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Full Length Research Paper

Tomato lycopene is a natural antioxidant and can alleviate hypercholesterolemia

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Lycopene of tomato wastes was extracted and determination. The level of tomato lycopene was 145.50 ppm. An aliquots of the concentrated tomato lycopene, represent 100, 200, 400 and 800 ppm; grade lycopene (200 ppm) and butylated hydroxyl toluene (BHT, 200 ppm) were investigated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. These compounds were administered to rats fed on hypercholestrolemic diet daily form 10 weeks by stomach tube. Serum lipid contents (total lipids, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol), oxidative biomarkers (glutathione peroxidase and malonaldhyde), the liver (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities) and kidney (uric acid, urea and creatinine) function testes were measured to assess the safety limits of the lycopene in tomato wastes. The data of the aforementioned measurements indicated that the administration of tomato lycopene did not cause any changes in liver and kidney functions. On the contrary, rats fed on hyperchoestrolemic diet induced significant increases in the enzyme activities and the serum levels of total lipids, total cholesterol, low and high density lipoprotein and decreased levels of the glutathione peroxidase and malonaldhyde.

Key words: Lycopene, antioxidant activity, serum analysis, oxidative biomarkers.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most popular of vegetables, used as a salad, in food pre-parations and as juice, soup, puree, ketchup or paste. Lycopene is the principle carotenoid, causing the characteristic red hue of tomatoes (Clinton, 1998; Shi and Le-Maguer, 2000). Tomato lycopene content varies considerably, reflecting the influence of variety (generally genetic factors), maturity, and both agronomic and environmental conditions during growing (George et al., 2004; Kaur et al., 2006).

Commercial processing of tomato produces a large amount of waste at various stages. Tomato pomace con-stitutes the major part of the waste that comes from the pulper. The wet pomace contains 33% seed, 27% skin and 40% pulp while the dried pomace contain 44% seed and 56% pulp and skin (Sogi and Bawa, 1998). Pomace consists of skin that could be utilized for extracting lycopene (Kaur et al., 2008).

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Lycopene is a lipobhilic, 40-carbom atom, and highly unsaturated, straight open chain hydrocarbon containing 11 conjugated and 2 non conjugated double bonds (Rao and Agarwal, 2000; Shi, 2000). The all trans isomer of lycopene is the most thermodunamically stable form (Livny et al., 2002) . During chemical reactions, light or thermoenergy, food processing and storage these bonds can undergo isomeriation form trans to mono or poly- cis isomers and the most commonly identified are all trans, 5-cis, 13-cis and 15- cis isomeric forms of lycopenel (Tapiero et al., 2004; Omoni and Aluko, 2005). 5-cis isomer of lycopene is more stable than the all- trans isomer (chasse et al., 2001). The many conjugated double bounds of lycopene make it a potentially powerful antioxidant (Arab and Steck, 2000), a characteristic believed to be responsible for its beneficial effects. The antioxidant activity of lycopene is high lighted by its singlet oxygen quenching property and its ability to trap peroxyl radicals (Kuhad et al., 2008). This singlet quenching ability of lycopene is twice as high as that of - carotene and 10 times higher than that of - tocopherol and butylated hydroxyl toluene (BHT) (Agarwal and Rao,

2000; Basuny et al., 2006). Lycopene is also a potent neuroprotective (Hisao et al., 2004), antiproliferative, anti-cancer (Gunasekera et al., 2007), antiflammatory, cogntion enhancer (Akboraly et al., 2007) and hypocholesterolemic agent (Riso et al., 2006). Lycopene also modulates cyclo-oxygenase synthesis pathway (Sengupta et al., 2006) and reduces mutagenesis in the Ames test (Matulka et al., 2004). Lycopene has been under considerable investigation for its antioxidant benefits in treating various chronic human diseases like cancer, cardiovascular diseases, osteoporosis and diabetes (Rao, 2007). The purpose of this study was designed to investigate the antioxidant activity of tomato lycopene using the 2,2-diphenyl-1-picrylhydazyl (DPPH) free radical scavenging capacity assay and its effect on hypercholesterolmic of rats and measurements of rat serum constituents such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) activates and the serum levels of total lipids, total cholesterol, high and low density lipoproteins, creatinine, urea and uric acid. Also, oxidative biomarkers (glutathione peroxidase and malonaldhyde) assay was conducted.

MATERIALS AND METHODS

Source of tomato waste

Tomato waste obtained from tomato processing line, Food Technology Research, Agric. Res. Center, Giza, Egypt.

Solvent, standard and reagent kits

Grade lycopene, DPPH free radical were purchased from Sigma Co. (Oakiville, Canada), BHT was purchased from Sigma Co. (St. Louis, Mo 63178, USA). Total cholesterol, HDL-cholesterol, LDL-cholesterol, total lipids, alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutathione peroxidase and malonaldhyde bis kits were obtained from Randox Laboratories Ltd, England.

Extraction and determination of lycopene

Lycopene was extracted from tomato waste according the method described by Chen and Tang (1998) . The color was measured in a 1 cm cell at 503 nm using petroleum ether as blank.

DPPH free radical-scavenging capacity assay

The radical-scavenging capacity was determined according to the procedure of Mensor et al. (2001) with slight modifications. To measure the scavenging capacity of a single antioxidant or a mixture of antioxidants, the required amount of antioxidant was pipetted into a cuvette, and then ethanol and hexane were added to maintain a constant ethanol–hexane ratio in the final reaction mixtures. These antioxidant mixtures were subjected to pre incubation at room temperature for 5 min in the dark. To start the reaction, 1 mL of DPPH in ethanol (0.3 mmol/L, stored at 30°C) was added to 2 mL of the antioxidant mixture in a teflon capped cuvette at room temperature. The mixtures were shaken quickly, placed into the cell holder and the absorbance was measured at 540 nm with a UV spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer UV-60). Each cuvette was removed from the spectrophotometer and incubated at room temperature. To make sure that they were not exposed to light, the cuvettes were covered with an opaque container. Absorbance at 540 nm was measured every 10 min over a 60 min time period.

Animal experimental

A total of fifty six Albinos male rats were raised the animal house of faculty of Agriculture, Cairo Universty, Giza, Egypt. The animals were fed on a basal diet for 7 days as an adaptation period. The basal diet was formulated according to A.O.A.C. method (2005) and consisted of casein (15%), corn oil (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%) and starch (65%). Water was available *ad libitum*. Known volumes of the concentrated tomato lycopene was dissolved in a mixture of distilled water and tween 20 (12: 1, v/v) to obtain lycopene concentrations of 100, 200, 400 and 800 ppm. BHT solution (200 ppm) was prepared exactly as mentioned above for tomato lycopene. The rats were divided into seven groups and each group comprised eight rats. The first group presents the control rats and the rats of six groups were allowed to feed on hypercholestrolemic diet (1% cholesterol + 0.2 bile salts) to induce hypercholesterolemia through the feeding period. Groups were given 100, 200, 400 and 800 ppm of tomato lycopene, respectively. The sixth and seventh groups were given grade lycopene (200 ppm) and BHT (200 ppm). Each rat group was stomach ingested by gavage daily (1 ml) from the tomato lycopene, grade lycopene and BHT solution for 10 weeks.

Blood samples were taken at the start of the experiment and after 10 weeks of the administration of the tomato lycopene, grade lycopene and BHT solution. The blood samples were obtained from orbital plexus venus by means of fine capillary glass tubes according to the method described by Schermer, (1967). It was not possible to collect 10 ml blood from a single rat, hence the blood of eight rats in each group was pooled. The blood samples were placed in dray and clean centrifuge tubes and allowed to clot for 1 - 2 h at room temperature. Sera were then removed using a Pasteur pipette and centrifuged for 20 min at 1100 g. The clean supernatant sera were then kept frozen until analysis.

Measurements of biochemical variables

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) activites were measured according to the methods described by Bergmyer and Harder (1986), Kachmar and Moss (1976) and Varley et al. (1980), respectively. Urea, uric acid and creatinine were determined according to the methods described by Doumons et al. (1987), Fawcett and Soctt (1960) and Barham and Trinder (1972), respectively. The levels of serum cholesterol, low and high density lipoproteins, and total lipids were determined according to the methods outlined by Roechlau et al. (1974), Assmann (1979) and Frings and Dunn (1979). Glutathione peroxidase activity and malonaldehyde were determined according to the methods described by Hu (1994) and Jentzsch et al. (1996). Each determination was carried out in triplicate and the mean values are presented in the text.

Statistical analysis

The present data were subjected to analysis of variance and the least significant difference test was calculated to allow comparison between the average values of the studied factors (Snedecor and Cochran, 1973).

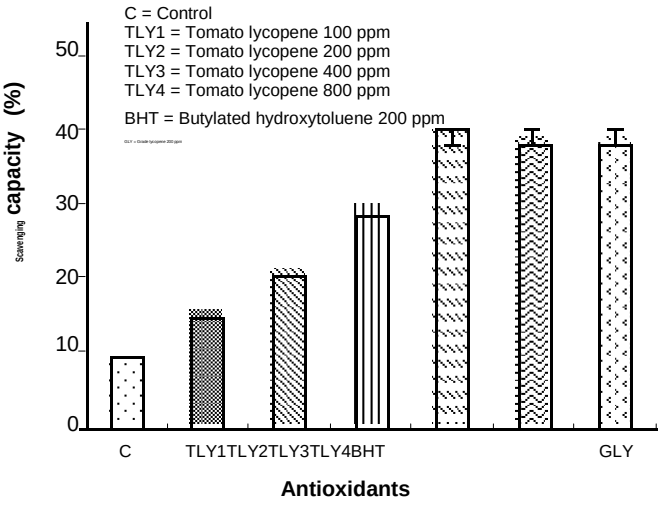

Figure 1. Scavenging capacity of DPPH free radicals by tomato lycopene, grade lycopene and BHT.

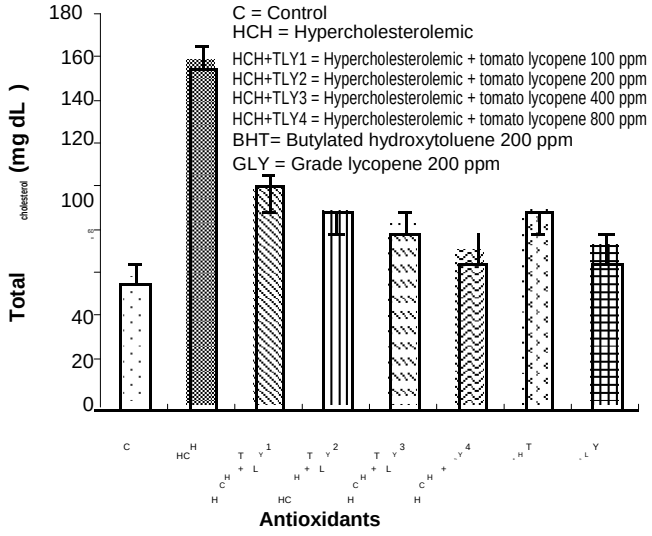

Figure 2. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of sera total cholesterol.

RESULTS AND DISCUSSION

Analysis of fresh tomato waste

Analysis of fresh tomato waste indicated that the lycopene content was 145.5 ppm. The changes in scavenging capacity of the lycopene antioxidant measured with the hydrophobic DPPH free radical is shown in Figure 1. Four levels of the concentrations of tomato lycopene were used tomato lycopene with a very high scavenging capacity of 40.00 after only 10 min. In all cases the scavenging capacity did not increase after the first 10 min of incubation. The reaction of grade lycopene and BHT with DPPH were similar to tomato lycopene with

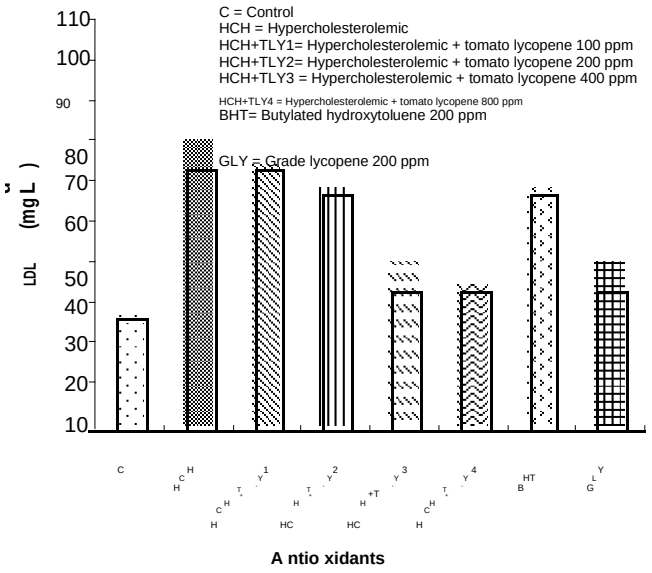

Figure 3. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum LDL.

DPPH; the scavenging capacities were similar.

Effect of tomato lycopene on serum lipids

Effect of tomato lycopene, grade lycopene and synthetic antioxidant (BHT) on serum lipoprotein cholesterol and total lipids of hyperchlosterolemic rats was studied and the results are shown in Figure 2. Total cholesterol of rats fed with high fat diet plus cholesterol was in high concentration (160.30 mg/dl). The groups of rats fed with high fat diet and given tomato orally in different concentration (100, 200, 400 and 800 ppm) induced significant decreases in serum total cholesterol. In addition, rats fed with high fat diet with grade lycopene and BHT caused significant decreases in serum cholesterol, indicating that lycopene can lower the concentration of serum total cholesterol.

Tomato lycopene could prevent an increase in total and LDL serum cholesterol in high cholesterol fed rats (Figure 3) as also reported by (Kuhad et al., 2001). These data are in agreement with Carrapeiro et al. (2007). The lowest level of HDL was in rats fed high fat diet with cholesterol (Figure 4). Groups of rats fed high fat diet with tomato lycopene recorded the highest concentration of HDL. Also, groups of rats which had grade lycopene or BHT (200 ppm) recorded the highest concentration of HDL.

Regarding total lipids of serum for different groups of rats, results in Figure 5 shows that the total lipids of group 2 rats fed high fat diet plus cholesterol was statistically higher (685.00 mg/dl). This low level of serum total lipids for other groups indicated the effect of tomato lycopene, grade lycopene and BHT in lowering lipid in rats blood.

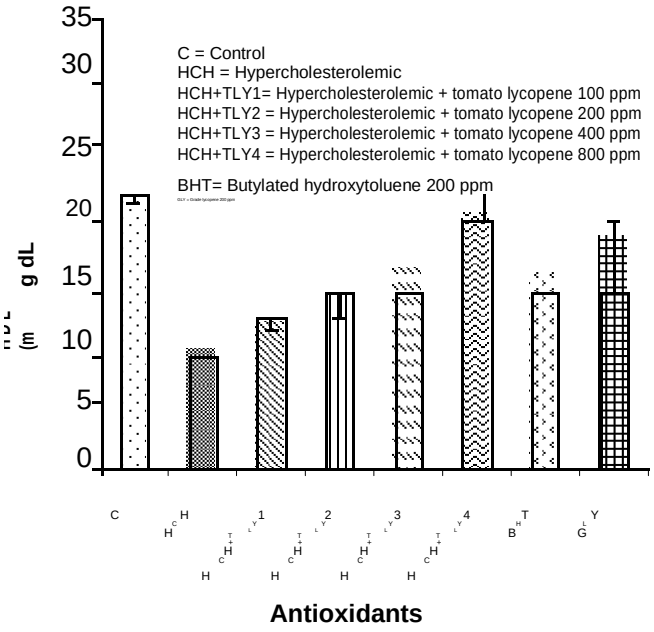

Figure 4. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum HDL.

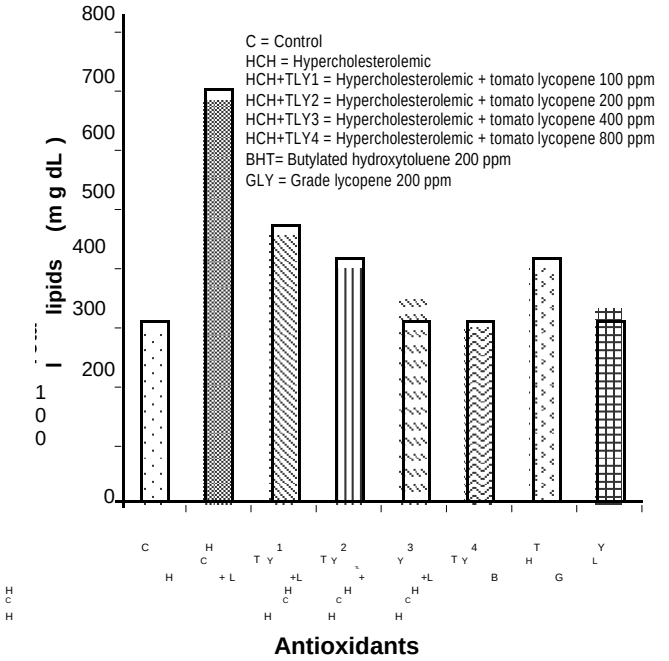

Figure 5. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum total lipid.

Glutathione peroxidase activity

The activity of glutathione peroxidase (GSH-px) enzyme in blood of different groups of rats fed with control diet or high fat diet with or without tomato lycopene, grade lycopene or BHT at different concentrations was measured and the results are Figure 6. The lowest value was found to be with group 2 which had high fat diet with cholesterol and which recorded only 25.00 U/ml. Meanwhile, this value was increased up to 63.10 U/ml for group 1 of rats which were fed basal diet. Moreover, the group of rats fed with high fat diet containing cholesterol and had tomato lycopene, grade lycopene or BHT by stomach tube at different concentration showed higher GSH-px activates than group 2. In this concentration, results show that, as the concentration of tomato lycopene increased the activity of GSH-px enzyme increased.

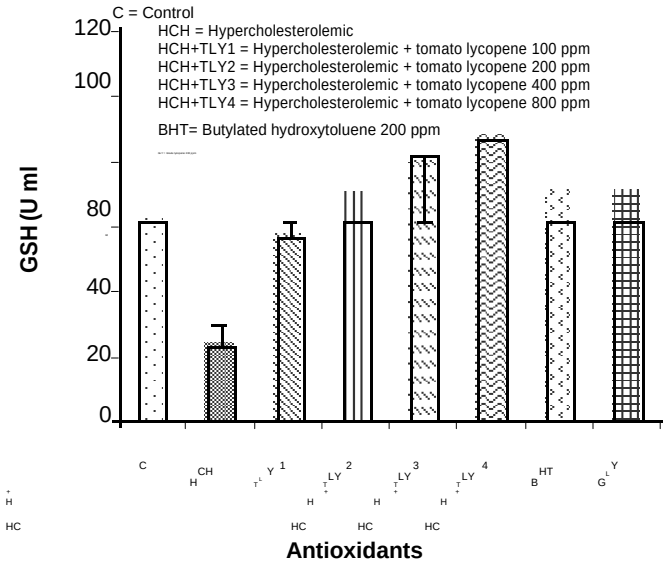

Figure 6. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum GSH.

Lipid peroxidation levels (malonaldehyde MDA)

Free radical-mediated lipid peroxidation has been implicated in a variety of pathological processes, especially in both the ignition and promotion of atherosclerosis (Kehrer, 1993). So, free radical involvement in such pathological stats occurs whenever a disturbance in the pro-oxidant – antioxidant balances is in flavor of the former, leading to potential damage. Increased superoxide anion (O_2) production in hypercholesterolemic contributes to the atherosclerotic process (Ohara et al., 1993). In this connection, it has been reported that hyperchlestrolemic atherosclerosis is associated with increased tissue content of a lipid peroixdation product, MDA. The effect of tomato lycopene at different concentrations (100, 200, 400 and 800 ppm) on lipid peroxidation as MDA in the blood of rats which fed a high fat diet with cholesterol was investigated and compared with the control group “basal diet” results are shown in Figure 7. The highest values of MDA were located for group 2 of rats which were feed high fat with cholesterol

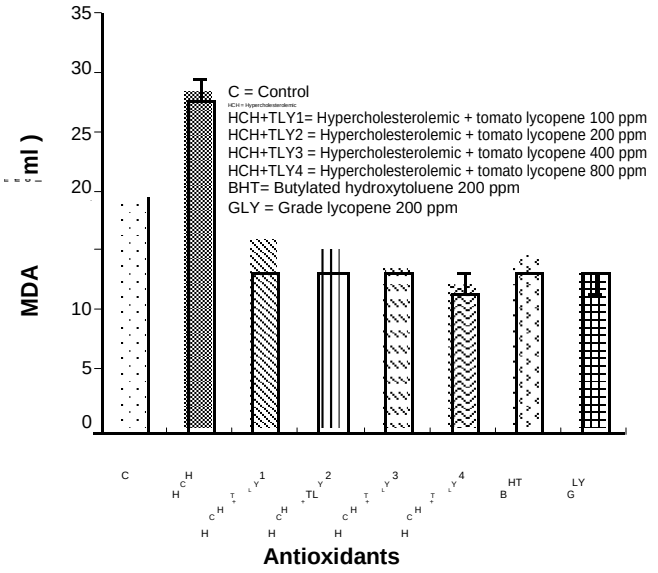

Figure 7. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum MDA.

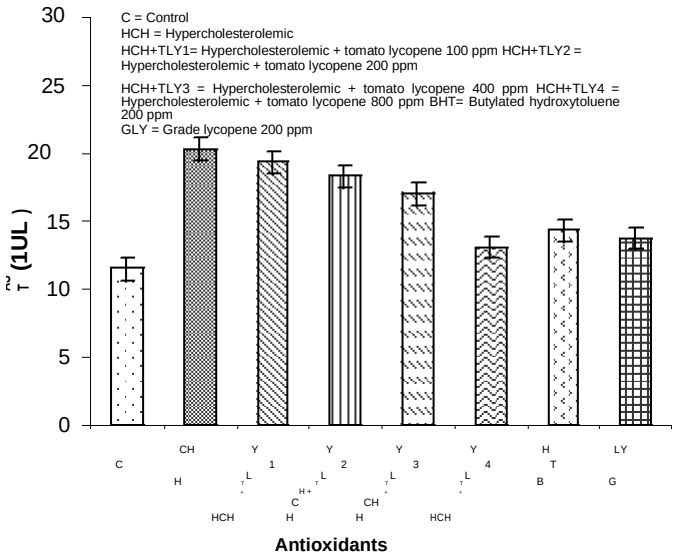

Figure 9. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum AST.

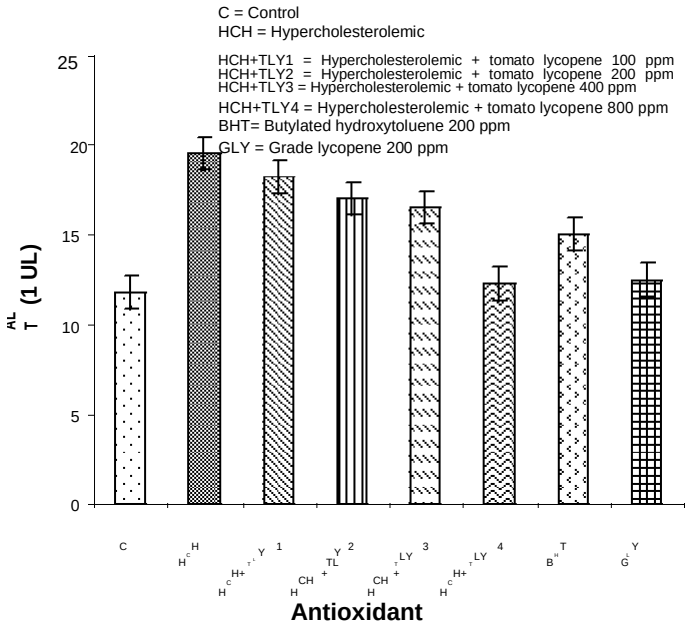

Figure 8. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum ALT.

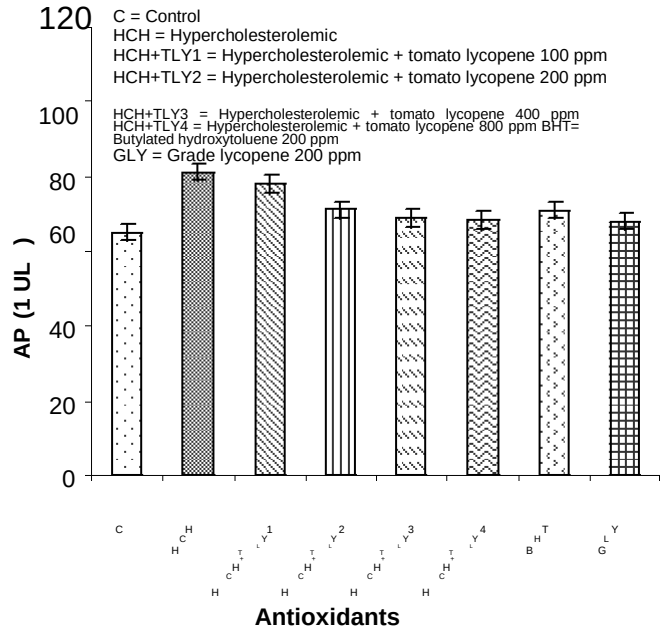

Figure 10. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum AP.

(28.50 nmol/ml). The group of rats fed with basal diet had lower value (19.30 nmol/ml) than group 2 which reflect the occurrence of more oxidation into the blood of group 2 of rats than group 1. On the other hand, the other groups of rats which fed high fat diet with tomato lycopene, grade lycopene or BHT were in less values of MDA, which indicate the importance of these antioxidants in lowering lipid peroxidation agents. This could indicate the ability of tomato lycopene as a natural antioxidant to reduce the lipid oxidation with the same efficiency of synthetic antioxidant (BHT).

Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities

Figures 8, 9 and 10 show the activities of ALT, AST and AP for control rats and the values were slightly increased

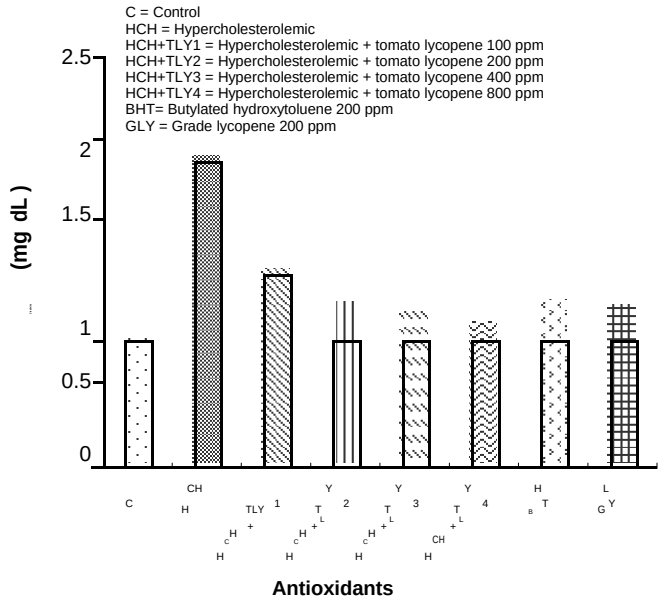

Figure 11. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum creatinine.

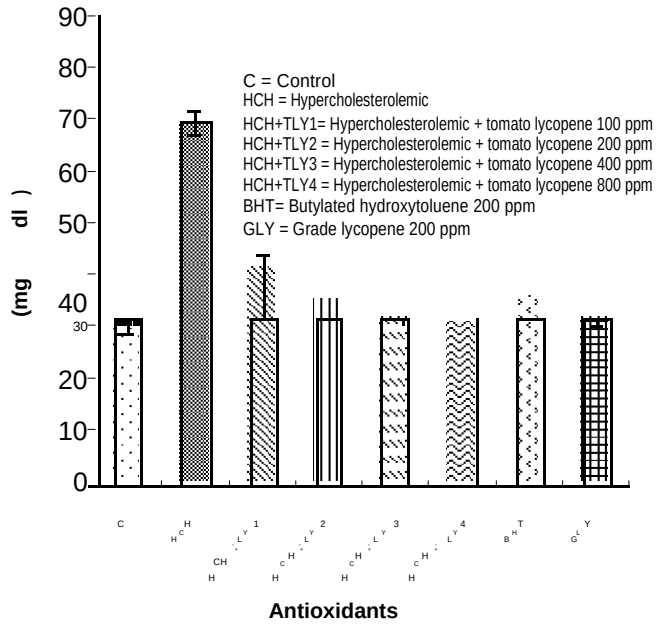

Figure 12. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum urea.

during the whole experiment. The administration of the high fat and cholesterol (group 2) induced significant increases in serum ALT, AST and AP activities. The administration of tomato lycopene at 100, 200, 400 and 800 ppm, grad lycopene 200 ppm and BHT at 200 ppm cause significant decreases in enzyme activities compared with the group 2. AST and ALT have long been

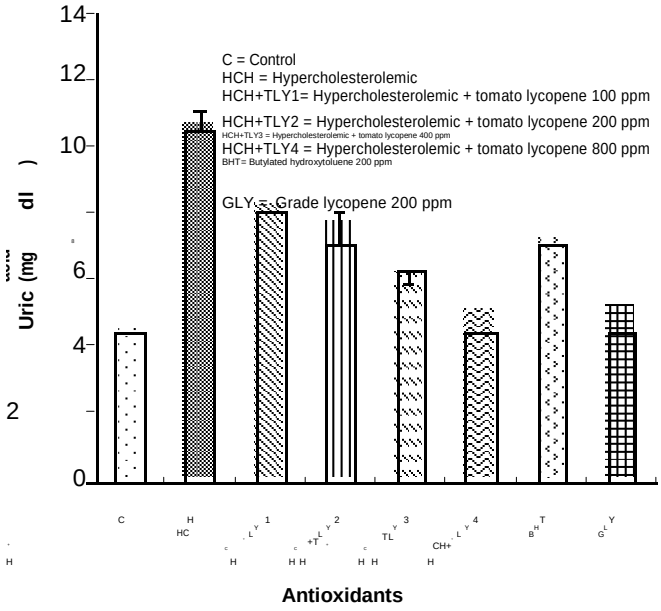

Figure 13. Effect of tomato lycopene, grade lycopene and BHT supplementation on the concentration of serum uric acid.

used as sensitive indicators of liver disease in humans and have regarded as being virtually liver specific (Wills, 1985). In this situation, Zamora et al. (1991) reported that lycopene could significantly decrease the serum level of ALT.

Serum uric acid, Urea and creatinine

Figures 11, 12 and 13 show the levels of uric acid, urea and creatinine for the control rats, rats administered high fat with cholesterol and rats administered various concentrations of tomato lycopene, grade lycopene and BHT. The results show significant decrease in the levels of uric acid, urea and creatinine. On the contrary, group 2 (hypercholesterolemia) exhibited increase on the levels of rat serum, uric acid, urea and creatinine compared with group 1 (control).

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