# Effects of *Solanum lycopersicum* L. on serum lipid profile and oxidative stress in liver tissue of high fat fed diet rats

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**Abstract:** The aim of this study was to assess the effects of *Solanum lycopersicum* L. (Tomato) pulp on oxidative stress of liver tissue in rats fed with high fat diet. For this end, male Wistar rats were treated in 4 experimental groups including: 1-healthy control group given standard diet, 2- high fat fed diet group for induction of oxidative stress in liver, 3- high fat diet plus Clofibrate (320 mg/kg) as positive control group, and 4- high fat diet plus Tomato pulp (20 ml/kg) for protection of oxidative stress, at a period of 6 weeks. At the end of experiment, the serum levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C) and high-density lipoprotein (HDL-C) were detected to determine deleterious metabolic effects. The lipid peroxidation product, malondialdehyde (MDA) and reduced glutathione (GSH) content was measured to assess free radical activity in the liver tissues. The enzymatic activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were measured as indicators of antioxidation in liver tissue. After 6 weeks treatment, After 4 weeks, high fat diet caused deleterious metabolic effects, including hypertriglyceridemia and hypercholesterolemia. Rats fed with high fat diet showed significant decline in antioxidants, and elevated lipid peroxidation product (MDA) in liver. Tomato pulp treatment significantly reduced MDA level and brought back the liver antioxidants near to normal. The results obtained showed that Tomato pulp exerts inhibitory effects against oxidative stress in liver tissue of high fat fed diet rats through its antioxidant activity.
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## 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury in many countries around the world. It has a broad pathologic spectrum which ranges from simple fatty infiltration of the liver or steatosis, to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis and to liver failure. (Assy et al., 2000). Nonalcoholic fatty liver disease (NAFLD) is now recognized as the most common type of liver disease and might lead to important public health problems (Clark et al., 2002).

Triglycerides and cholesterol are of important biological lipids of body that excess get them through the diet is resulted in hypertriglyceridemia (Hokanson, 2002; Kametani et al., 2002) and hypercholestrolemia (Walldius et al., 2004). NAFLD is diagnosed by accumulation of triglycerides in the hepatocytes in consequence of the esterification of free fatty acids and glycerol. Increase in free fatty acids in the liver is driven from three separate sources includes lipolysis (hydrolysis of glycerol and fatty acid from triglycerides) in adipose tissue, high fat diet and de novo lipogenesis (Postic and Girard, 2008). In contrast, fatty acids may used through β-oxidation, de novo esterification to triglycerides and store as fat droplets or excretion in the form of VLDL. Thus, accumulation of fat in the liver can occurs in results of increase the synthesis of fat, reduce in fat excretion or reduce in them oxidation. Donnelly et al., 2005 showed that 60% of liver triglyceride content is driven from influx of fatty acids from adipose tissue, 26% from de novo lipogenesis, and 15% from the diet (Donnelly et al., 2005). Nonalcoholic fatty liver is associated with some histopathologic changes, which is different from steatosis to cirrhosis (Dixon et al., 2001; Angulo and Lindor, 2002; Clark et al., 2002 and Farrell, 2003). It was formerly believed that steatosis is a simple phenomenon and has no complications. However, nowadays it is known that fatty liver is vulnerable to factors such as oxidative stress and can lead to Steatohepatitis, which is associated with necrosis, inflammation, fibrosis and cirrhosis (James and Day, 1999, Orrenius et al., 2007). In the pathogenesis of nonalcoholic steatohepatitis is assumed that the accumulation of triglycerides in the liver or steatosis will yield to increases the susceptibility of liver to the damages caused by inflammatory cytokines and lymphokines, mitochondrial dysfunction and oxidative stress (Day, 2006; Day and James, 1998). Barbuio et al., 2007

showed that oxidative stress is effective in alteration of steatosis to steatohepatitis (Barbuio et al., 2007). However, although liver steatosis may lead to complete hepatic failure, but appropriate and ideal treatment is not established (Angulo and Lindor, 2002). Biological materials with plant origin forms modern branch pharmacotherapy of disease. Although various pharmacologic agents exist to treat various diseases, but most patients cannot tolerate the side effects of chemical drugs from one hand and plants have very few side effects on patients from other hands. Obviously, it is necessary that several studies must be done on the new drugs in several stages before their entrance to the field of medicine.

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components. The consumption of tomatoes has been proposed to reduce the risk of several chronic diseases such as cardiovascular diseases and certain types of cancer and especially prostate cancer (Hollman et al.,1996; Rao and Agarwal, 1999). In addition, tomato consumption leads to decreased serum lipid levels and low density lipoprotein oxidation (Agarwal et al., 2001). These health protective effects have been widely attributed to the presence of key antioxidants such lycopene, beta-carotene, vitamin C, quercetin glycosides, naringenin chalcone and chlorogenic acid. All of these are known to contribute significantly to the antioxidant activity of tomato fruit (Rao and Agarwal, 1999; Abushita et al., 2000). Among the various protective mechanisms, the antioxidant activity of Tomato is considered responsible for its pharmacological effects. By consideration of antioxidant and hypolipidemic activity of Tomato pulp, this matter it will probably be able to protect the liver from steatosis.

To our knowledge, no other biochemical investigations have so far been carried out concerning the effect of Tomato pulp on the liver oxidative stress in high fat diet fed-rats are available in the literature. Therefore, present study examined the hypothesis that Tomato pulp supplementation prevents liver oxidative stress in a high fat diet model.

## 2. Materials and methods

This study carried out during 2012 in the research center of Islamic Azad University. All procedures were conducted under supervision of Animal Rights Monitoring Committee of Islamic Azad University Research Center.

### 2.1. Extract Preparation

Fresh ripe tomato fruits (500g) were washed, seeds removed and the pulp homogenized using a blender. The homogenate was then stored in a refrigerator at 4°C until used.

### 2.2. Animals

Forty male Wistar rats, weighted 180±20 gr and aged 10 weeks old were obtained from the animal breeding center of Islamic Azad University. The rats were divided into 4 equal groups of 10 animals including: 1- normal control, 2- normal rats fed high-fat diets, 3- normal rats fed high-fat diets plus Clofibrate (320 mg $kg^{-1}$/day) and 4- rats which are fed high-fat diets plus Tomato pulp (20 ml/kg). Management and husbandry conditions were identical in all groups with 12/12 h light/dark cycle at 21 ± 2°C. Food and water were provided ad libitum.

### 2.3. Experimental plan

In rats were fed with high-fat diets used of high-fat emulsion, which its formula is mentioned in table 1, to induce hepatic steatosis based on Zou et al., 2006 method (Zou et al., 2006). All treatment groups received high-fat emulsion at the dose of 10 ml kg-1 daily at morning 8 o'clock for 6 weeks. In groups 4 beside of high-fat emulsion, Tomato pulp (20 ml/kg) was given through the gavage. Simultaneously, control group received normal saline in same dosage. Group 3 beside of high-fat emulsion received Clofibrate at the dose of 320 mg $kg^{-1}$/day through gavage as suspension in the 2 ml $kg^{-1}$ methylcellulose 0.5% (Sheng et al., 2006). Control group received 2 ml $kg^{-1}$ methylcellulose 5%.

**Table 1:** Composition of high-fat emulsion gavaged to rats

| Constituents | Amount |
|---|---|
| Corn oil | 400 g |
| Sacarose | 150 g |
| Milk powder | 80 g |
| Cholesterol | 100 g |
| Sodium deoxy colat | 10 g |
| Tween 80 | 36.4 g |
| Propilen glikol | 31.1 g |
| Multi vitamin | 2.5 g |
| Salt | 10 g |
| Minerals | 1.5 g |
| Normal saline | 300 ml |

### 2.4. Serum lipid measurement

Serum triglyceride (TG), total cholesterol (TC), LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) level were measured enzymatically with commercial assay kits (Nanjing, China). Serum VLDL cholesterol (VLDL-C) was calculated by subtracting LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) from total cholesterol (TC).

### 2.5. Measurement of antioxidant activity

All experimental rats were euthanized by cervical dislocation. The rat's Liver were removed immediately and washed in normal saline and

homogenate 10% prepared in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 7000 ×g for 10 minutes at 4°C and supernatant were used for measurement of Oxidative stress by determination of malondialdehyde (MDA) as well as antioxidant enzymes (AOE) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX) and glutathione reductase. MDA, SOD, CAT and GSH-PX, GR were measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Liver homogenate MDA levels were expressed as nmol MDA per mg protein and antioxidant activity was expressed as arbitrary units per mg protein.

Degree of lipid peroxidation in kidney tissue homogenates was determined in terms of thiobarbituric acid reactive substances (TBARSs) formation by following the protocol of Esterbauer and Cheesman (Esterbauer and Cheesman, 1990). SOD activity was measured by Nishikimi method (Nishikimi et al., 1972) and was modified by Kakkar method (Kakkar et al., 1984). Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 minute, under study conditions. CAT activity was measured by Claiborne method (Claiborne, 1985) and was based on hydrogen peroxide breakdown. GPX activity was measured by Rotruck method (Rotruck et al., 1973) and was expressed as micromole of GSSG /minute/milligram of protein, based on blew reaction:

$2H_2O+GSSG \longrightarrow H_2O_2+2GSH$

GR activity was measured by Mohandas method (Mohandas et al., 1984), based on blew reaction:

$NADPH+H^+ +GSSG \longrightarrow NADP^+ +2GSH$

### 2.6. Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

## 3. Results

### 3.1. Effect of Tomato pulp on metabolism of fat due to high-fat diet

Clofibrate in groups 3 significantly ($p<0.001$) decreased, markedly increased serum levels of TG, total cholesterol, LDL and VLDL compared with group 2 and significantly ($p<0.01$) increased slightly decreased serum levels of HDL than group 2. In group 4, Tomato pulp significantly ($p<0.01$) decreased serum levels of total cholesterol, LDL and VLDL compared with group 2 and significantly ($p<0.05$) increased serum levels of HDL than group 2. (Table 1).

**Table 1:** Effect of Tomato pulp on lipid levels in rats fed high-fat diet

| Groups | Biochemical parameters | | | | |
|---|---|---|---|---|---|
| | TG mg/l | Total cholesterol mg/l | LDL mg/l | VLDL mg/l | HDL mg/l |
| control | 88.68±4.21 | 83.65±3.58 | 13.69±0.83 | 19.45±1.16 | 50.51±3.26 |
| high-fat diet | 233.61±6.90 | 218.14±7.81 | 122.72±4.75 | 49.52±2.21 | 45.90±2.34 |
| high-fat diet+Clofibrate | 95.87±3.42[c] | 110.28±4.29[c] | 25.54±1.09[c] | 31.32±1.15[c] | 53.42±4.38[b] |
| high-fat diet+Tomato pulp | 182.52±4.61[b] | 139.35±5.16[b] | 52.65±2.88[b] | 35.16±2.34[b] | 51.54±3.95[a] |
| ANOVA | P=0.000 | P=0.000 | P=0.000 | P=0.000 | P=0.000 |

Values are presented as mean ± SEM for 10 rats in each group.
a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ in compared with high-fat fed diet group.

**Table 2:** Effect of Tomato pulp on anti-oxidative activity of rat livers in steatosis induced by high fat diet
Values are presented as mean ± SEM for 10 rats in each group

| Groups | Biochemical parameters | | | | |
|---|---|---|---|---|---|
| | MDA nmol/g protein | SOD U/mg protein | CAT U/mg protein | GPX U/mg protein | GR U/mg protein |
| 1-control | 3.54±0.16[bd] | 13.64±0.54[bd] | 64.66±2.13[bd] | 22.84±1.65[bd] | 123.37±5.65[bd] |
| 2-high-fat diet | 5.18±0.21[acd] | 9.13±0.32[acd] | 41.74±1.15[acd] | 17.49±0.83[acd] | 88.85±3.52[acd] |
| 3-high-fat diet+Clofibrate | 3.59±0.18[b] | 12.53±0.52[b] | 60.84±1.74[b] | 21.95±1.54[b] | 116.13±3.42[b] |
| 4-high-fat diet+Tomato pulp | 4.78±0.24[ab] | 10.62±0.81[ab] | 54.14±1.35[ab] | 19.73±1.28[ab] | 108.79±4.11[ab] |

a, significant difference with group 1; b, significant difference with group 2; c, significant difference with group 3; d, significant difference with group 4 ($p<0.05$).

### 3.2. Effect of Tomato pulp on anti-oxidative activity of liver in damage induced by high fat diet

In group 2, Hepatic levels of antioxidant enzymes superoxide dismutase, catalase, glutathione

peroxidase and glutathione reductase compared with group 1 (normal control), significantly ($p<0.01$) reduced and the levels of malondialdehyde significantly ($p<0.01$) increased. Clofibrate in groups 3 significantly ($p<0.01$) increased, markedly decreased levels of SOD, CAT, GPX and GR compared with group 2 and significantly ($p<0.01$) decreased slightly increased levels of malondialdehyde than group 2. In group 4, Tomato pulp significantly ($p<0.05$) decreased levels of ALT, AST, ALP and TB compared with group 2 and significantly ($p<0.05$) increased levels of TP and Alb but not reached to normal levels. Data are showed in table 2.

## 4. Discussion

In order to, analyze the possible role of *Solanum lycopersicum* L. in lipid metabolism which is the key factor in steatohepatitis, serum TG, TC, VLDL-C, HDL-C and LDL-C were investigated. After 6 weeks of treatment, the serum levels of TG, TC, VLDL-C, and LDL-C was markedly increased in the high fat diet fed group compared to those in the control group. This finding was parallel to the previous study (Zou et al., 2006). Treatment of high fat diet fed rats with Tomato pulp caused considerable restoration of lipid levels to that of control. The increased serum levels of TG, TC, VLDL-C and LDL-C were significantly suppressed, whereas the decreased serum HDL-C level was obviously elevated by Tomato pulp treatment in high fat diet fed rat. This result suggests that Tomato pulp can prevent hepatosteatosis via downregulation of accumulation of lipid in serum and liver. Liver plays a key role in lipid metabolism. Hepatic steatosis refers to the excessive accumulation of lipids within hepatocytes due to imbalance between lipid formation and lipid degradation (Burt et al., 1998). Hypercholesterolaemia, hypertriglyceridaemia, low level of HDL-C and high level of LDL-C are the most common impairments in lipid homeostasis in patients with steatosis (Angulo and Lindor, 2002). Previous study has showed Tomato has hypolipidemic effects (Agarwal et al., 2001). In this study, Tomato pulp significantly improved the serum lipid profile through down-regulation the levels of TG, TC, VLDL-C and LDL-C and elevation HDL-C synthesis. These results indicate that Tomato pulp attenuates the disorder of lipid metabolism resulted from high fat diet fed. This change is associated with attenuation of oxidative stress by Tomato pulp treatment. Our results show that high fat diet caused significant decreases in SOD, CAT, GPx and GR activities. The derangement in enzymatic antioxidant potential indicates that high fat diet fed rats is unable to cope up with excess free-radical formation which leads to tissue damage. A body of evidence indicates that accumulation of fat in the liver increases the susceptibility to other insults such as oxidative stress that results in the progression of steatosis to steatohepatitis, fibrosis and cirrhosis (Koteish and Diehl, 2002). Considering the recently recognized association between oxidative stress and inflammation (Chidambarama and Venkatraman, 2010), the present experiment confirms that high fat diet could result in oxidative liver injury. Induction of oxidative stress is evident from the increased peroxidation marker (MDA) and inadequate antioxidant enzymes status in liver of rats fed high fat diet. We estimated antioxidant activities of Tomato pulp by determination of hepatic MDA content and antioxidant enzymes activity. High fat diet fed caused an increase in liver MDA content but a decrease in liver antioxidant enzymes activity compared with normal control group. Tomato pulp significantly improved the antioxidant defense mechanisms in high fat diet fed rats.

These results suggest that the imbalance between oxidative stress generation and antioxidants formation could occur after high fat diet fed, and Tomato pulp could prevent this pathological process, indicating its therapeutic and preventive effect on hepatosteatosis induced by high fat ingestion. Antioxidant activity of Tomato pulp is concordant with those of other investigators (Sharma, 1976; Sreejayan and Rao, 1994). The findings suggest that Tomato pulp treatment prevents oxidative stress and the effects are comparable with that of Clofibrate.
These results demonstrate that *Solanum lycopersicum* L. exerts preventive effects against high fat diet induced liver tissue oxidative stress through its antioxidant activity. It is noteworthy that this experiment has been performed on animal, so further studies are needed to examine whether similar findings would be obtained in humans.

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