

# Protective effect of citrus lemon on inflammation and adipokine levels in acrylamide-induced oxidative stress in rats

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The high exposure to acrylamide (AA) due to smoking and increased consumption of processed and fast foods in recent years, has become one of the health threatening problems. This study examined the effect of lemon juice on inflammation and adipokines in acrylamide-induced oxidative stress in rats. Forty animals were divided into five groups. Toxicity was induced by AA (35mg/kg) for two weeks in all groups except normal control group. After that, lemon juice in three doses was administrated to treatment groups for 4 weeks. Serum levels of adipokines and inflammatory parameters and both serum and liver levels of oxidative stress parameters were measured. The results showed groups were received AA had significant higher levels of malondialdehyde, tumor necrosis factor alpha, leptin and C-reactive protein and lower levels of total antioxidant capacity compared to the negative control group. Lemon juice in all three doses significantly improved serum levels of TAC, MDA, TNF $\alpha$  and hs-CRP in treated groups. Also, 7.5 ml/kg lemon juice significantly decreased leptin levels. However, lemon juice had no significant effect on adiponectin levels. This study suggests lemon juice as a potential dietary alternative could attenuate leptin levels and manage oxidative and inflammatory damages in acrylamide- induced toxicity in rats.

**Keywords:** Citrus lemon. Acrylamide. Inflammation. Leptin. Adiponectin.

## INTRODUCTION

Acrylamide (AA) or 2-propenamide is an unsaturated and reactive compound (Schwend *et al.*, 2009). According to the International Agency for Research on Cancer (IARC), AA is a probable human carcinogen factor (Besaratnia, Pfeifer, 2007). It is not found naturally in food sources. It is produced in starchy foods such as crisps, bread, crackers, biscuits and breakfast cereals during high-temperature cooking by Maillard reaction (Richarme *et al.*, 2016). Cigarette and tobacco smoke are also one of the main routes of exposure to AA (Diekmann, Wittig, Stabbert, 2008). After AA absorption in the body, it is metabolized by direct conjugation with glutathione or oxidized to glycidamide resulted in the production of reactive oxygen species (ROS) and induction of lipid peroxidation and apoptosis in cells (Kocadağlı,

Gökmen, 2015). Moreover, this deleterious process has demonstrated related to inflammatory damages in various tissues. (Zhang *et al.*, 2013).

Leptin and adiponectin are two major adipocytokines which have pro-inflammatory and anti-inflammatory roles, respectively (Ohashi *et al.*, 2010; Shen *et al.*, 2009). Increased oxidative stress could be involved in cytokines and adipokines dysregulations, including decreased adiponectin transcription and increased levels of leptin, TNF- $\alpha$  and hs-CRP (Ejaz *et al.*, 2009; Tang *et al.*, 2012).

Citrus lemon (*Citrus limon*Burm.F) is a source of vitamin C, flavonoids and carotenoids (González-Molina *et al.*, 2010). Eriocitrin and hesperidin are the main flavonoids in lemon. The antioxidant activity of eriocitrin is more potent than other citrus flavonoids (Miyake *et al.*, 2007), so we conducted the current study on the lemon as a rich source of this antioxidant. In recent years, antioxidant and anti-inflammatory effects of citrus flavanone, eriocitrin, and hesperidin began to receive attention among researchers. It has been suggested that eriocitrin has

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protective effect on exercise-induced oxidative damage in rat liver (Minato *et al.*, 2003). In another study, the intake of 600 mg/day hesperidin increased adiponectin level and decreased the endothelial inflammation in patients with myocardial infarction (Haidari *et al.*, 2015). A recent study showed fresh lemon juice attenuated liver function biomarkers including bilirubin and liver enzymes (Wang *et al.*, 2017).

Increased consumption of fast food and canned food especially among young people and in western countries unavoidably associated with continuous and ubiquitous exposure to AA (Braithwaite *et al.*, 2014). Food and dietary compounds with antioxidant and anti-inflammatory properties may help body to cope with the adverse effects of AA. To the best of our knowledge, no previous study has reported the effects of citrus lemon on inflammatory biomarkers and adipocytokines in AA exposure. Therefore, this study was designed to investigate the effect of lemon juice on inflammation and adipokine levels in acrylamide-induced oxidative stress in rats.

## MATERIAL AND METHODS

### Animals

Forty male Wistar rats aged 6-8 weeks old (150-200 g) were obtained from animal house of Ahvaz Jundishapur University of Medical Sciences (AJUMS). The animals were housed in cages in an air conditioned room with a controlled temperature ( $22 \pm 3^\circ\text{C}$ ),  $55 \pm 5\%$  humidity, and a 12-hour light/dark cycle; and allowed standard *ad libitum* feed access and tap water. General guidelines for the use and care of animals for scientific purposes were considered, and this study was approved by the Research Ethics Committee of AJUMS, Iran (NCR-9305).

### Experimental protocol

After an acclimatization period of 2 weeks, the experimental animals were randomly divided into five groups (n=8) and treated as follows:

- I) Negative control (sham) received distilled water
  - II) Positive control received AA
  - III) AA- intoxicated rats treated with 2.5 ml/kg lemon juice
  - IV) AA- intoxicated rats treated with 5.0 ml/kg lemon juice
  - V) AA- intoxicated rats treated with 7.5 ml/kg lemon juice
- Oxidative stress in rats was induced orally by gavage

AA (35 mg/kg) daily (800830, Merck, Germany) for 2 week (El-Mehi, El-Sherif, 2015; Ghorbel *et al.*, 2017). Lemon was supplied from a local market collecting in Shush, a city located in Khuzestan province, Iran in December 2017. For removing the pulp; lemons were squeezed and filtered through What-man papers. After induced oxidative stress, treatment groups were received lemon juice in three doses orally with gavage tubes for 4 weeks. The dose of lemon juice was chosen based on the previous study (Chike, Dede, Otiede, 2011).

### Preparation of serum and liver samples

Twenty-four hours after the last treatment animals were anesthetized, and fasting blood samples were collected directly from the heart. Blood samples were centrifuged at  $4000 \times g$  for 10 min, and then serum samples were stored at  $-70^\circ\text{C}$  for further analysis of parameters. The livers of animals were also removed, weighed and rapidly washed in cold saline (0.9%) and then placed in ice-cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA. The livers were then chopped into 4-5 volumes of 50 mM phosphate buffer (pH=7.4) and homogenized by a homogenizer fitted with a Teflon pestle. For providing supernatant, the homogenate was then centrifuged at several times and the lipid layer was carefully removed and the. The supernatant was stored at  $-70^\circ\text{C}$  until use (Haidari *et al.*, 2013).

### Assessment of the Biochemical Parameters

TNF- $\alpha$ , hs-CRP, leptin, adiponectin and total antioxidant capacity (TAC) were measured using an enzyme-linked immunosorbent assay (ELISA) method and with commercial kits (Orgenium laboratories-Finland for TNF- $\alpha$ ; Boster- China for leptin and adiponectin; Randox labs- UK for TAC and Labor Diagnostika Nord for hs-CRP) according to the manufacturer's instructions. The concentration of malondialdehyde (MDA) was assayed by spectrophotometry. The method is based on the reaction of MDA with thiobarbituric acid (Janero, 1990).

### Statistical analysis

All data were expressed as mean  $\pm$  standard deviation (SD). To examine the normality of distribution, Kolmogorov-Smirnov test was used. Data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Tukey test using SPSS version 17 (SPSS Inc, Chicago, Illinois) software.  $P < 0.05$  was defined as statistically significant.

## RESULTS AND DISCUSSION

In recent years the high exposure to AA has become one of the health threatening problems because of increased consumption of processed and fast foods, and smoking (Braithwaite *et al.*, 2014; Ng *et al.*, 2014). So, it is noteworthy to find effectively non-drug treatment with natural food ingredients dealing with noxious effects of AA.

### Effect of citrus lemon administration on serum and liver oxidative stress

The results showed that the AA administration decreased both serum and liver TAC levels significantly in the positive control group compared to the negative control group ( $P < 0.01$  and  $P < 0.01$ , respectively). In contrast, both serum and liver MDA levels significantly increased in positive control group compared to the negative control group ( $P = 0.05$  and  $P < 0.05$ , respectively). The administration of lemon juice in 5.0 and 7.5 ml/kg doses caused to a significant increase in serum and liver TAC levels ( $P < 0.05$ ). The results also showed that the AA-

intoxicated rats treated with 5.0 and 7.5 ml/kg lemon juice had significantly lower serum and liver levels of MDA compared to the positive control group at the end of the study ( $P < 0.05$ ) (Table I).

In the present study, citrus lemon juice treatment in a dose-dependent manner improved AA-induced oxidative stress biomarkers, including MDA and TAC. Several studies have demonstrated that Citrus lemon and its bioactive components like flavonoids and vitamin C with antioxidant properties could suppress oxygen-free radicals. Olukanni *et al.* (2013) showed that administration of lemon juice (10%) for 5 weeks increased serum glutathione levels significantly, but did not have significant effects on antioxidant enzymes in rats (Olukanni *et al.*, 2013). Lemon flavonoids have been also shown improved antioxidant status. Improving levels of MDA and glutathione redox status in rat liver during acute exercise by eriocitrin administration prior to exercise has been reported (Minato *et al.*, 2003).

Elavarasan *et al.* (2012) demonstrated that the administration of hesperidin (100 mg/kg/day) for 90 days increased catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione

**TABLE I** - Effect of citrus lemon supplementation on serum and liver oxidative stress biomarkers<sup>a</sup>

Groups	Serum TAC (mmol/L)	Liver TAC (u/mL)	Serum MDA (μmol/L)	Liver MDA (nmol/mg)
Negative control(sham)	9.46±0.58	9.73±0.81	5.24±2.29	4.95±2.45
Positive control	7.88±1.17 <sup>##</sup>	7.86±1.00 <sup>##</sup>	8.72±3.24 <sup>#</sup>	9.28±4.13 <sup>#</sup>
AA + lemon juice (2.5 ml/kg)	9.21±1.02 <sup>*</sup>	8.49±1.02	5.86±1.41	6.21±1.49
AA + lemon juice (5.0 ml/kg)	9.59±0.97 <sup>*</sup>	10.20±1.50 <sup>**</sup>	4.74±1.23 <sup>*</sup>	5.13±1.43 <sup>*</sup>
AA + lemon juice (7.5 ml/kg)	9.45±0.66 <sup>**</sup>	10.58±1.58 <sup>**</sup>	5.17±1.13 <sup>*</sup>	5.60±1.13 <sup>*</sup>

a: All values are expressed as mean ± SD. ANOVA followed by Tukey test was used for statistical analysis. \*: Indicates  $P < 0.05$ ; \*\*:  $P < 0.01$  vs. positive control group; #:  $P < 0.05$  and ##:  $P < 0.01$  vs. negative control group. Acrylamide (35mg/kg). TAC: total antioxidant capacity; MDA: malondialdehyde. AA: Acrylamide

**TABLE II** - Effect of citrus lemon supplementation on serum TNF- $\alpha$  and hs-CRP levels<sup>a</sup>

Groups	TNF- $\alpha$ (pg/mL)	hs-CRP (mg/L)
Negative control(sham)	31.13±13.72	631.37±111.64
Positive control	56.13±18.61 <sup>#</sup>	895.25±82.90 <sup>##</sup>
AA + lemon juice (2.5 ml/kg)	26.25±11.08 <sup>**</sup>	737.28±56.73 <sup>**</sup>
AA + lemon juice (5.0 ml/kg)	27.15±13.43 <sup>*</sup>	738.28±60.95 <sup>**</sup>
AA + lemon juice (7.5 ml/kg)	21.09±7.70 <sup>**</sup>	721.00±123.47 <sup>**</sup>

a: All values are expressed as mean ± SD. ANOVA followed by Tukey test was used for statistical analysis. \*: Indicates  $P < 0.05$ ; \*\*:  $P < 0.01$  vs. positive control group; #:  $P < 0.05$  and ##:  $P < 0.01$  vs. negative control group. Acrylamide (35mg/kg). TNF $\alpha$ : tumor necrosis factor  $\alpha$ ; hs-CRP: high sensitive C-reactive protein. AA: Acrylamide

reductase (GR) levels and decreased serum MDA in the heart tissue in senescent rats. Other components of citrus lemon such as carotenoids showed the antioxidant effect. Kobori *et al.* (2014) in an animal study revealed  $\beta$ -Cryptoxanthin suppressed inflammation through preventing lipopolysaccharide (LPS) and TNF $\alpha$  genes in NASH (Kobori *et al.*, 2014). Previous studies also indicated synergistic properties of polyphenols with together in fruits and vegetables (Liu, 2003). So, in the same line with our findings, it seems that citrus containing flavonoids could enhance the ability to scavenge superoxide radicals formed during AA-induced oxidative stress (Elavarasan *et al.*, 2012).

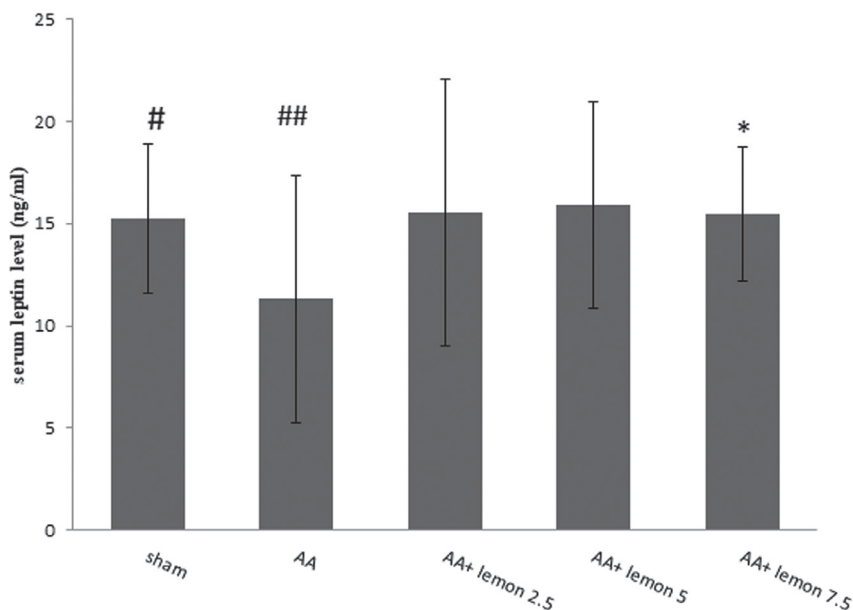
### Effect of citrus lemon administration on serum TNF $\alpha$ , hs-CRP leptin and adiponectin

The results revealed that the positive control group had significant higher levels of TNF $\alpha$ , hs-CRP and leptin compared to the negative control group at the end of study ( $P < 0.05$ ,  $P < 0.01$  and  $P < 0.01$ , respectively). The Lemon juice administration in all three doses significantly decreased the serum levels of TNF $\alpha$  and hs-CRP compared to the positive control group ( $P < 0.05$ ). The administration of 7.5 ml/kg lemon juice also decreased leptin levels in the treated group compared to the positive control group ( $P < 0.05$ ) (Figure 1).

TNF- $\alpha$  as a cytokine mediate the inflammatory pathway through the transcription of NF- $\kappa$ B, mitogen-activated protein kinase (MAPK), and inducing ROS production (Jeon *et al.*, 2013; Zhou, Mrowietz, Rostami-

Yazdi, 2009). Moreover, ROS can trigger p38 which regulate cell death and phosphorylation in many cells causing the ability to activate the inflammatory cascade (Bao, Wu, Lu, 2010; Bai *et al.*, 2013). It is suggested that p38 is a pro-inflammatory mediator. Oxidative stress could lead to increase NF- $\kappa$ B activation and TNF- $\alpha$  gene transcription; in addition, TNF- $\alpha$  induces p38 and c-Jun NH2-terminal kinase (JNK) (Chandel *et al.*, 2000; Daniele *et al.*, 2015).

Increased inflammatory factors during AA-induced toxicity have been previously reported (Zhang *et al.*, 2013). However, little is known about the anti-inflammatory effects of lemon juice, and its effects on AA-induced inflammatory damages remain unclear. A study on lemon fruit indicated the anti-inflammatory and protective effect of this fruit extract against cyclophosphamide-induced intestinal and pancreatic injury in mice (Quita, Balbaid, 2015). Pantsulaia *et al.* (2014) suggested the citrus peel extract could prevent the development of liver injury with reduction of inflammatory biomarkers, TNF- $\alpha$  and interferon (Pantsulaia *et al.*, 2014). In another study oral administration of hesperidin, one of the main flavonoids in Citrus lemon, (500 mg/day for 3 weeks) reduced hs-CRP levels in subjects with the metabolic syndrome (Rizza *et al.*, 2011). In the present, the significant higher levels of TNF- $\alpha$ , hs-CRP and leptin in the positive control group compared to the negative control group study might be due to oxidative damage of AA. Consequently, the decreased levels of inflammatory factors in the treatment groups might be down to the antioxidant properties of lemon juice and its inhibitory effects on the generation of TNF- $\alpha$ .



**FIGURE 1** - Effect of citrus lemon supplementation on serum leptin levels.

This study is one of the first to investigate changes in serum levels of leptin associated with AA toxicity. Also, the effect of lemon juice on serum levels of leptin has not been studied yet. In the present study, administration of the higher dosage of lemon juice (7.5 ml/kg/day) could reduce the elevated level of leptin in AA-intoxicated rats. Previous studies revealed the protective role of adiponectin and the destructive effect of high amounts of leptin level on liver (Machado *et al.*, 2012; Matsunami *et al.*, 2010). In addition, hepatotoxicity effect of AA has been demonstrated in several studies (Seydi *et al.*, 2015; Zhang *et al.*, 2013). From present findings, we can conclude AA-induced hepatotoxicity may impair hepatic leptin signaling resulting in increased levels of leptin. Leptin signaling could be regulated by the nutritional status, which is sensed by AMPK in rat liver (Brabant *et al.*, 2005). There is another possible hypothesis that both leptin and AA in different mechanisms but synergistic or additive effects exacerbate hepatotoxicity. Therefore, it seems that increased levels of leptin may be one of the mechanisms contributing to AA-induced hepatotoxicity.

Available evidence suggests the antioxidant components may involve in modulating leptin levels in oxidative stress (Lin *et al.*, 2015). In a study, naringin, a citrus antioxidant, suppressed leptin-induced activation of the p38 MAPK pathway in cardiomyoblasts (Chen *et al.*, 2014).

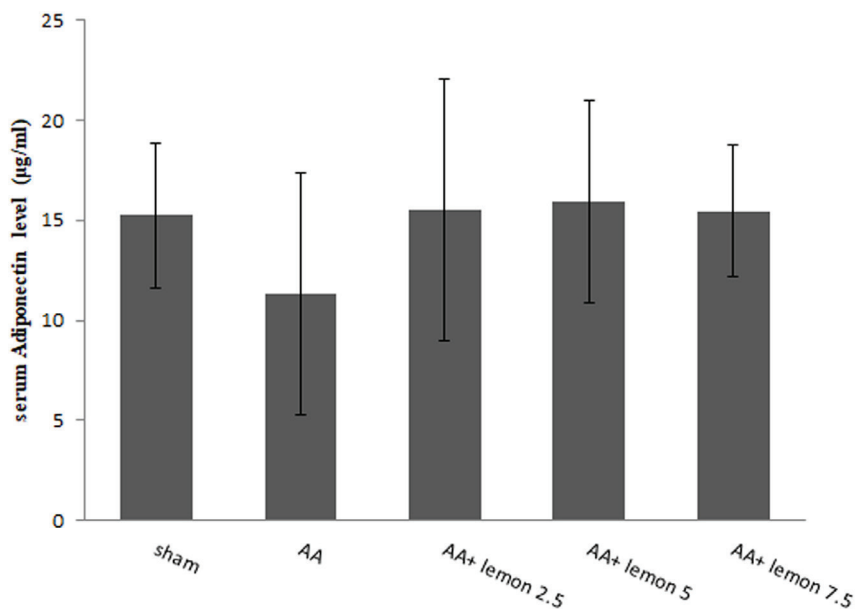
Although few studies have reported the modulator effect of citrus fruits or their flavonoids on adiponectin levels, in the present study citrus lemon juice did not alter adiponectin levels in the treated groups (Figure 2). It should be noted that studies with a higher dosage of lemon extracts have reported different results on adiponectin levels (Mahmoud, 2013).

## CONCLUSION

In conclusion, our results suggest that lemon juice as a potential dietary alternative may be helpful to manage oxidative and inflammatory damages and regulate leptin abnormalities in AA intoxication. Moreover, the results give a major support of reported anti-inflammatory activity of citrus that may be of importance in the treatment of other immune and inflammatory diseases. Additional studies should be performed to determine the influence of citrus consumption in people at risk of high AA exposure.

## ACKNOWLEDGMENT

This paper is issued from the MSc. thesis of Mojdeh Fathi. This work was financially supported by a grant from Nutrition and Metabolic Disease Research Center, Ahvaz Jundishapur University of Medical Sciences (Grant Number: NRC-9305).



**FIGURE 2** - Effect of citrus lemon supplementation on serum adiponectin levels. ANOVA followed by Tukey test was used for statistical analysis. \*: Indicates  $P < 0.05$ ; \*\*:  $P < 0.01$  vs. positive control group; #:  $P < 0.05$  and ###:  $P < 0.01$  vs. negative control group. Acrylamide (35mg/kg).



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Received for publication on 21<sup>th</sup> June 2018

Accepted for publication on 20<sup>th</sup> December 2018