b}	283.27 ± 1.85^{a}
	After 4 months	278.63 ± 2.85^{a}	241.89 ± 2.41^{c}	242.83 ± 2.54^{b}	252.04 ± 1.64^{a}
GST U/g. tissue	After 2 months	259.42 ± 4.06^{a}	207.00 ± 2.23^{b}	296.32 ± 9.20^{a}	272.47 ± 21.24^{a}
	After 4 months	246.44 ± 3.85^{a}	192.51 ± 2.07^{b}	269.65 ± 8.37^{a}	242.49 ± 18.90^{a}
MDA nmol/g. tissue	After 2 months	3.72 ± 2.25^{c}	31.05 ± 2.27^{a}	24.30 ± 0.97^{ab}	22.95 ± 3.04^{b}
	After 4 months	3.53 ± 2.13^{c}	28.87 ± 2.11^{a}	22.11 ± 0.88^{b}	20.42 ± 2.70^{b}
H_2O_2 mM/g. tissue	After 2 months	11.92 ± 0.72^{b}	41.15 ± 0.94^{a}	11.07 ± 2.16^{b}	12.17 ± 0.58^{b}
	After 4 months	11.32 ± 0.68^{b}	38.26 ± 0.87^{a}	10.07 ± 1.97^{b}	2.79 ± 0.51^{b}

Data are presented as (Mean ± S.E), S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P<0.05$).***** Means a significant difference between periods of treatment ($P<0.05$).

Table (6): The effect of pomegranate peel extract on antioxidant and lipid peroxide parameters in *HEART* tissues of male rabbits exposed to sucrose-induced oxidative stress for 2 and 4 months.

Parameters	Animal groups / Durations	Control	Sucrose Stressed	Pomegranate only	Pomegranate + Sucrose
GSH mg/g. tissue	After 2 months	32.62 ± 1.17^{b}	26.77 ± 0.76^{c}	41.85 ±1.18^{a}	38.70 ±1.32^{a}
	After 4 months	30.98 ±1.11^{b}	24.89 ±0.70^{c}	38.08 ±1.07^{a}	34.44 ±1.17^{a}
Catalase U/g. tissue	After 2 months	72.00 ± 2.87^{a}	54.67 ± 1.48^{b}	63.22 ±0.76^{a}	64.80 ±4.32^{a}
	After 4 months	68.40 ±2.72^{a}	50.84 ±11.37^{b}	57.53 ±0.69^{a}	57.67 ±3.84^{a}
GST U/g. tissue	After 2 months	135.55 ± 3.93^{a}	109.12 ±3.84^{b}	135.45 ±3.42^{a}	117.90 ±3.69^{b}
	After 4 months	128.77 ±3.73^{a}	101.48 ±3.57^{b}	123.25 ±3.11^{a}	104.94 ±3.28^{b}
MDA nmol/g. tissue	After 2 months	622.57 ±18.34^{b}	715.50 ±6.26^{a}	535.72 ±13.17^{c}	681.75 ±7.68^{a}
	After 4 months	591.44 ±17.42^{b}	665.41 ±5.82^{a}	487.50 ±11.98^{c}	606.75 ±6.83^{a}
H_2O_2 mM/g. tissue	After 2 months	0.70 ± 0.05^{b}	0.94 ± 0.05^{a}	0.74 ± 0.04^{b}	0.72 ±0.05^{b}
	After 4 months	0.66 ±0.04^{b}	0.87 ±0.04^{a}	0.67 ±0.03^{b}	0.64 ±0.04^{b}

Data are presented as (Mean ± S.E), S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P<0.05$).***** Means a significant difference between periods of treatment ($P<0.05$).

4. DISCUSSION:

The major target of this research was to evaluate the biochemical benefit of pomegranate peel powder supplementation on sucrose induced oxidative stress of liver and heart antioxidant parameters of male rabbits. This study investigated the ability of pomegranate to counteract sucrose induced oxidative stress and lipid peroxidation in rabbits.

The results presented in Table (5) demonstrated that the rabbits kept on sucrose high diet possess the highest values of hepatic MDA, while it possesses the lowest levels of hepatic GSH, Catalase and GST. Most of these criteria were greatly improved to these in control group after adding of Pomegranate peel to sucrose. These results are confirmed by Nandhini et al., (2005) who reported that, male wister rats received a fructose-enriched diet (greater than 60 percent of total calories for 30 days), resulted in increased lipid peroxidation and impaired antioxidant status. Moreover, Srividhya and Anuradha (2002)

reported that increases in the levels of the thiobarbituric acid-reactive substances (TBARS) and hydroperoxides were observed in the liver of fructose-fed rats.

Fructose feeding can induce free radical formation by a number of mechanisms. It causes down regulation of the key enzymes of the hexose monophosphate pathway, namely glucose-6-phosphate dehydrogenase and 6- phosphogluconate dehydrogenase that generate a reduced environment in the form of NADPH and NADH, impaired regeneration of NADPH could result in an increased oxidative state of the cell (Srividhya and Anuradha 2002).

Huang et al; (2011) mentioned that, increased extracellular oleate concentrations in the fructose-treated liver cells, indicates that, in addition to increasing de-novo hepatic fatty acid synthesis, fructose-treatment also leads to enhanced triglyceride secretion, as when triglycerides are assembled for release, they require oleate to provide fluidity. The combination of increased fatty acid synthesis and triglyceride release provides novel insight into the hepatic steatosis that has been demonstrated in animal models following dietary fructose and in humans where fructose intake correlates with prevalence of fatty liver (Watt 2008).

The obtained data were agree with El-Hafidi et al., (2011) who reported that, the excess H_2O_2 in the intermembrane space may cross the external mitochondrial membrane and affect the redox state of the entire liver by decreasing the concentration of reduced GSH. Additionally, the reduced activity of catalase observed in the SFR liver homogenate may contribute to increased levels of lipid peroxidation and protein carbonylation in whole liver cells.

Moreover, Bialonska, et al (2010): reported that, ethanol-induced oxidative stress and lipid peroxidation possibly by two pathways; First, by rapid conversion of H_2O_2 to H_2O and preventing H_2O_2 accumulation and second, by quenching the hydroxyl radicals in which trapping HO° leads to oxidative breakdown of the phenolic compounds (Lan, 2009). Indeed, Pomegranate peel has been shown to be scavengers of superoxide anions, and inhibitors of the respiratory burst of neutrophils and hypochlorous acid-derived radicals (Seeram et al 2005).

Table 6 reveals that, the highest values of GSH were noticed in rabbits kept on Pomegranate peel plus sucrose while the lowest values were observed in those kept on sucrose. On the other hand, the highest values of MDA was recorded in sucrose fed group, while the same group possess the lowest values of Catalase and GST and the feed of Pomegranate peel success for extent in increasing the values of their two enzymes. These result were agree with study stated that, increased production of ROS-mediated NO inactivation has been suggested in several models of rats fed a high fructose diet for 8 weeks (El- Gharawi 2018). The obtained result was supported by Kaplan, et al (2001) who reported that the antioxidant enzyme CAT is known to be inhibited in diabetes mellitus as a result of non-enzymatic glycosylation and oxidation. In addition, Pomegranate peel is able to chelate metal ions, such as Cu^{2+} and Fe^{2+}, which catalyse free radical generation reactions it also possess the structural requirement (a catechol group) needed for optimum antioxidant and/or scavenging activity (Seyedeh et al, 2017), As demonstrated by Maha, (2017) who reported that pomegranate peel has a positive impact on these enzymes observed could be explained with two possible mechanisms. Firstly their

antioxidative effects which may prevent further glycosylation and peroxidation of proteins by interacting with free radicals and hence minimizing their noxious effects. The high content of Pomegranate peel may induce protein synthesis of these enzymes that, explains the observed elevated activities of these enzymes as provide by Wang, et al., (2013) who found that, Pomegranate peel increased the expression of glutathione-related enzymes at the transcriptional level. It was shown that total olive leaves extract had antioxidant activity higher than vitamin C and vitamin E, due to the synergy between flavonoids, oleuropeosides and substituted phenols (Seeram, et al 2005)

Moreover, Pomegranate peel inhibited superoxide anion-mediated impairment of endothelium , improved endothelium-dependent relaxation in rat aorta , inhibited angiotensin- converting enzyme and nuclear factor κ light chain enhancer of activated B cell activation, and decreased interleukin-6 and tumor necrosis factor- α a release both in vitro and in vivo (Maha ,2017).

It is well known that oxidative stress is one of the main factors that link hypercholesterolemia with atherosclerosis (Young and Eneny, 2001). Hyper-cholesterolemia leads to increased cholesterol accumulation in cells thereby activating the production of reactive oxygen species (ROS), and coronary vessels are extremely vulnerable to these oxidative challenges and hypercholesterolemia (Kay et al., 1991).

5. CONCLUSIONS AND RECOMMENDATIONS:

In conclusion, the present study demonstrated that pomegranate provided an antioxidant effect against oxidative stress induced by sucrose stress in rabbits, since this compound was able to ameliorate serum biochemical parameters. We recommended the great therapeutic effects of pomegranate by its administration for patients suffering from hyperlipidemia.

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