Hepato-Protective Effect of Vitamin C Extract from Kiwifruit on Hepatic Injury Induced by Monosodium Glutamate
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Abstract
Human and animal studies have shown that some drugs and chemical agents have potential hepatotoxic effects. The hepatotoxic effect of drugs and some chemical agents like MSG as flavor enhancer is reported to be associated with the generation of reactive oxygen species (ROS) and lipid peroxidation in the liver. This mechanism has led to continuous evaluation of the hepatoprotective effect of antioxidants like vitamin C in humans and animals.

This study included 36 adult male albino rats divided into six experimental groups. Liver function tests, oxidative stress and antioxidant status were estimated. Moreover, histopathological investigations of liver tissues before and after treatment. There are significant changes in liver function tests, oxidative stress and antioxidant parameters noted after treatment with vitamin C. Moreover, the photomicrograph of liver tissue section show significant improvement in-group treated with vitamin C after MSG administration.

Vitamin C is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent. In this study it is observe that vitamin C has hepatoprotective effect through antioxidant and anti-lipid peroxidation mechanisms.

Key words: Vitamin C, Hepatic Injury, Monosodium glutamate, Oxidative Stress

Introduction
Monosodium glutamate (MSG) produces a flavor that cannot provided by other foods. MSG toxic effects have raised the increasing interest in MSG intake as flavor enhancer. Despite its taste stimulation and improved appetite enhancement, reports indicated that MSG is toxic to human and experimental animals. Its pathological influence on liver, neurotoxic effects in brain, obesity and metabolic defects, and detrimental effects on sex organs are the most discussed in the connection
with MSG intake. MSG may have some deleterious effect on the liver of adult rats at higher dose and it may affect the liver functions (Nwaopara et al., 2004). On the contrary, U.S. Food and Drug Administration FDA lists it as a GRAS (generally recognize as safe) and limits its use only in baby food (Singh et al., 2004).

Vitamin C (ascorbate) is an essential micronutrient nutrient in humans (Burri and Jacob 1997), and it must obtained on a daily basis, through the diet, in order to prevent hypovitaminosis C and the life-threatening deficiency disease scurvy (Sauberlich, 1997). Vitamin C has a number of important functions in vivo. It is an essential cofactor for a variety of dioxygenase enzymes that hydroxylate amino acids in the synthesis of procollagen, carnitine, hormones and neurotransmitters (Tsao, 1997), and is also a highly effective water-soluble antioxidant, scavenging both one-electron and two-electron oxidants (Carr and Frei, 1999). However, the significance of the antioxidant activity in vivo remains to be determined.

Recent research has uncovered vital novel functions for members of the dioxygenase family, including roles in gene regulation and signaling pathways (Loenarz and Schofield, 2008 and Arrigoni and De Tullio, 2002). Vitamin C is now known to be a cofactor for the hydroxylases responsible for the regulation of the transcription factor hypoxia-inducible factor 1 (Vissers et al., 2007), a metabolic sensor that has been implicated in a number of conditions such as cancer, ischaemic cardiovascular disorders and inflammation (Gao et al., 2007). As hypoxia-inducible factor 1 is ubiquitously expressed throughout the body, there is a requirement for adequate levels of vitamin C in all tissues.

In the present study, we explore the therapeutic manipulation of antioxidant potential by vitamin C through preventing liver injury and fibrosis. Moreover, the study extended to give awareness of MSG harmful to human health.

**Materials and methods**

**Experimental Animals:** Adult male albino rats of Wistar strain, weighing 130-150 g, were obtained from the animal house of the Biological Products & Vaccines (VACSERA) Cairo, Egypt, and acclimatized for one week in a specific area where temperature (25±1°C) and humidity (55%). The standard diet consists of protein 21.27%, fat 2.83%, and fiber 2.46%, and rats allowed drinking tap water during the study. Rats kept in good ventilation with 12hr light/dark cycle.

**Experimental Set-up:** This study included 36 adult male albino rats divided into six experimental groups (6 per group) as follow: Control group: animals of this group fed on a standard diet, Vitamin C treated group: animals of this group were given 30 mg/kg body weight vitamin...
C for one month (Sanghishetti et al., 2014). MSG treated group: animals of this group were given 4 mg/Kg.b.w I.P (Nayanatara et al., 2008), MSG+Vit C treated group: animals of this group were given vitamin C and MSG at same time for one month. MSG+Vit C15 treated group: animals of this group given MSG for 2 weeks and then given vitamin C for other 2 weeks, Vit C15+ MSG treated group: animals of this group were administered vitamin C for 2 weeks and then give MSG only for other 2 weeks. All treatments were administered for 30 days.

**Samples Collection:** At the end of the experimental period, samples were collected and all animals were fasted for 12 hours and blood samples were collected from retro-orbital venous plexus under diethyl ether anesthesia (Schermer, 1967). The blood samples were left to clot and the sera were separated using cooling centrifugation (4 ºC) at 3000 rpm for 10 min and then stored immediately at -20 ºC in clean plastic Eppendorf until analyzed. Kiwifruit fruit ascorbic acid extraction. The vitamin C content was obtained by extracting a sample of kiwifruit in 0.54 M-perchloric acid containing the metal chelator diethylene triamine pentaacetic acid (DTPA) (100 µmol/l) followed by centrifugation (24). The supernatant was analysed by HPLC with electrochemical detection. There was 89 (SD 8) mg vitamin C/100 g fruit (n4), which is equivalent to about 80 mg vitamin C per kiwifruit.

**Biochemical Analyses:** Liver function tests including serum glutamate-pyruvate transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (Alp), bilirubin (total and direct), total protein and albumin were estimated using auto analyzer A15 (Biosystem, Barcelona, Spain). Moreover, oxidative stress and antioxidant status including serum superoxide dismutase (SOD), catalase (CAT), and Protein Carbonyl Content (PCO) were estimated using kits purchased from BioVision Co., USA according to the manufacturer’s instructions. In addition, Malondialdehyde (MDA) was assayed by enzyme linked immunosorbent assay (ELISA) procedure using kits purchased from Mybiosource Co., USA, according to the manufacturer’s instructions.

**Histopathological investigation:** After fixation of liver samples in 10% formalin saline for twenty-four hours, washing was applied in tap water, and then serial dilutions of alcohol were used for dehydration. Specimens were then cleared in xylene and embedded in paraffin at 56 degrees in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by microtome. The obtained tissue sections were collected on glass slides deparaffinized and stained by hematoxylin and eosin (H&E) stains for histopathological examination through the electric light microscope (Banchroft et al., 1996).
Statistical analysis: In the present study, all results were expressed as Mean ± standard error (S.E) of the mean. Data were analyzed by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 17 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when P value was < 0.05.

Results
Table 1 represents liver function tests of all groups including control group, MSG group and treated groups with vitamin C, MSG group show a significant increase (P< 0.05) in liver enzymes, bilirubin, and significant decrease in sera of total protein and albumin in comparison with MSG group and vitamin C treated groups. Moreover, groups treated with vitamin C have significant changes depending on time and period of vitamin C treatment. when comparison of (MSG+VIT C15) and (VIT C+MSG15) with (MSG+VIT C) treated groups, data of this study show significant increase (P< 0.05) of antioxidant parameters and significant decrease of MDA and PCO as represent of oxidative stress of MSG+VITC15 group in compression with other vitamin C treated groups.

Histopathological investigations:
The photomicrograph of liver tissue section of negative control group showed normal histological structure with normal hepatocytes and normal hepatic architecture. MSG group: liver is showing aggregation of lympho-plasmocytic exudate in hepatic tissue and hepatic cell degeneration. VitC group: Liver is showing normal hepatocytes and normal hepatic architecture. MSG+VITC group: liver is showing aggregation of round cells in portal vein (arrow). MSG+VITC15 group: liver is showing mild focal lymphocytic infiltration in hepatic tissue. VitC+MSG15: liver is showing focal necrosis of hepatocytes and neutrophil infiltration.
Table 1. Serum glutamate-pyruvate transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (Alp), bilirubin (total and direct), total protein and albumin of all groups after and before treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>GPT (IU/ml)</th>
<th>GOT (IU/ml)</th>
<th>Alkaline Phosphatase (IU/ml)</th>
<th>Bilirubin (Total) (mg/dL)</th>
<th>Bilirubin (Direct) (mg/dL)</th>
<th>Total Protein (g/dL)</th>
<th>Albumin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.2±5.3*</td>
<td>38.5±2.1*</td>
<td>44.2±6.2*</td>
<td>0.4±0.03*</td>
<td>0.17±0.02*</td>
<td>6.4±0.7*</td>
<td>3.1±0.39*</td>
</tr>
<tr>
<td>MSG</td>
<td>86.4±10.0*</td>
<td>117.4±50.4*</td>
<td>179.1±6.05*</td>
<td>1.4±0.09*</td>
<td>0.82±0.05*</td>
<td>4.6±2.7*</td>
<td>2.1±0.32*</td>
</tr>
<tr>
<td>VitC</td>
<td>23.4±5.3*</td>
<td>31.3±5.6*</td>
<td>49.4±9.1*</td>
<td>0.3±0.04*</td>
<td>0.17±0.02*</td>
<td>6.2±0.6*</td>
<td>2.9±0.25*</td>
</tr>
<tr>
<td>MSG+VITC</td>
<td>35.9±4.07*</td>
<td>44.6±3.7*</td>
<td>59.2±7.01**</td>
<td>0.4±0.04*</td>
<td>0.22±0.01*</td>
<td>7.9±1.2*</td>
<td>3.8±0.53*</td>
</tr>
<tr>
<td>MSG+VITC15</td>
<td>27.4±6.8*</td>
<td>37.3±2.27*</td>
<td>45.9±6.87*</td>
<td>0.4±0.02*</td>
<td>0.19±0.008*</td>
<td>6.5±0.8*</td>
<td>2.8±0.15*</td>
</tr>
<tr>
<td>VitC+MSG15</td>
<td>36.5±4.3*</td>
<td>46.5±6.1*</td>
<td>55.6±5.3*</td>
<td>0.4±0.07*</td>
<td>0.20±0.01*</td>
<td>8.2±0.8*</td>
<td>3.9±0.61*</td>
</tr>
</tbody>
</table>

*: Significant change at P< 0.05 in comparison with the control group.  α: Significant change at P< 0.05 in comparison with the MSG group.  °: Significant change at P< 0.05 in comparison of (MSG+VIT C15) and (VIT C+MSG15) with (MSG+VIT C) treated groups

Table 2. Serum superoxide dismutase (SOD), catalase (CAT), Malondialdehyde (MDA) and Protein Carbonyl Content (PCO) of all groups after and before treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD Inhibition rate %</th>
<th>CAT mU/mL</th>
<th>MDA ng/mL</th>
<th>PCO nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.6±1.3*</td>
<td>42.2±1.9*</td>
<td>67.8±4.6*</td>
<td>142.3±2.2*</td>
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<tr>
<td>MSG</td>
<td>30.4±2.9*</td>
<td>17.8±1.5*</td>
<td>166.4±2.4*</td>
<td>264.9±6.1*</td>
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<tr>
<td>VitC</td>
<td>55.3±3.3*</td>
<td>47.1±2.02*</td>
<td>55.7±7.6</td>
<td>131.8±7.2</td>
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<tr>
<td>MSG+VITC</td>
<td>45.9±2.3*</td>
<td>39.4±2.1</td>
<td>74.2±4.6</td>
<td>147.5±4.4</td>
</tr>
<tr>
<td>MSG+VITC15</td>
<td>51.1±3.2*</td>
<td>44.7±1.4</td>
<td>59.6±5.6</td>
<td>137.6±5.6</td>
</tr>
<tr>
<td>VitC+MSG15</td>
<td>42.7±2.9**</td>
<td>36.8±2.2</td>
<td>80.7±10.3</td>
<td>154.4±7.6**</td>
</tr>
</tbody>
</table>

*: Significant change at P< 0.05 in comparison with the control group.  α: Significant change at P< 0.05 in comparison with the MSG group.  °: Significant change at P< 0.05 in comparison of (MSG+VIT C15) and (VIT C+MSG15 with (MSG+VIT C) treated groups
Fig 1. Photomicrograph of liver sections of (A) negative control group showed normal histological structure with normal hepatocytes (arrow) and normal hepatic architecture. (B) MSG group liver is showing aggregation of lymphoplasmocytic exudate in hepatic tissue (arrow) and hepatic cell degeneration. (C) VitC group liver is showing normal hepatocytes (arrow) and normal hepatic architecture. (D) MSG+VITC group liver is showing aggregation of round cells in portal vein (arrow). (E) MSG+VITC15 group liver is showing mild focal lymphocytic infiltration in hepatic tissue (arrow). (F) VitC+MSG15 group liver is showing focal necrosis of hepatocytes and neutrophil infiltration (arrow). (HE, 100x)

Discussion

Human and animal studies have shown that some drugs and chemical agents have potential hepatotoxic effects. The hepatotoxic effect of drugs and some chemical agents like monosodium glutamate in the present study is reported to be associated with the generation of reactive oxygen species (ROS) associated with lipid peroxidation in the liver. This mechanism has led to continuous evaluation of the hepatoprotective effect of antioxidants in humans and animals. Among the antioxidants been evaluated is vitamin C which is a water-soluble antioxidant. In the present study, vitamin C with hepatoprotective property in rat is demonstrated through different supposed mechanisms. Reported researches showed that, vitamin C was reported to attenuate hepatic damage induced by some chemical agents especially in animals. This is supported by the work of Bashandy and Alwasel, (2011). The results of the present study shows that vitamin C normalized levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, catalase and malondialdehyde in sera of monosodium glutamate intoxicated
rats. Moreover, these results were agreeing with reported by some authors (Ademuyiwa et al., 1994), (Kataoka et al., 2012). In addition, ascorbic acid was also able to preserved 100% of cell integrity and modulated alanine aminotransferase and aspartate aminotransferase (Grajeda-Cota et al., 2004) as well as reduced cypermethrin induced cytotoxicity in rat hepatocytes by recovering 60% of glutathione and 54% decrease in gamma glutamyl transpeptidase. Pretreatment with vitamin C (200 mg/kg) normalized the abovementioned parameters (Mongi et al., 2011).

These observations are contrary to the work of Kamel et al. (2012). On the level of liver histopathology our results showed centrilobular necrosis, hepatic cell degeneration and necrosis with loss of nucleus in MSG treated group compared with vitamin C treated group. The administration of vitamin C attenuated hepatotoxicity via normalization of biochemical and histopathological changes induced by MSG. Moreover, it was observed that vitamin C pretreatment gave a better protection than post treatment with MSG in VitC15+MSG versus MSG15+VitC treated group which animals of this group were given MSG for fifteen days only and Vitamin C for another fifteen days as a treatment. This agreed with the report of Eissa (2004), who observed mild recovery in the liver of Japanese quail treated with vitamin C. Moreover, Supplementation with vitamin C, 25 mg/100gram body weight for 3 days in male guinea pigs ameliorated ethanol-induced hepatotoxicity (Abhilash et al., 2012).

Dietary vitamin C supplement may have protective effect on the liver and improve hepatic function as reported by some researchers. This is supported by findings from the evaluation of vitamin C on lipid peroxidation and glutathione system in the normal guinea pig heart. It was observed that dietary vitamin C supplementation is able to increase global antioxidant capacity of guinea pig heart tissues (Rojas et al., 1994). Dietary vitamin C supplement was also reported to confer protective effect against endotoxin induced oxidative damage to protein in guinea pig liver. This seems mainly due to a direct increase in hepatic ascorbate levels in vitamin C exposed animals (Cedenas et al., 1998). Dietary vitamin C supplement was reported to decrease endogenous protein oxidative damage and lipid peroxidation in the guinea pig liver (Barja et al., 1994). Further study showed that administration of monosodium glutamate (food additive) at a dose level of 0.6, 6 and 60 mg/kg for 14 days increased serum alanine aminotransferase and aspartate aminotransferase dose dependently. These elevated parameters were reduced after pretreatment with vitamin C. Histopathological changes induced by monosodium
glutamate were also ameliorated (Ibrahim et al., 2011).

In conclusion, the hepatoprotective effect of vitamin C is said to be associated with it oxidative property. Vitamin C is a watersoluble antioxidant which decreases lipid peroxidation. In this work, Vitamin C was also reported to scavenge aqueous reactive oxygen species (ROS) by rapid election transfer that inhibits lipid peroxidation. Moreover, it is observed that vitamin C may have hepatoprotective effect on the level of histopathology of the liver tissues.

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The abstract in Arabic:

أظهرت الدراسات على حيوانات التجارب وكذلك الإنسان أن بعض الأدوية والمركبات الكيميائية لها ضرر بالغ على الصحة العامة وخاصية الكبد ومن ضمن المركبات أحادي جلوتاميت الصوديوم والذي يستخدم كمحسن طعم في بعض الأغذية وقد وجد أنه أيضاً يزيد من شفافية الأكسجين الحرة وأكسدة الدهون في خلايا الكبد مما دفعنا إلى إكتشاف ومتابعه هذه الأدوات الضارة في خلايا الكبد واستعمال فيتامين سي المستخلص من فاكهة الكيوي كعامل مقاوم للأكسدة الناتجة عن هذه المركبات الضارة وقد تضمنت الدراسة 36 جرذًا ذكرًا قسمت إلى 6 المجامع

المجموعة الأولى مجموعه ضابطه المجموعه الثانية مجموعه فيتامين سي فقط لمدة 30 يوم والمجموعه الرابعة حققت بألذابي جلوتاميت الصوديوم ومجموعه فيتامين سي معاً لمدة 15 يوم وبدأت السادسة حققت بألذابي جلوتاميت الصوديوم لمدة 15 يوم وتم أيضًا قياس وظائف الكبد والضغط التأكسدي وCORDA صفات الأكسدة في كل الجرذان لكل المجموعات وتتوافق مع اختبارات خلايا الكبد في كل المجموعات قبل وبعد العلاج بفيتامين سي المستخلص من فاكهة الكيوي وقد أظهرت النتائج اختلاف واضح في وظائف الكبد والضغط التأكسدي وعود التأكسد في مجموعات الانتاج التي عولجت بفيتامين سي عن مجموعات التي لم تعرض كما أظهرت النتائج وجدت تحسن واضح على مستوى خلايا الكبد للجرذان التي عولجت بفيتامين سي خاصة المجموعه الخامسة

بالخلاصة:

ثبت من هذه الدراسة أن فيتامين سي عامل مساعد قوي يدخل في كثير من التفاعلات البيوكيميائية التي تحمي خلايا الجسم وخاصة الكبد من تأثير عوامل مؤكسدة يتم استخدامها في كثير من المنتجات الغذائية ومنها أحادي جلوتاميت الصوديوم حيث يعتبر فيتامين سي مركب يحمي خلايا الكبد وكذلك مضاد للأكسدة خاصة أكسدة الدهون في هذه الخلايا وتوصي هذه الدراسة باستخدام فيتامين سي كعلاج للأشخاص الأكثر استلباكاً لأحادي جلوتاميت الصوديوم في أغذيتهم.