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EXPERIMENTAL STUDY

Effects of tomato pulp on hepatic steatosis in the rats fed with high fat diet

Mohajeri D, Monadi A

Department of Pathobiology, College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
daryoushmohajeri@yahoo.com

ABSTRACT

BACKGROUND:Hepatic steatosis is one of the most common causes of chronic liver injury**.**

OBJECTIVES: This study was aimed to evaluate the protective effects of *Solanumlycopersicum* (tomato) pulp on high fat diet-induced hepatic steatosis in rats.

METHODS:Male Wistar rats were treated in 4 experimental groups including: healthy control group given standard diet, high fat diet group for induction of hepatic steatosis, high fat diet plus Clofibrate as positive control, and high fat diet plus tomato pulp for protection of liver steatosis. Finally, the groups were compared considering serum lipid profile, serum biomarkers of liver tissue injury and liver histopathological changes. The lipid peroxidation product and the activities of antioxidant enzymes were measured as the indicators of antioxidation in liver.

RESULTS:Rats fed with the high fat diet showed hypertriglyceridemia, hypercholesterolemia, increased activities of hepatocellular enzymes, significant decline in antioxidants, and elevated lipid peroxidation indices in liver. Tomato pulp treatment significantly reduced elevated markers of liver injury and malondialdehyde level, as well as brought back the liver antioxidants and the excessive accumulation of lipids in serum towards normal.

CONCLUSION: The results showed that tomato pulp exerted protective effects against hepatic steatosis in rats fed with high fat diet, possibly through its antioxidant actions *(Tab. 5, Fig. 2, Ref. 40)*. Text in PDF *www.elis.sk.*

KEY WORDS:high fat diet, *Solanumlycopersicum* L., hepatic steatosis, rat.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is emerging as the most common liver disease in industrialised countries. It has a broad pathologic spectrum, which ranges from simple fatty infiltration ofliver or steatosis, to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis and to liver failure (1).Nonalcoholic fatty liver disease (NAFLD) is now recognized as the most common type of liver disease and might lead to important public health problems (2).

NAFLD is diagnosed by accumulation of triglycerides in the hepatocytes in consequence of the esterification of free fatty acids and glycerol (3, 5). Increase in free fatty acids in 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 (6). In contrast, fatty acids may be used through β-oxidation, de novo esterification to triglycerides and store as fat droplets or excretion in the form of VLDL.Thus, accumulation offat inlivercanoccuras the results of increasedsynthesisoffat, reducedfat excretionorreduced oxidation inthem. Donnelly et al (7) showed that 60%oflivertriglyceridecontentwas driven from influx of fatty acidsfromadipose tissue, 26%from*de novo* lipogenesis, and 15% from the diet. Nonalcoholic fatty liver is associated with some histopathologic changes, whichdiffer from steatosis to cirrhosis (2, 8–10). 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 (11, 12). In the pathogenesis of nonalcoholic steatohepatitisit is assumed that the accumulation of triglycerides inliver or steatosis will yield to increase the susceptibility of liver todamage caused by inflammatory cytokines and lymphokines, mitochondrial dysfunction and oxidative stress (13, 14).Barbuio et al (15) showed that oxidative stress was effective in alteration of steatosis to steatohepatitis. However,liver steatosis may lead to complete hepatic failure, but appropriate and ideal treatment is not established (9). Biological materials with plant origin form modern branch pharmacotherapy of the disease. Although various pharmacologic agents exist to treat various diseases, most patients cannot tolerate the side effects of chemical drugs,on the other hand,plants have afew side effects on patients. 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.

Department of Pathobiology,College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Address for correspondence: D. Mohajeri, PhD, Department of Pathobiology, College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, P.O. Box: 5157944533, Iran.
Phone: +98.0413.6373339, Fax: +98.0413.6373935

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Tomato (*Solanumlycopersicum* 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 (16,17). In addition, tomato consumption leads to decreased serum lipid levels and low densitylipoprotein oxidation (18). These health protective effects have been widely attributed to the presence of key antioxidants such as: lycopene, beta-carotene, vitamin C, quercetin glycosides, naringeninchalcone and chlorogenic acid.All of these are known to contribute significantly to the antioxidant activity of tomato fruit (17,19). Among the various protective mechanisms, the antioxidant activity of tomato is considered responsible for its pharmacological effects.Consideringthe antioxidant and hypolipidemic activity of tomato pulp, this matterwill probably be able to protect the liver from steatosis.

The objective of this study was to evaluate the protective effects of *Solanumlycopersicum* L. (tomato) pulp on high fat diet-induced hepatic steatosis in rats.The results of this study demonstrated that tomato supplementation prevented liver steatosis and decreased oxidative stress in hepatocytes exposed to high levels of lipids.

Materials and methods

Animals and experimental design

Forty male Wistar rats, 200–250 gr obtained from Pasteur Institute of Iran, were housed in colony rooms with 12/12 h light/dark cycle at 21 ± 2°C and fed with laboratory pellet chow and given water ad libitum. Animals were acclimatized to their environment one week prior to experimentation. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for the laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health publication no. 85–23, revised in 1985) and our Ethical committee on animal care approved the protocol.

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/day) and 4– rats, which are fed high-fat diets plus tomato pulp(20ml/kg).

Tab. 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 deoxycolat	10 g
Tween 80	36.4 g
Propylenglykol	31.1 g
Multi vitamin	2.5 g
Salt	10 g
Minerals	1.5 g
Normal saline	300 ml

For induction of liver steatosis,high-fat emulsion was used based on Zou et al (20) method, which formula is mentioned in Table 1. All treatment groups received high-fat emulsion at the dose of 10 ml/kg daily at morning 8 o'clock for 6 weeks. Inthe group 4, beside of high-fat emulsion, tomato pulp (20 ml/kg) was given through the funnel.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/day through gavage as suspension in the 2 ml/kgmethylcellulose 0.5% (21). Control group received 2 ml/kg methylcellulose 5%.

Plant

The tomato used in this study was prepared from local market (Tabriz, Iran) and was identified by the Department of Cultivation and Development of Institute of Medicinal Plants, Tehran, Iran.

Extract preparation

Five hundred of fresh ripe tomato fruits were washed, seeds removed and the pulp homogenized using a blender. The homogenate was then stored in a refrigerator at 4 °C until used.

Biochemical factors evaluation

Blood samples were collected from the retro-orbital plexus and the sera prepared through centrifuging at 2500 ×gfor 15 minutes at 30 °C. Serum biomarkers of liver function including alanine aminotransferase (ALT), aspartate aminotransferase (AST) (22),alkaline phosphatase (ALP) (23), albumin, total protein (24) and total bilirubin (25) were measured using commercially available kits.

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).

Measurement of antioxidant activity

All experimental rats were sacricified by cervical dislocation. The rat's livers 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 °Cand supernatant were used for the measurement ofoxidative stress by determination of malondialdehyde (MDA) as well as antioxidant enzymes (AOE) such as: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). MDA, SOD, CAT,GPx and 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.

A degree of lipid peroxidation in liver tissue homogenates was determined in terms of thiobarbituric acid reactive substanc-

Tab. 2.Effect of tomato pulp on serum biochemical parameters in hepatic steatosis consequence of high-fat diet.

Group	Biochemical parameters					
	ALT (U/L)	AST (U/L)	ALP (IU/L)	TB (Mg/dl)	Alb (g/dl)	TP (g/dl)
1	54.82±2.36^{bd}	68.90±1.71^{bd}	194.87±9.03^{bd}	0.81±0.03^{bd}	4.38±0.42^{bd}	8.28±0.58^{bd}
2	71.25±3.12^{acd}	93.21±2.96^{acd}	281.67±11.25^{acd}	1.29±0.07^{acd}	3.12±0.21^{acd}	5.56±0.45^{acd}
3	55.90±2.47^{b}	68.21±1.34^{b}	208.92±8.09^{b}	0.87±0.06^{b}	4.32±0.36^{b}	7.26±0.47^{b}
4	62.56±2.95^{ab}	78.30±2.61^{ab}	235.62±6.91^{ab}	1.06±0.05^{ab}	3.73±0.29^{ab}	6.16±0.43^{ab}

Values are presented as the mean ± SEM for 10 rats in each group. ALT– Alanine aminotransferase; AST – Aspartate aminotransferase; ALP – Alkaline phosphatase; TB – Total bilirubin; Alb – Albumin; TP – Total protein. a – Significant difference with group 1; b– Significant difference with group 2; c– Significant difference with group 3; d– Significant difference with group 4 ($p<0.05$).

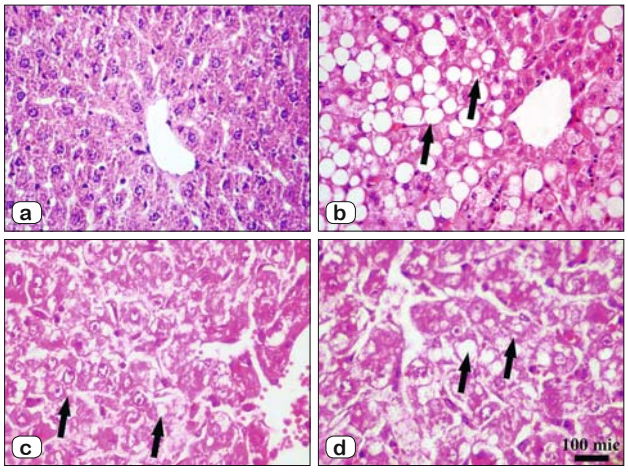

Fig. 1.Photomicrographs of H&E stained sections of rat liver. (a) Group 1: Control group. (b) Group 2: High-fat diet group. (c) Group 3: High-fat diet+Clofibrate. (d), Group 4: High-fat diet+tomato pulp. Arrows show micro and macrovesicular lipid droplets(×250; Bar = 100 µm).

es (TBARSs) formation by following the protocol of Esterbauer and Cheesman(26). SOD activity was measured by Nishikimiet al (27),method and was modified by Kakkaret al (28) method. Each unit of the 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 (29) method and was based on hydrogen peroxide breakdown. GPX activity was measured by Rotrucket al (30) method and was expressed as micromole of GSSG /minute/milligram of protein, based on blew reaction:

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

GR activity was measured by Mohandas et al (31) method, based on blew reaction:

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

Tab. 3.Effect of tomato pulp on the hepatic steatosis in rats fed with high-fat diet.

Groups	hepatic steatosis grading					p
	0	I	II	III	IV	
1	10	0	0	0	0	
2	0	0	1	2	7	a
3	7	2	1	0	0	c
4	6	2	1	1	0	bd

Each group contains 10 rats. a – $p<0.01$; b – $p<0.05$ in compared with control group. c – $p<0.01$; d– $p<0.05$ in compared with high-fat fed diet group.

Microscopic studies

To prepare liver sections for light microscopy, a small piece of hepatic tissue from the anterior portion of the left lateral lobe was removed and was fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin.

Frozen tissue samples were prepared in cryostat. The sample was then sliced into 5 µm sections, and stained with Oil-Red-O for blinded histological assessment (32). Hepatocytes were assayed from fatty changes aspect using the method by Wang et al (33) and steatosis were degreed from 0 to 4 (0: without steatosis, 1: <25% steatosis, 2: approximately 26–50% steatosis, 3: approximately 51–75% steatosis, 4: >76% steatosis). The stained 5 µm sections were graded as following: 0, absent; I, minimal; II, mild; III, modest; IV, severe. The histological changes were evaluated in nonconsecutive, randomly chosen ×200 histological fields using light microscope, NIKON ECLIPSE E200 (34).

Statistical analysis

All biochemical results were expressed as the mean±SEM. Significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test, using the SPSS statistical analysis program. Statistical significance was considered at $p<0.05$.

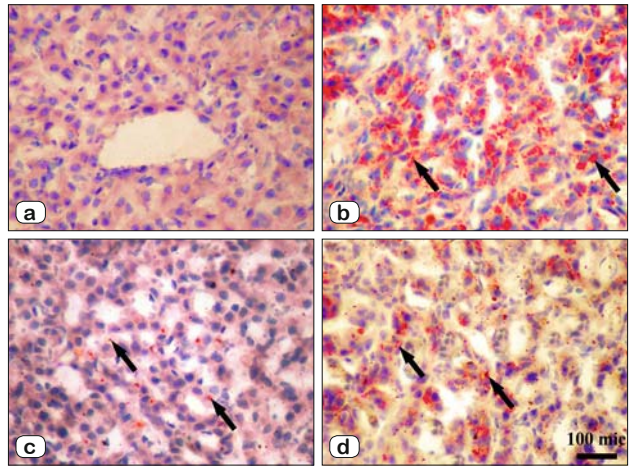

Fig. 2.Photomicrographs of Oil-Red-O stained sections of rat liver. (a) Group 1: Control group. (b) Group 2: High-fat diet group. (c) Group 3: High-fat diet+Clofibrate. (d), Group 4: High-fat diet+tomato pulp. Arrows show micro and macrovesicular lipid droplets(×250; Bar = 100 µm).

Results

Effect of the tomato pulp on biochemical parameters

The results of the effect of tomato pulp on biochemical parameters are presented in the Table 2. In high-fat diet group (group 2),ALT, AST, ALP and TB increased and TP and Alb decreased significantly ($p<0.01$) in comparison with the control group. In high-fat diet+Clofibrate group (group 3), high levels of ALT, AST, ALP and TBsignificantly decreased ($p<0.01$) to normal values and levels of TP and Alb increased to their normal boundaries. In high-fat diet+Tomato pulp group (group 4), levels of ALT, AST, ALP and TB significantly decreased ($p<0.05$) and levels of TP and Alb significantly increased ($p<0.05$) but did not reach normal values.

Histopathological findings

The effects of the tomato pulp on the pathologic grading of hepatic steatosis in rats fed high-fat diet are listed in the Table 3. In histopathological examinations, no abnormalities wereobserved in the livers of rats inthe control group (Fig. 1a). But in rats in the group 2, which were fed with high-fat diet, sever steatosis was found as micro and macrovesicular fatty changes accompanied by hepatitis (Fig. 1b). Clofibrate prevented steatosisin rats in the group 3(Fig. 1c). In the group 4, tomato pulp obviously prevented fatty changes in hepatocytes(Fig. 1d). Figure 2 showsmicroscopic view of hepatic tissue stained by oil red-O method.

Effect of the tomato pulp on metabolism of fat due to high-fat diet

The results of the effects of the tomato pulp on metabolism of fat due to high-fat diet are presented in the Table 4. Clofibrate in the high-fat diet+Clofibrate group (group 3) significantly ($p<0.001$) decreased markedly elevated serum levels of TG, total cholesterol, LDL and VLDL in comparison with the high-fat diet group (Group 2) and significantly ($p<0.01$) increased slightly diminished serum levels of HDL in comparison with the Group 2. In the high-fat diet+Tomato pulp group (Group 4), tomato pulp significantly ($p<0.01$) decreased serum levels of total cholesterol, LDL and VLDL in comparison with the Group 2 and significantly ($p<0.05$) increased serum levels of HDL.

Effect of the tomato pulp on anti-oxidative activity of liver in damage induced by high fat diet

The results of the effects of the tomato pulp on anti-oxidative activity of liverare presented in the Table 5. In high-fat diet group (group 2), the levels of SOD, CAT, GPx and GRwere significantly ($p<0.01$) reduced in comparison with the normal control group (Group 1), and the values of MDA significantly ($p<0.01$) increased. In the high-fat diet+Clofibrate group (Group 3),Clofibratesignificantly ($p<0.01$) increased markedly diminished levels of SOD, CAT, GPX and GR in comparison with the high-fat diet group (Group 2) and significantly ($p<0.01$) decreased slightly elevated levels of MDA.In the high-fat diet+Tomato pulp group (Group 4),tomato pulp significantly ($p<0.05$) decreased the serum levels of ALT, AST, ALP and TB in comparison with the high-fat diet group (Group 2) and significantly ($p<0.05$) increased serum levels of TP and Alb,althoughdid not reach normal levels.

Discussion

In the current study, the results of biochemical and histopathological assessments reflected liver injuries in rats fed with high fat diet. Significant elevations in markers of liver injury (ALT, AST and ALP) as well as bilirubin and significant decrease in serum albumin and total protein in high fat fed diet rats were observed in comparison with normal healthy rats.

The increased levels of biomarker enzymes, AST, ALT and ALP are indicative of liver tissue injury (35). Sincethese serum liver biomarkers derangement have been evidenced in fatty liver disease (33, Chitturi and Farrell, 2001 S. Chitturi and G.C. Farrell, Etiopathogenesis of nonalcoholic steatohepatitis, *Seminars in Liver Disease***21** (2001), pp. 27–41. View Record in Scopus | Cited By in Scopus (310)35, 36), their levels were studied. Increased serum activities of AST, ALT and ALP were observed in high fat diet fed rats, indicating damage to hepatocytes. These results were consistent with the findings reported by Chidambarama and Venkatraman (35).Treatment with the tomato pulp notably prevented the elevation of these enzymes to an extent that was similar to the Clofibrate.

The biochemical results were in accordance with the histopathological findings. The rats fed with high-fat emulsion for 6 weeks developed a higher degree of steatosis. However, histopathological assessment of liver tissues from high fat induced hepatic steatosis displayed the antihepatosteatosis effects of the tomato pulp. Administration of the tomato pulp resulted in the inhibition of fat deposition in hepatocytes. Histopathological changes in agreement with biochemical findings were concordant with those previously reported (33).

Our results showed 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 areunable to cope up with excessive 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,which results in the progression of steatosis to steatohepatitis, fibrosis and cirrhosis (37).

Considering the recently recognized association between the oxidative stress and inflammation (35), 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 with high fat diet. We estimated antioxidant activities of the 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 to the normal control group. Thetomato 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

the tomato pulp is concordant with those of other investigators (38,39). The results of biochemical tests together with histological observations suggest that tomato pulp treatment lowers steatosis and prevents peroxidative damage and the effects are comparable with that of Clofibrate.

To analyze the possible role of the tomato pulp in lipid metabolism, which is the key factor in fatty liver formation, 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 were markedly increased in the high fat diet fed group compared to those in the control group. This finding was parallel to the previous study (20). Treatment of the high fat diet fed rats with the 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.

The results of the histological changes in high fat diet fed rats, widespread deposition of lipid droplets inside the parenchymal cells, are consistent with the result of the biochemical analysis. 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 an excessive accumulation of lipids within hepatocytes due to imbalance between lipid formation and lipid degradation (40). 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 (9). Previous study has showed that tomato hadhypolipidemic effects (18). In this study,the tomato pulp significantly improved both the biochemical and histological evidence of hepatic lipid accumulation. These results indicate that tomato pulp attenuates the disorder of lipid metabolism in liver resulted from high fat diet fed.

Taken together, tomato pulp may have a value as a safe preventive or therapeutic agent against hepatic steatosis and the use of this plant in fatty liver disease is then supported, but more work is warranted to elucidate the precise active substance(s), site(s) and myriad mechanism(s) of this pharmacological effect.

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