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Protective effects of tomato consumption against the oxidative damage caused by CCl_4 in rat's liver

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Objective: The aim of the study was to examine the effects of tomato juice, which is as a rich source of lycopene, on acute liver injury caused by the oxidant CCl_4.

Background: Lycopene is the mostly encountered carotenoid in Lycospersicum esculentum. Like tomato, pink guava, papaya and apricot also involve lycopene which is a more effective antioxidant than the other carotenoids. CCl_4 is a xenobiotic that causes hepatotoxicity. In daily life it is used as organic solvent in oil products, insecticides, resin, wax, organic diluent in rubber products and as a transfer agent in cooling equipments. Because of its solvent property it is used as stain remover for furniture and carpets, as well as an anesthetic in extraction of plants. Because of these reasons CCl_4 is contagious to human by breathing, swallowing and contact.

Methods: 26 Wistar albino rat were separated into 3 groups; To Group I (Control group) physiologic serum equal to the amount of CCl_4 volume administered to other groups' animals was injected, To Group II (CCL_4 Group) CCl_4 was injected to the animals, to Group III (CCL_4+Lycopene) CCl_4 was injected to the animals, and only to the animals of this group, starting 20 days before the first administration of CCl_4 and throughout the study period, 12.5 ml. of squeezed fresh tomato juice was given daily by their water bottles throughout the study. At the end of the study animals were sacrificed and tissue samples from their liver were investigated to establish MDA, SOD, GSH, GPx values.

Results: MDA levels were higher in CCl_4 administered group than the control group; in the group which received tomato juice, those levels were close to the control group. In the group which received CCl_4 + tomato juice SOD, GSH and GPx enzyme values were

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meaningfully higher when compared with the group which only received CCl_4 (For CCl_4 + tomato juice group P<0.0001). Those three enzyme levels were statistically significant when compared to the control group and CCl_4 + tomato juice receiving group 3. (P<0.05, P<0.0001, P≤0.01 respectively).

Conclusion: According to the result of our study, tomato juice containing lycopene and ascorbic acid, which have antioxidant properties, possess a strong effect on oxidative damage of CCl_4 in liver.

Key words: Tomato juice, carbon tetrachloride, rat, liver, antioxidant

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Introduction

Oxidative damage biomolecules have widely been implicated in the etiology of degenerative diseases such as coronary heart disease, cancer and aging.[1,2] Antioxidants including vitamins and carotenoids providing protection against oxidative damage and aging have an increasing importance, also they are potential chemo-preventive agents. Lycopene which is the major carotenoid in tomato, is known to have the highest antioxidant activity compared to other carotenoids.[3] Lycopene is a dietary carotenoid component which can accumulate in human tissues and which can be found in fruits such as ripe tomato, watermelon, papaya guava and grapefruit.[4] In recent studies it has been indicated that lycopene has antioxidant and potential anticarcinogenic properties.[5] In another contemporary study a significant correlation between intake of tomato products and lowered risk for prostate cancer has been indicated.[6] With in vitro studies it is also shown that lycopene is more effective antioxidant than B- caratone.[7]

Carbon tetrachloride (CCl_4) effects human by breathing, swallowing and contact. It is used as organic solvent in oil products, insecticide, resin, wax and organic diluent in rubber products and as a transfer agent in cooling equipments, again because of its solvent property it is used as stain remover for furniture and carpets, also it is used in extraction of plants. CCl_4 is a xenobiotic that causes hepatotoxicity in humans as well as animals,[8] and Carbon tetrachloride (CCl_4) is frequently used as experimental hepatotoxin.[9] The hepatotoxic effect of CCl_4 is thought to result from its reductive dehalogenation by cytochrome P-450 to the highly reactive trichlorometyl radical and further to the trichlorometyl peroxyl radical in the presence of oxygen.[4]

Although many studies investigated the in vivo properties of lycopene and its levels under different conditions of oxidative stress, few studies examined the in vivo features of tomato juice. Thus, in this study, we examine the effects of tomato juice, which is as a rich source of lycopene, on acute liver injury caused by the oxidant CCl_4.

Materials and Methods

Animals

For the study 28 male, 3 months old Wistar albino rats, weighting 160 g, raised in Istanbul University, Cerrahpaşa Medical School, Experimental Animals Investigation and Production Laboratory. To the animals, which were kept in polypropylene cages, food and water were provided ad libitum, and controlled conditions of temperature and humidity with 12-hours light/dark cycle were maintained. All animals were fed with standard rat pellet diet. The Faculty Animal Care and Use Committee approved all animal procedures.

Groups and the administration methods were as follows

To Group I (Control group, n=8) physiologic serum equal to the amount of CCl_4 volume adminis-

tered to other groups' animals was injected, To Group II (CCL4 Group, n=10) CCl4 was injected to the animals, to Group III (CCL4+Lycopene, n=10) CCl4 was injected to the animals, and only to the animals of this group, starting 20 days before the first administration of CCl4, squeezed fresh tomato juice was given daily by their water bottles throughout the study.

Experimental liver damage induced by carbon tetrachloride

The suspension was made from 0.2-mg/kg b.w./animal CCl4 (Sigma 5331) and olive oil and this mixture was administered 3 times a week intraperitonaly to the animals' of Groups 2 and 3 in order to cause hepatotoxicity till the day of sacrification.[10]

Tomato juice preparation and administration

Tomato juice given to Group 3, was obtained from tomatos raised in Fethiye region of Turkey (Lycospersicum esculentum) and bought daily. After those tomato were processed with mixer their pulps were taken away and brought to a consistency which animals can drink. In each application 12.5 ml freshly prepared tomato juice was given to each animal after diluting with the same amount of water (25 ml-[tomato juice + tab water]). 20 days before the onset of CCl4 administration Group 3 started to receive tomato juice. It has been observed that amount of liquid intake by these animals has reached the levels of the control group water intake after the first week. Administration of tomato juice was continued 12 weeks after CCl4 administration.

48 hours after the end of 12 weeks the animals in all groups were sacrified under ether anesthesia. Left lobules of liver were taken, and wrapped with aluminum foil and transferred immediately to –70 0C for the analysis of biochemical parameters.

Biochemical assessment of the liver

MDA,[11] SOD,[12] GSH[13] and GPx (Commercial kits-Randox-Ransel, cat no: RSo 505) were analyzed with standard methods in liver tissue.

Statistical analysis

The mean value ± standard error was calculated for each parameter. Results were statistically analyzed by one-way ANOVA, Bonferoni and Dunnet T-test.[14]

Results

No significant changes were observed in feed consumptions of both control and experimental groups' animals. It has been observed that each animal in Group 3 have orderly consumed the daily given 12.5 ml/day fresh tomato juice (25 ml-[tomato juice + tap water]) which is equal to other groups' water consumption. Only 2 animals from Group 2 whom did not receive tomato juice and were administered with CCl4, died 4 weeks after the onset of the experiment. In contrast, there was no death in the group receiving tomato juice with CCl4 through the experiment. Although there were no significant differences in the weights of the animals at the beginning of the experiment 3-4 weeks after the onset of CCl4 injections there was an evident weight lost in Group 2, which did not receive tomato juice. In Table 1 mean body weights of the control and experimental groups' animals at the end of the study are shown. While mean body weight of Group 2 animals was 191.25 gr., the difference between this group and Group 3 was considered as insignificant (P>0.05). The differences between Group 1 (254.37gr.) and the other groups Group 2 and 3 (210.55 gr.) were found to be statistically significant (P<0.01).

Table 1
The mean animal weights at the end of the study are shown

Mean animal weights (g)			
Group 1 Control	Group 2 CCL4 Group Juice Group	Group 3 CCL4+Tomato	F
254.37 ± 6.78[a]	191.25 ± 15.75[b]	210.55 ± 9.44[b]	8.078**

a,b: The differences between the groups' mean values with different letters are significant (**P<0.01).

Table 2
MDA, SOD, GSH and GPx values in liver tissue:

Enzyme Levels	Group 1 (n=8)	Group 2 (n=10)	Group 3 (n=10)	Significance Group 1x2	Group 1x3	Group 2x3
Liver Tissue MDA Nmol/gw.w	97.96±11.74	128.38±11.77	87.13±6.99	P<0.0001	P>0.05	P<0.0001
Liver Tissue SOD U/mg protein	9.20±0.80	7.37±0.56	10.23±0.80	P<0.0001	P<0.05	P<0.0001
Liver Tissue GSH nmol/mgprotein	26. 41±1.38	21.24±1.87	30.02±1.41	P<0.0001	P<0.0001	P<0.0001
Liver Tissue GPx mU/mgprotein	104.80±9.63	82.89±4.98	116.50±8.65	P<0.0001	P≤0.01	P<0.0001

In Table 2 MDA, SOD, GSH and GPx enzyme concentrations in liver tissues for the control and experimental groups are given. According to those results, liver MDA level which is an indication for lipid peroxidation, showed a significant increase in the group which received CCl_4 administration. While the control group liver MDA level was 97.96±11.74 nM/g w.w., in Group 2 it has raised to 128.38±11.77 nM/g w.w. (P<0.0001) (Table 2). Liver MDA level of Group 3 was 87.13±6.99 nM/g ww and this result was close the control group's result (P>0.05) (Table 2).

SOD, GSH and GPx enzyme activities of control and experimental groups' animals' liver tissues were also investigated. According to those results while administration of CCl_4 alone caused a significant decrease in mean liver SOD concentration of liver (7.37±0.56 U/mg, (p<0.0001), the SOD concentration in Group 3 indicated a statistically significant increase when compared with the control group (10.23±0.80 U/mg protein). This value was even higher than the level determined for the control group (9.20±0.80 U/mg protein) (Table 2). In addition to that, when the Group 2 SOD values compared with those of Group 3 they were considered to be meaningful (p<0.0001).

Another meaningful result was the value of GSH, which is one of the major antioxidant soluble in water. While in the control group the mean GSH level of liver tissue was 26.41± 1.38 nmol/mg protein, in Group 2 this value was lower than the other two groups GSH levels and was equal to the level of 21.24±1.87 nmol/mg protein. At the same time in Group 3 the mean liver GSH level was higher than the other two groups and was 30.02 ±1.41 nmol/mg protein. The difference between three groups was shown in Table 2. From these, liver tissue GSH level showed a decrease in CCl_4 receiving group when compared with the control group (p<0.0001). When the results of GPx levels were investigated; the mean value was 104.80±9.63 mU/mg protein in the control group. Mean value of group 2 was lower than the other two groups and was close to the mean value of 82.89±4.98 mU/mg protein. The mean value for Group 3 was higher then other groups and has reached 116.50±8.65 mU/mg protein (Table 2). The results, which belong to the Group 3 was also meaningful when compared with control group (p<0.01). Likewise, a significant difference was determined when the values of Group 3 was compared with Group 2 (p<0.0001). The decrease in the same value of group 2 was not meaningful when compared with the control group (p<0.0001).

Discussion

According to the findings obtained from epidemiological studies it has been reported that the good consumption of vegetables and fruits which contain high levels of carotenoids provide protection against colon, stomach and prostate cancers which are commonly observed in human. Lycopene is known to have the highest antioxidant activity when compared with other carotenoids.[15] It is the major carotenoid of tomato, which is also a good ascorbic acid source.[16]

The reasons for affecting the biosensivity and absorption of lycopene are plentiful. All-trans is the predominant isomeric form of lycopene and is found in unripe tomato. According to the performed studies chemical construction of lycopene

changes when tomato is processed into tomato juice, sauce, paste or ketchup, with the effects of temperature alterations, its biosensivity and absorption increase when all-trans form transferred into cis conformation during the process. Also, existence of lipids taken with diet affects the biosensivity and absorption. Tomato juice, ketchup, pizza with tomato ingredient, spaghetti sauces are the major supporters of lycopene diet. Lycopene combines with the lipid micelles and is absorbed in intestinal mucosa even with passive diffusion. Lycopene is secreted into lymphatic system after combining with chylomicrons and transported to liver.[17]

Recent studies show that lycopene found in squashed tomato has greater biosensivity than the fresh tomato.[18] The explanation is that cis isomer form has higher biosensivity than trans form.[17]

Beside the researchers considering that lycopene is a strong antioxidant, Pelegrini N et al.[19] reported that daily consumption of tomato can increase lycopene and B carotene levels of plasma, but consumption mashed tomato does not have any effect on the total plasma antioxidant capacity.[19]

It is known that lycopene accumulates in tissues.[17] Hence, in order to enable lycopene to reach designated levels, we started to give tomato juice 20 days earlier than the administration of CCl_4 to the tomato juice receiving group. Velmurgan et al[3] investigated the effects of administration of 2.5 mg/kg lycopene 3 times a week against acute tissue damage formed by MNNG. In this study's experimental group which received lycopene + MNNG mean body weight was higher in statistically meaningful manner when compared with the group which MNNG administered alone.[3] Hence when the animal weights we obtained in our study lasting 12 weeks were investigated statistically with ANOVA, the weights of animals in the control group were higher than the animals in experimental groups. Although the animals in CCl_4 + tomato juice receiving group were heavier, the difference between them was not statistically meaningful. Animals receiving tomato juice being only heavier than the CCl_4 receiving animals support the results of Velmurgan et al.[3] However, 3 animals died in CCl_4 receiving group throughout the experiment period. We think that encountering no deaths in group 3 which received tomato juice in addition to CCl_4 and animal weights being closer to the control group in that group is welded from the protective role of lycopene, showing high antioxidant property against the damage of CCl_4 as reported by Mascio et al[15] In addition to that, as Kim et al[20] which conducted a similar study to our with tomato juice reported other antioxidants such as beta carotene and ascorbic acid present in tomato. Those ingredients could have assist protection of lycopene.

Leal et al[16] reported that MDA concentrations were decreased in the group they administered T-2 toxin together with 25 mg lycopene/kg b.w. /day and increased in the group they only administered T-2 toxin. The MDA concentrations were similar to the control group in the group they only administered lycopene.[16] In this study, CCl_4 administered tomato juice receiving group showed MDA levels which were close to the control group levels, whereas in the group which received only CCl_4 they were quite higher in comparison with the control group.

Parallel to the results we obtained, Bhuvaneswari et al[21] demonstrated that administration of 2.5 mg/kg lycopene has a chemo-preventive effect against DMBA in rats and reported that lycopene has increased liver tissue GSH levels in a statistically meaningful manner.

Besides Velmurgan et al[3] also reported that blood lipid peroxidation levels have increased, and enzyme activity of GPx which is an antioxidant enzyme has decreased in 6-8- weeks Wistar albino rats which developed tumors with MNNG inductions. After the administration of lycopene lipid peroxidation level has decreased and GPx activity has increased. We also used tomato juice instead of synthetic lycopene in our study. With this administration we have seen a meaningful increase in GSH and GPx levels. We think that strong antioxidant effect that we established is because of tomato's content of lycopene being responsible for the increase of GSH, GPx levels as Velmurgan et al[3] explained.

Breinholt et al[22] investigated the regulating roles and effects on enzyme activities of different lycopene doses; given daily to rats approximately 3 weeks old and established that lycopene increases SOD activity statistically. Those results are parallel to our SOD levels results of the group to which received tomato juice.

Kim Y et al[20] investigated the effects of synthetic lycopene and tomato extract on serum activity of aspartate aminotransfertase and sorbitol dehydrogenase in hepatic damage they induced with CCl_4 in Spraque dawley rats. They observed that tomato extract partially inhibits the activity of those enzymes whereas synthetic lycopene could not. The reasons for that can be the absorption of tomato extract is being better than synthetic lycopene and tomato extract including other substances like ascorbic acid together with lycopene. According to the result of that study tomato extract protected rats partially from the acute liver damage formed because of chemical oxidant stress whereas synthetic lycopene could not.[20]

In our findings collected at the end of our study; no death was observed in CCl_4 + tomato juice group, animal body weight was closer to the control group, there was a decrease in free radicals, and an increase in antioxidant enzymes, proving that daily tomato consumption has a strong protective effect on liver.

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