Mega-dose Vitamin C: Is it Harmful to the Liver? Biochemical and Histological Study in Rats

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ABSTRACT

Aim: To investigate if mega-doses of vitamin C would have deleterious effects on the liver in an animal model.
Methods: A mega-dose of vitamin C (1000 mg/kg/day) was administered by oral gavage to male Wistar rats for 60 days. Both biochemical and histopathological measures were undertaken.
Results: The results showed that a mega-dose of vitamin C significantly elevated lipid peroxidation and transaminase activity level in addition to the significant suppression of antioxidant enzymes activities. These results were consistent with the presence of histological lesions.
Conclusion: A mega-dose of vitamin C is not safe and can cause liver injury.
Keywords: Mega-dose, vitamin C, liver lesions.

INTRODUCTION

Vitamin C (Ascorbic Acid) is a powerful water soluble antioxidant and reducing agent.[1] It is an essential vitamin that is not synthesized by the human body. It is obtained mainly from a diet of fruits and vegetables.[2] The intake of one cup of orange juice, one cup of strawberries or half a cup of red peppers can provide more than twice the current Dietary Reference Intake (DRI) for vitamin C (75 mg per day for women and 90 mg per day for men).[3] In addition, vitamin C is an over-the-counter drug that is intensively advertised as a health promoter. It is available to the public in doses beyond the current DRI.

As an electron donor, vitamin C acts as a cofactor for several enzymes that are involved in hydroxylation reactions including collagen synthesis, production of certain hormones, neurotransmitters, and bile salts. Besides that, ascorbic acid has an important role in maintaining a proper immune system. [2, 4]

Vitamin C has been implicated in a wide range of related and unrelated aspects of health and disease. Considerable information has been presented during the last thirty to forty years describing the beneficial effect of a high dose of vitamin C (200 mg to 10 gm). There is a strong supported movement for using large doses of vitamin C in an attempt to obtain specific therapeutic effects. Several researchers have used high doses of vitamin C in the treatment of cancer[5], common cold[6-9], heart diseases[10], acute pancreatitis[11], Helicobacter pylori infection[12] and wound healing[13].

On the other hand, possible potential harmful effects of a high dose of vitamin C may also be present. The combination of chronic psycho-social stress and vitamin C causes liver injury that is correlated with the presence of histological lesions.[4] The effects of chronic stress and vitamin C on the liver were dependent on the dosing level of vitamin C, where only high dose vitamin C (500

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mg/kg/day) was associated with liver injury during chronic stress conditions. We believe that reduced blood flow to the liver caused by exaggerated levels of stress hormones in relation to both chronic stress and high dose vitamin C is a possible mechanism. High intake of vitamin C (500 mg/day) by humans and rats also been found to exhibit a pro-oxidant activity that is associated with the production of a reactive oxygen species (ROS) or an inhibiting antioxidant system. [14-17] A dose of one gram of vitamin C a day or higher was reported to increase the risk of kidney stones[18, 19], reduce plasma ceruloplasmin levels similar to those caused by copper deficiency[20], impair bactericidal activity[21], and cause gastrointestinal symptoms such as stomach upset and diarrhea[22, 23]. Vitamin C is an essential nutrient that is required by humans in a small amount, but conclusive medical evidence for the use and safety of a mega-dose of vitamin C in treatment of specific conditions is still controversial. A pharmacologic dose of vitamin C (approximately 0.25-0.5mg/gm of body weight) shows no apparent adverse effects when patients are properly screened for normal renal function and absence of glucose-6-phosphate dehydrogenase deficiency, iron overload, and oxalate nephropathy. [24, 25] However, other studies showed that a mega-dose (dose well beyond the current DRI) of antioxidant vitamins impairs myocardial perfusion and coronary endothelial function as vitamins shift from antioxidant to pro-oxidant activity.[26]

Based on our previous findings and the limited data that is available on the effects of the chronic use of mega-doses of vitamin C on the liver under normal conditions, this research was undertaken to test the hypothesis that chronic use of mega doses of vitamin C would have deleterious effects on liver.

Materials and Methods

Animals

Adult male Wistar rats weighing 120-150 g were housed in metal cages at 23^0C and fed *ad libitum* control pellet diet that contains barley 50%, corn 21%, soya bean meal 20%, concentrated (crude) protein 7.0%, wheat bran 0.5%, salt 0.5%, and vitamins and minerals 1%. All animals were maintained on a 12/12 hour light/dark cycle (lights on at 8 am). They were allowed two weeks to acclimate before experimental manipulations began. Experiments were performed between 8 am and 5pm. The research was approved by the Animal Care and Use Committee of Jordan University of Science and Technology.

Rats in the experiment group (six rats) were supplemented with 1000mg of vitamin C/kg body weight daily by oral gavage for 60 days, while the control group were supplemented with distilled water.

At the end of the experiment, the animals were sacrificed at 9 am by deep anesthesia after an overnight fast. Blood was obtained by external heart puncture, and centrifuged at 3000 rpm to obtain serum. The serum was stored at –18°C until analysis. Livers were removed, and 3–4 small pieces were fixed immediately in 10% formalin solution for 24 hours and processed routinely for histological examination. Examinations were performed by a pathologist who had no prior knowledge of the treatment groups.

Biochemical Parameters

Lipid Peroxidation

Malondialdehyde (MDA), the index of lipid peroxidation, was measured in ten percent of 1.15% KCl liver homogenate according to the method of Mihara and Uchiyama.[27] MDA reacts in an acidic media with thiobarbituric acid (TBA) to produce a pink color, which is measured spectrophotometrically at 535 nm. The results were not calculated as a concentration of MDA but were presented in the form of absorbance, since other products of lipid peroxidation which react with TBA absorb light in a similar wavelength.

Antioxidant Enzymes

Ten percent of liver homogenate was prepared by homogenizing the liver tissue in ice-cold 1.15% KCI after washing liver slices three times with ice-cold phosphate buffer saline to remove blood. The homogenate was then centrifuged at 14000 rpm (18620 X g) at 4°C for 10 minutes. The supernatant was used for measuring the antioxidant enzymes activities (superoxide dismutase

(SOD), catalase (CAT), glutathione peroxidase (GSHpx)). Antioxidant enzymes activities were measured as described previously.[28] In brief, SOD activity was measured based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide. One unit of the SOD activity is defined as the amount of enzyme causing half the maximum inhibition of NBT reduction (units/mg protein). CAT catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. The enzyme activity was followed a decrease in absorbance at 240 nm at 15 second intervals. One unit of CAT activity is defined as the one micromole of H_2O_2 decomposed per minute per milligram of protein sample. Oxidized glutathione formed during a GSHPX reaction is instantly and continuously reduced by an excess of glutathione reductase activity for a constant level of glutathione. The concomitant oxidization of NADPH is monitored spectrophotometrically at 340 nm. One unit of GSHPX activity was defined as one micromole of NADPH oxidized per minute/mg protein (unit/mg protein/min).[28]

Estimation of Protein in the Supernatant of Liver Homogenate

The total protein in liver homogenate supernatant was estimated by the method of Lowry *et.al*.[29]

Alanine Transaminase

The activity of alanine transaminase (ALT) was estimated kinetically using a commercial kit (BIOSYSTEMS Spain).

Statistical Analysis

Minitab 14 statistical software was used for analysis of the data. A student's t test was used to compare the experimental group to the control group. Results were expressed as a mean ± SD. P value < 0.05 was considered statistically significant.

Results

Biochemical Parameters

Significant changes in ALT, MDA and antioxidant enzymes were observed in rats that were administrated 1000mg/kg/day of vitamin C as compared to the control group. Serum ALT activity was significantly increased in animals that received 1000mg/kg/day vitamin C compared to the control ($P<0.0001$) (Figure1). Administration of vitamin C also significantly ($P=0.007$) enhanced lipid peroxidation as indicated by an elevated MAD level (Figure 2). However, animals administrated 1000mg vitamin C exhibited significant reduction in SOD, CAT and GSHPX activities in liver homogenate compared to the control group ($P=0.001$, 0.012, 0.004, respectively) (Figures 3,4,5, respectively).

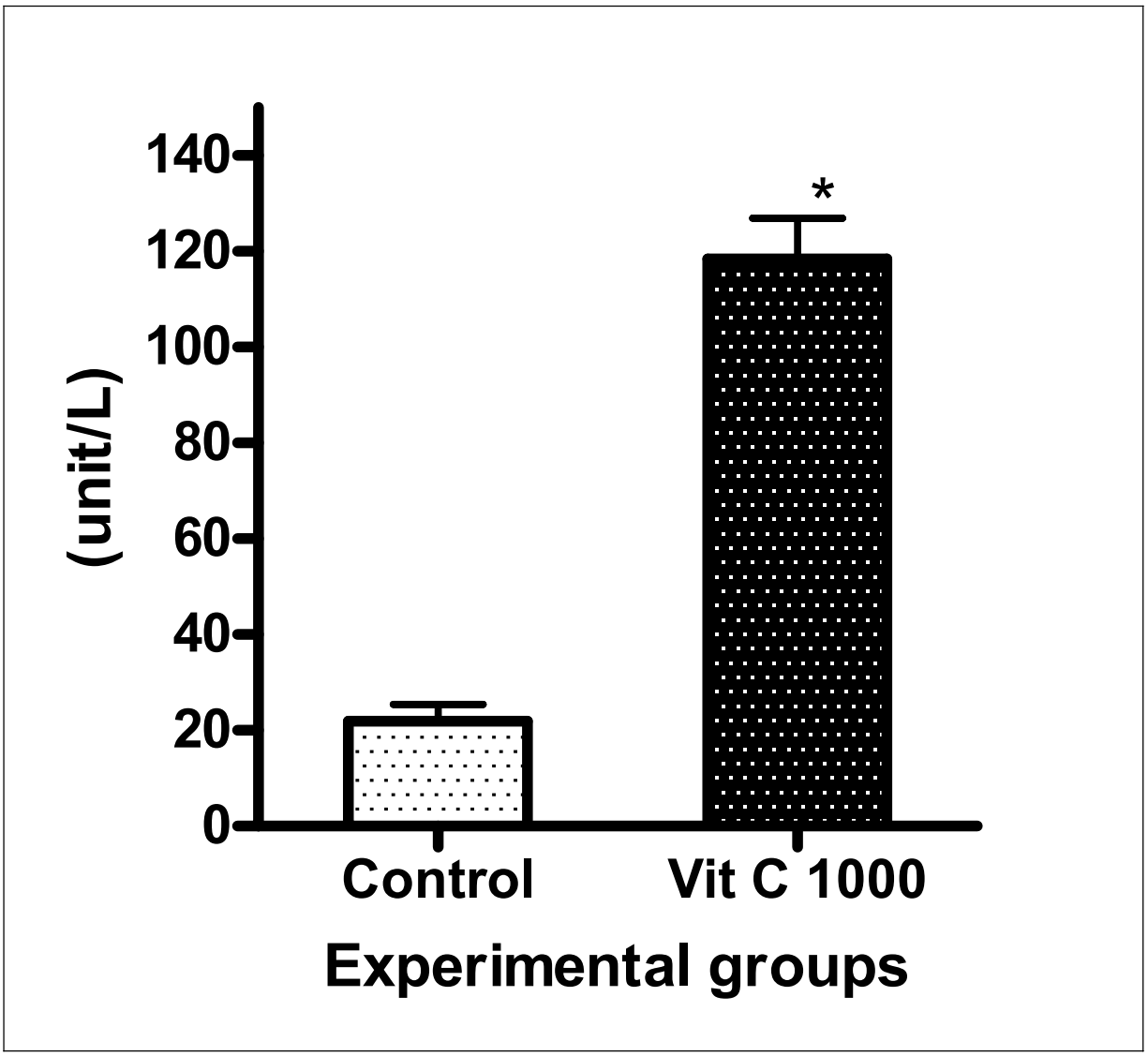

Figure 1: The level of alanine transaminase (unit/L) of control (22 ±3.4, n=6) and experimental group (118.5 ±8.5, n=6). Vitamin C 1000:1000 mg/kg/day of vitamin C, n: number of animals. Data are expressed as mean ± SD. *P<0.0001 (compared to control)

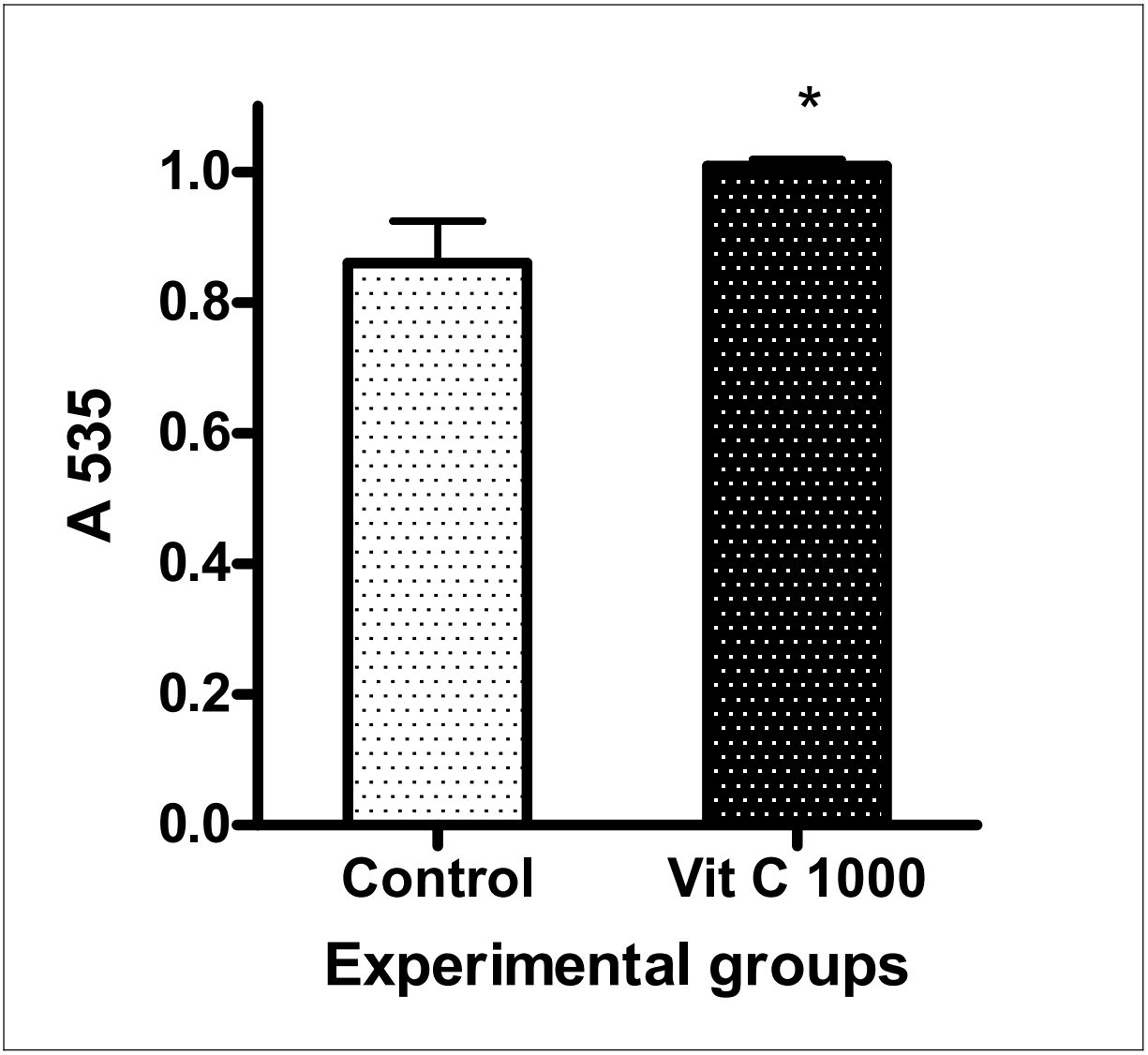

Figure 2: The level of hepatic malondialdehyde of control (0.86 ±0.06, n=6) and experimental group (1.01 ±0.01,n=5). Vitamin C 1000:1000 mg/kg/day of vitamin C, n: number of animals. Data are expressed as mean ± SD. *P=0.007 (compared to control).

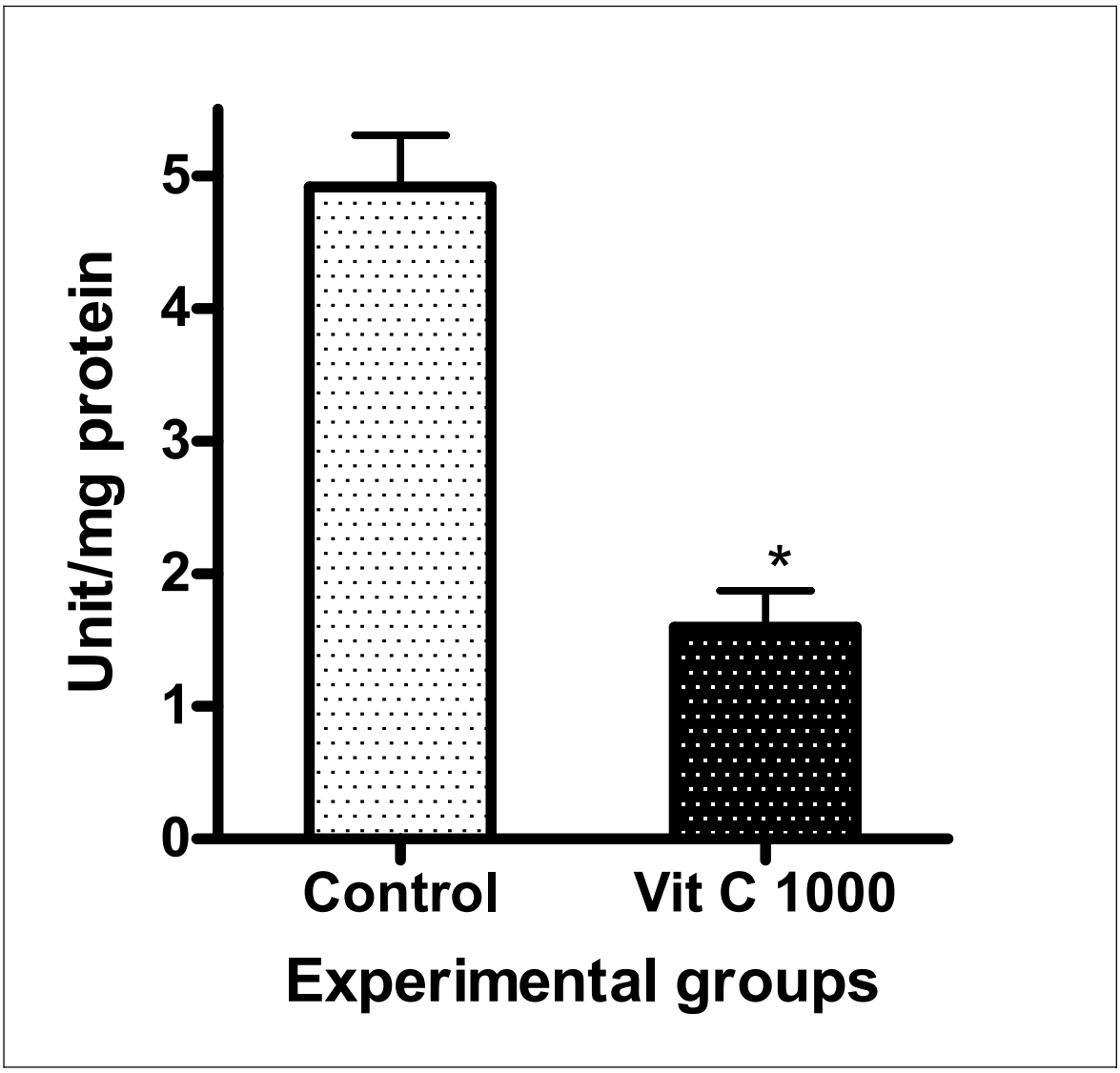

Figure 3: The level of hepatic superoxide dismutase (unit/mg protein) of control (4.91±1.1, n=6) and experimental group (1.6 ±0.56, n=6). Vitamin C 1000:1000 mg/kg/day of vitamin C. n: number of animals. Data are expressed as mean ± SD.
***P=0.007 (compared to control).**

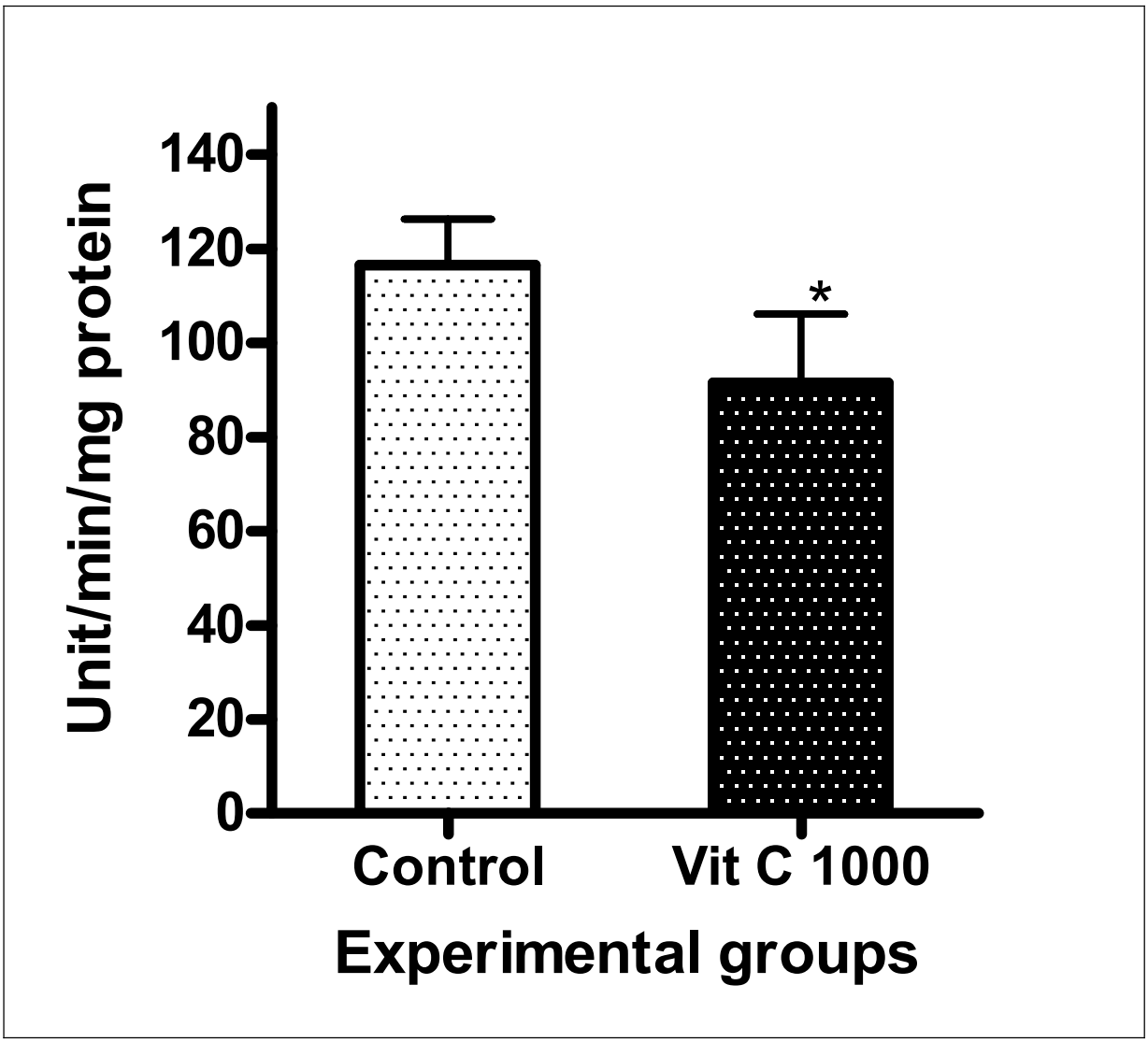

Figure 4: The level of hepatic catalase (unit/min/mg protein) of control (116.6 ±9.73, n=6) and experimental group (91.48 ±14.69, n=6). Vitamin C 1000:1000 mg/kg/day of Vitamin C, n: number of animals. Data are expressed as mean ± SD.
***P=0.012 (compared to control).**

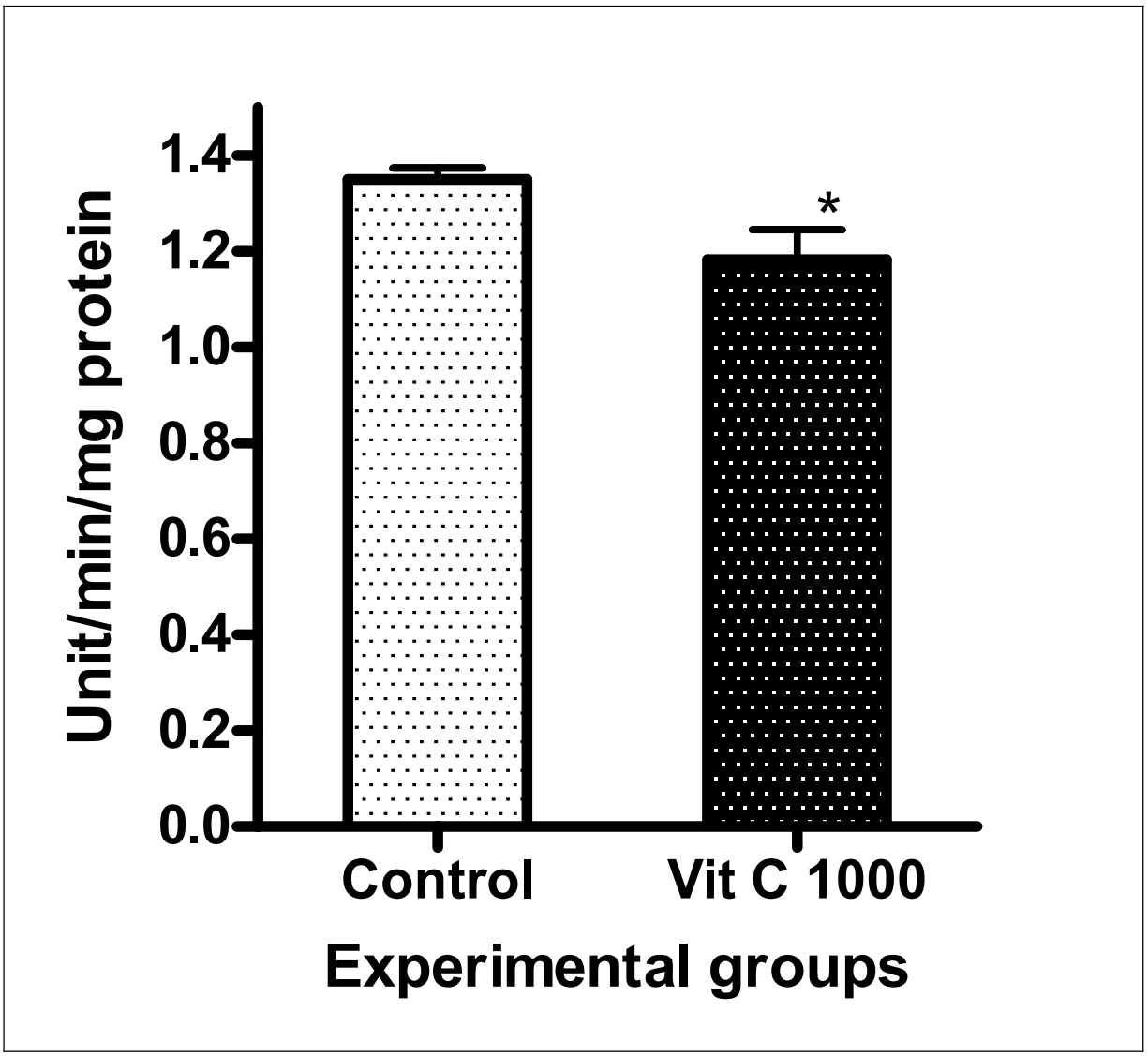

Figure 5: The level of hepatic glutathione peroxidase (unit/min/mg protein) of control (1.35 ±0.02, n=6) and experimental group (1.18 ±0.06, n=6). Vitamin C 1000:1000 mg/kg/day of vitamin C, n: number of animals. Data are expressed as mean ± SD.
***P=0.004 (compared to control).**

Histopathology

All animals of the control group showed normal liver morphology (Figure 6). While all animals in the group supplemented with 1000mg vitamin C/kg/day had similar hepatic lesions. The lesions were moderate diffuse hepatocellular vacuolar degeneration with occasional areas of hepatocyte necrosis (Figure7).

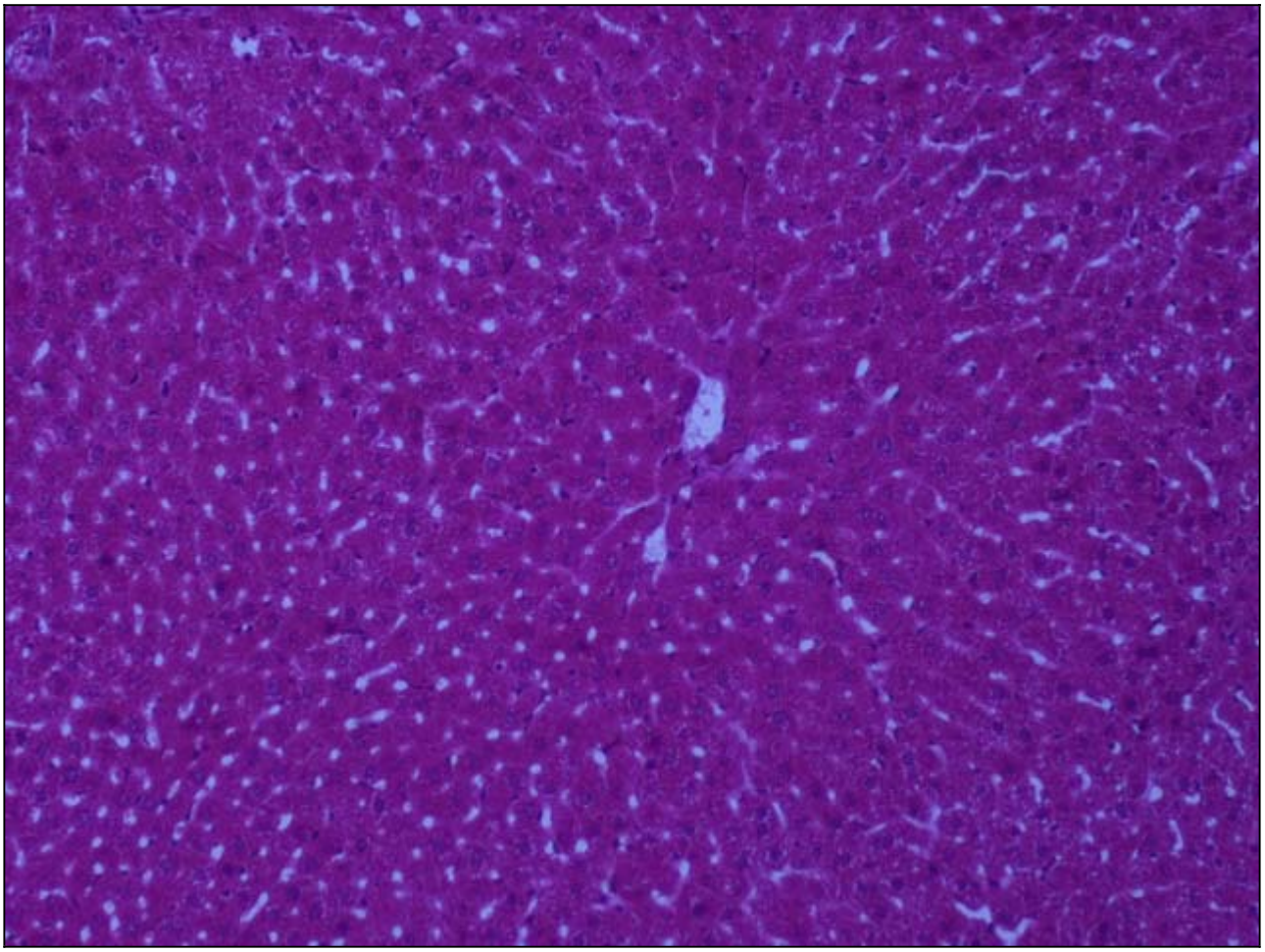

Figure 6: Liver of the control group. All animals of the control group showed normal liver morphology.

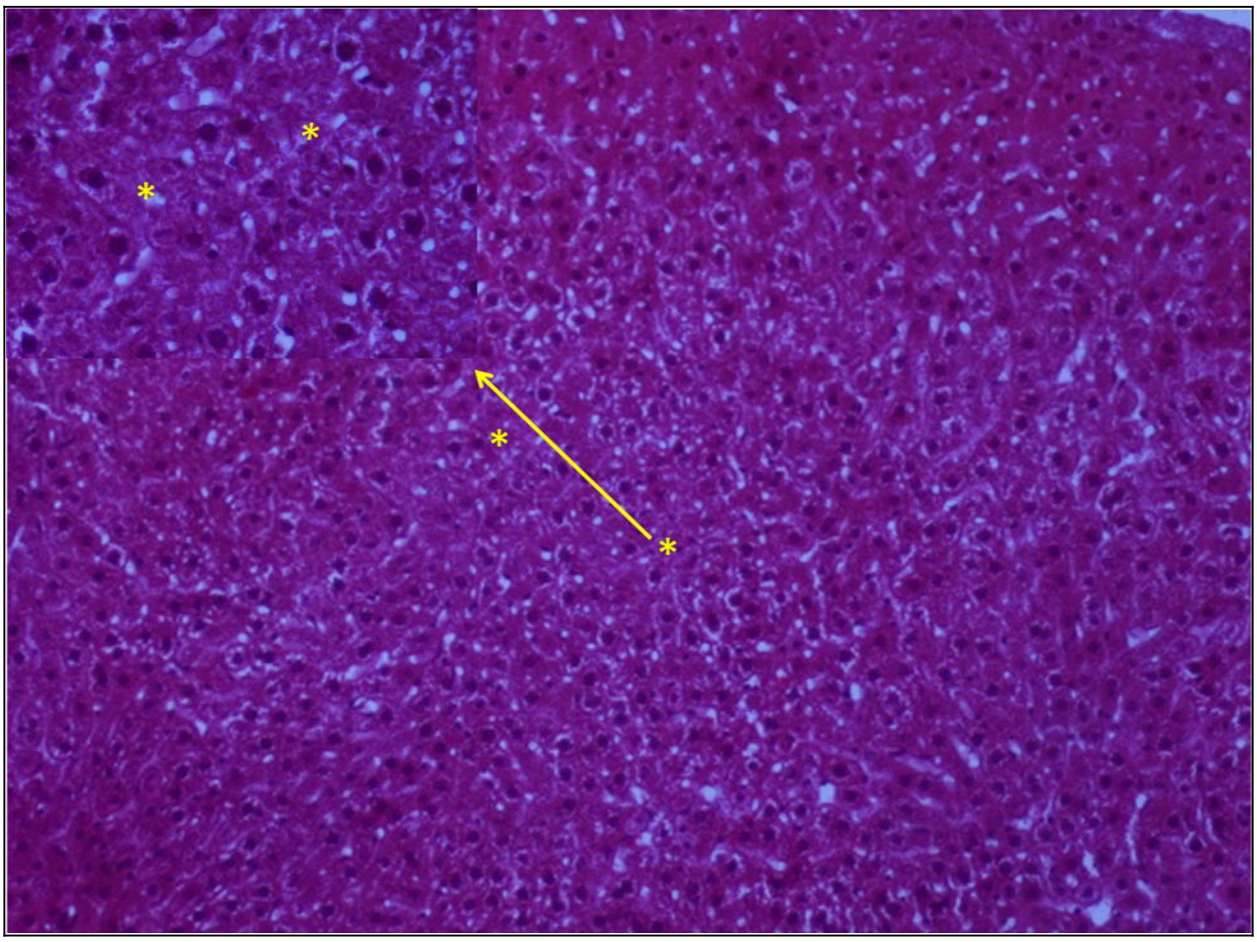

Figure 7: Liver of vitamin C group. The figure shows moderate diffuse vacuolar degeneration and occasional areas of necrotic hepatocytes (*). H&E 10x. The inset shows higher magnification of the necrotic hepatocytes.

Discussion

The major finding of this study is that the administration of 1000 mg of vitamin C/kg/day for 60 days to rats induced histopathological lesions, including moderate diffuse hepatocellular vacuolar degeneration with occasional areas of hepatocyte necrosis. This finding was supported by a significant increase in serum ALT activity, the marker of liver cell damage; there was also a significant enhancement in lipid peroxidation and a significant reduction in liver antioxidant enzyme activities.

Previously, we reported that the combination of chronic psycho-social stress and large doses of vitamin C (500 mg/kg/day) caused liver injury. We attributed these effects to reduced blood flow to the liver caused by exaggerated levels of stress hormones in relation to both chronic stress and high dose vitamin C as a possible mechanism. However, this was not the case in the current research. Histopathological lesions were associated with a significant increased level of serum ALT activity, a significant increase of MDA and a significant reduction of liver antioxidant enzyme activities. This indicated enhanced oxidative stress as a result of the pro-oxidant properties of vitamin C when administered in mega-doses. These findings are in agreement with other previously reported findings.[4] Parenteral administration of mega-dose vitamin C was found to produce both ascorbate radical and hydrogen peroxide in liver cells.[30]

Chronic supplementation of mega-doses of antioxidant vitamins to pigs were found to impair myocardial perfusion and coronary endothelial function by the increased level of oxidative stress in the arterial wall. [26] While a high intake of vitamin C (500 mg/ day) to humans and rats were found to exhibit a pro-oxidant activity that is associated with the production of the anion radical superoxide.[17]

The mechanism of pro-oxidant activity of high dose vitamin C apparently occurs through the ability of vitamin C to reduce transition metals, Fe^{+3} to Fe^{+2} or Cu^{+2} to Cu^{+1} [31] and also through induction of hepatic cytochrome P4502E1-linked monooxygenases (CYP2E1), which was associated with the generation of the anion radical superoxide.[17] CYP2E1 is a class of heme-containing proteins that has a unique ability to convert many substrates to cytotoxins[32] and can produce ROS which can damage liver cells.[33]

Conclusion

In the light of the results of this work, it can be concluded that supplementation of mega- dose vitamin C (1000mg/kg/day) for sixty days had pro-oxidant activity that resulted in histopathological lesions on a rat's liver.

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