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# The protective effects of eugenol on metabolic-syndrome, renal damages

Fatemeh Kourkinejad Gharaei<sup>1,2</sup>, Halimeh Lakzaei<sup>3</sup>, Abbass Ali Niazi<sup>4</sup>, Mehdi Jahantigh<sup>4</sup>,  
Mohammad Reza Shahraki<sup>5</sup>, Tahereh Safari<sup>5,6\*</sup>

<sup>1</sup>Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>2</sup>Student Research Committee, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>3</sup>School of Medicine, Department of Physiology, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>4</sup>School of Medicine, Department of Pathology, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>5</sup>School of medicine, Department of Physiology, PhD, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>6</sup>Pharmacology Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

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## ABSTRACT

**Introduction:** Metabolic syndrome consists of a group of abnormalities which is involved with chronic kidney disease and nephropathy. Eugenol is an important phenolic component, which is present in many plants' essential oils such as cloves oil with antioxidant effects.

**Objectives:** Our study planned to demonstrate eugenol's effects over nephrotoxicity derived from metabolic syndrome.

**Materials and Methods:** Thirty-five male Wistar rats were picked accidentally and then divided into five groups including 1) tap water; 2) water with fructose 10%; 3) water with fructose + sweet almond oil and administered intraperitoneally; 4) water with fructose+ eugenol 50 mg/kg/d and administered intraperitoneally; 5) water with fructose+ eugenol 100 mg/kg/d administered intraperitoneally. This regime lasted for 60 days, and at the beginning of day 31st, injections started for 30 days. Assessment of serum, urine and renal parameters (in homogenized kidney tissue) were conducted in the last step.

**Results:** The results argued that the induction of metabolic syndrome following renal injury has significantly increased serum blood urea nitrogen (BUN) and creatinine (Cr) levels in the fructose group. Consumption of eugenol resulted in a significant reduction in the level of these two biochemical factors ( $P < 0.05$ ). The renal level of malondialdehyde (MDA) increased in the fructose group while treatment with a dose of 50 eugenol decreasing its level ( $P < 0.05$ ). Proteinuria and kidney tissue damage score (KTDS) increased in the fructose group compared with the tap water group ( $P < 0.001$ ). It is noteworthy that treatment with eugenol did not affect the level of proteinuria and KTDS with any of the used doses.

**Conclusion:** Our results indicated the improvement of renal functioning and decrease in lipid peroxidation, although eugenol doses used in this study did not reduce proteinuria and KTDS.

### Implication for health policy/practice/research/medical education:

In the current study, 35 male Wistar rats were selected. Metabolic syndrome was induced using a fructose-rich diet and then the protective effects eugenol 50 and 100 mg/kg/d doses on renal damage due to metabolic syndrome were investigated. The results of this study indicated the improvement of renal functioning and decrease in lipid peroxidation, although eugenol doses used in this study did not reduce proteinuria and kidney tissue damage score.

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## Introduction

Metabolic syndrome includes a group of disorders such as abdominal obesity, hyperglycemia, dyslipidemia and hypertension (1-3). They are associated with insulin resistance, often together with weight gain and obesity. People suffering from metabolic syndrome are at increased

risk of diabetes, renal and cardiovascular diseases (3,4). New evidence suggests that the imminence of chronic renal illness is higher in people with metabolic syndrome (5, 6). According to recent studies, prevalence of metabolic syndrome is 34% globally (7). Important factors in the development of this disorder are high carbohydrate and

\*Corresponding author: Tahereh Safari, Ph.D, Email: tahereh\_safari@yahoo.com

high-fat diets (8,9). Studies have shown that application of fructose as a refined sweetener in modern nutrition is increasing, with daily intake amounting from 16-20 g to 85-100 g (10,11).

Various studies have suggested different pathways for tissue damage in the metabolic syndrome. One of the pathways is inducing and producing oxidative stress and free radicals respectively. The metabolic syndrome increases free radicals, namely, reactive oxygen species that lead to imbalances in antioxidant activities leading to cellular dysfunctioning (12,13). According to the current studies, oxidative stress plays some important roles in the happening of different diseases including kidney damage (14).

4-allyl-2-methoxyphenol also called as eugenol is a phenolic compound which is utilized being an additive in food production and fragrant (15). Many plants like clove, basil, and nutmeg extracts are saturated with eugenol. Biological activities of eugenol include antioxidant, neuroprotective, anti-inflammatory etc (16). Sheen and colleagues demonstrated eugenol protective effects against oxidative stress and gentamicin-induced nephrotoxicity (2).

### Objectives

Considering the importance of the metabolic syndrome and the protective role of eugenol, the subsequent questions are propounded: 1) Do biochemical parameters' levels in serum and kidney tissue vary after the injection of eugenol in rats affected with metabolic syndrome? 2) Are there any pathological alterations in nephric parenchyma after the application of eugenol in rats with induced metabolic syndrome? Thus this study was designed for assessment of eugenol's role in prevention of renal damages because of metabolic syndrome.

### Materials and Methods

#### Animals

In the current study, 35 Wistar rats which all had male gender and accidentally selected in the weight range of  $184.4 \pm 7.2$  g which were prepared from Zahedan University of Medical Sciences' animal center. The environmental temperature of storage place was 23 to 25°C. Rats had freeform water reaching, standardized rat food nourishment, and 12 hours light as daylight exposure and 12 hours darkness as night time. One week before the test they also adapted to their diet.

#### Medications

D-fructose >99% was purchased from Syarikat System Malaysia Company. Eugenol was gained from Sigma (St. Louis, MO, USA). Sweet almond oil was procured from Kimiagartoos Pharmaceutical Company in Iran.

#### Experiment's protocol

The animals randomly were allocated into five groups

including 1) tap water; 2) water with fructose 10%; 3) water with fructose 10% plus sweet almond oil and administered intraperitoneally; 4) water with fructose 10% plus eugenol 50 mg/kg/d (17) dissolved in sweet almond oil and administered intraperitoneally; 5) water with fructose 10% plus eugenol 100 mg/kg/d (17) dissolved in sweet almond oil and administered intraperitoneally. The above rats consumed the water saturated with fructose for 60 days (18). After 30 days, on the next day, eugenol and almond oil injection started for duration of 30 days. Throughout the survey, every day the animal's net weight was determined and registered. One day after the administration completion, anesthetizing with the mixture of ketamine (75 mg/kg, intraperitoneally) and 10 mg/kg intraperitoneally xylazine occurred and then blood samples from the heart of individual animal were taken.

### Measurements

Utilization of Pars Azmoon's kits (from Iranian company) the levels of serum creatinine (Cr), blood urea nitrogen (BUN) and proteinuria were measured quantitatively. We determined nitrite oxide stable metabolite and nitrite level in serum and supernatant via the Griess reaction of colorimetric assay kit (ZellBio, Germany). Homogenized tissue supernatant and serum level of malondialdehyde (MDA) were calculated manually (19, 20). Before tissue staining we use 10% formalin for fixation and paraffin. We colored tissue sections with hematoxylin-eosin staining prior to their examination. Then two pathologists assessed the slides blindly. Tissue pathology was assessed by vehemence of distal tubular damages, transparent renal cast, wastages and debris, flattening and demolition of tubular cell, vacuolization and widening of tubular lumen and scored from 1 to 4. Overall score was determined as kidney tissue damage score (KTDS) which was reported 1 to 4, whereas natural tubes without any damage considered zero scores.

### Ethical issues

This study was confirmed by the Ethics Committee of Zahedan University of Medical Sciences. As an experiment, the protocols were approved to be in accordance with the guidelines of Animal Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMS.REC.1397.258). This study was extracted from the MD thesis of Fatemeh Kourkinejad at this University (Thesis #1397-8997).

### Statistical analysis

By means of mean  $\pm$  SEM the data are reported. One-way analysis of variance (ANOVA) was applied in analysis if MDA, nitrite, Cr, BUN laboratory test results and kidney weights. Then the post hoc Tukey test also has been done. Evaluation of histopathological results was conducted with Kruskal-Wallis and Mann-Whitney U tests. We invoked SPSS version 16<sup>th</sup> for data analysis. *P* values lower

than 0.05 were cogitated significant statistically.

## Results

Collation of mean delta weight of animals in different groups showed a significant difference amongst the group receiving fructose besides tap water group ( $P < 0.05$ ). Treatment with eugenol significantly reduces body weight in comparison to fructose ones ( $P < 0.05$ ) as revealed in Figure 1A. It should be noted that the mean left kidney weight in the dissimilar groups did not demonstrate a significant difference (Figure 1B).

The results showed that the induction of metabolic syndrome following renal injury has significantly increased serum BUN and Cr levels in fructose in comparison with tap water group ( $P < 0.001$ ). Applying eugenol at dosages of 50 and 100 resulted in a significant reduction in serum BUN and Cr levels in comparison with the fructose group ( $P < 0.05$ ). This two administrations of eugenol (50 and 100 mg/kg/d) have the same effects on BUN and Cr levels (Figure 1C and D). The mean serum nitrite and MDA levels did not display any significant differences amongst the studied groups (Figure 1A and B). However, the kidney level of MDA and nitrite increased with the induction of metabolic syndrome in the fructose group and treatment with a dose of 50 eugenol decreased MDA level ( $P < 0.05$ ) as shown in Figure 2C and D. On the other hand, the levels of renal nitrite among different groups are not significantly different (Figure 2). The amount of urine protein suggests a kidney damage following the induction of a metabolic syndrome in fructose comparison to the tap water recipient group ( $P < 0.001$ ) as shown in Figure 3. It is noteworthy that treatment with eugenol did not affect

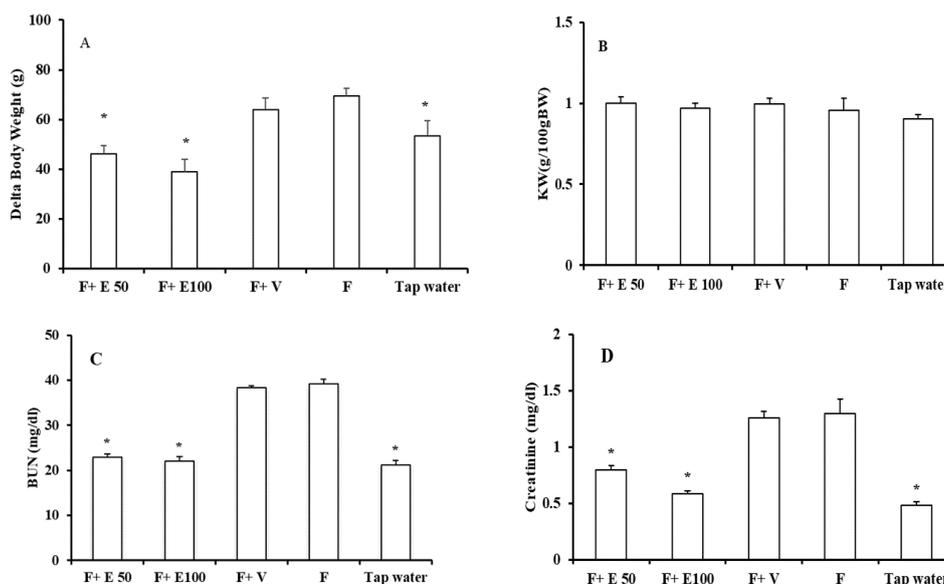
the level of this factor with any of the used doses. Tissue damage assessment by KTDS among the groups suggests that the metabolic syndrome caused kidney damage ( $P < 0.01$ ), but treatment with eugenol did not affect the KTDS (Figure 3).

## Discussion

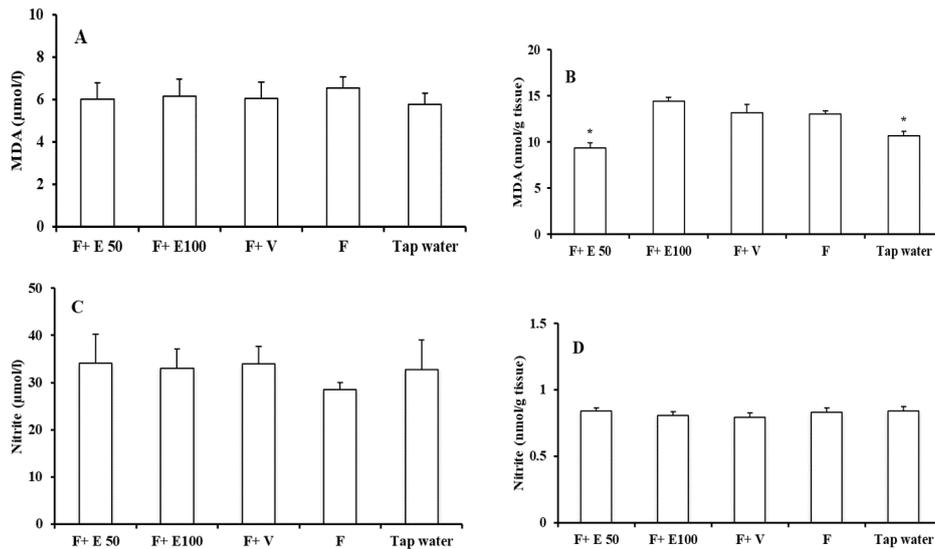
The results of this study indicate renal injury after induction of metabolic syndrome. Despite improvements in renal function, factors such as serum BUN and Cr levels by eugenol, KTDS and proteinuria are not affected by treatment with different eugenol doses.

Several studies approve the antioxidant and anti-inflammatory properties of eugenol (1,16,21). Their reports also indicate that eugenol performs as a free radical collector and reduces oxidative stress, lipid peroxidation, and improves antioxidant defense. The results of their studies indicate an enhancement in the glutathione's level, superoxide dismutase, reducing tumor necrosis factor alpha and prostaglandin E2. Eugenol can also reduce MDA by decreasing lipid peroxidation. Thus, the effects of eugenol are the improvement of antioxidant enzymes together with decreasing the inflammatory factors (15).

Another study has reported that eugenol improves the antioxidant capacity and arsenic trioxide -induced nephrotoxicity (21). In this regard, there is evidence that eugenol improved gentamicin nephrotoxicity by reparation oxidative stress, collecting free radicals and diminishing peroxidation of lipid and improving antioxidant defense. In fact, eugenol has its own effects by improving lipid peroxidation, increasing antioxidant defense, eventually improving tissue hypoxia (1).



**Figure 1.** Evaluation of body weight (A), left kidney weight, g/100g BW (B), BUN, serum level of blood urea nitrogen (C) & creatinine (D). The groups taken tap water, fructose (F), Fructose+ eugenol 50 mg/kg (F+E 50), Fructose+ eugenol 100 mg/kg (F+E 100) and Fructose+ almond oil (F+V). \* Significant differences compared to fructose and fructose+ almond oil group ( $P$  value  $< 0.05$ ).



**Figure 2.** Evaluation of MDA's serum level (A), kidney level of MDA (B), serum level of nitrite (C) and kidney level of nitrite (D). The groups received, tap water, fructose (F), Fructose+ eugenol 50 mg/kg (F+E 50), Fructose+ eugenol 100 mg/kg (F+ E100) and Fructose+ almond oil (F+V). \* Significant differences compared to fructose and fructose+ almond oil group ( $P<0.05$ ).

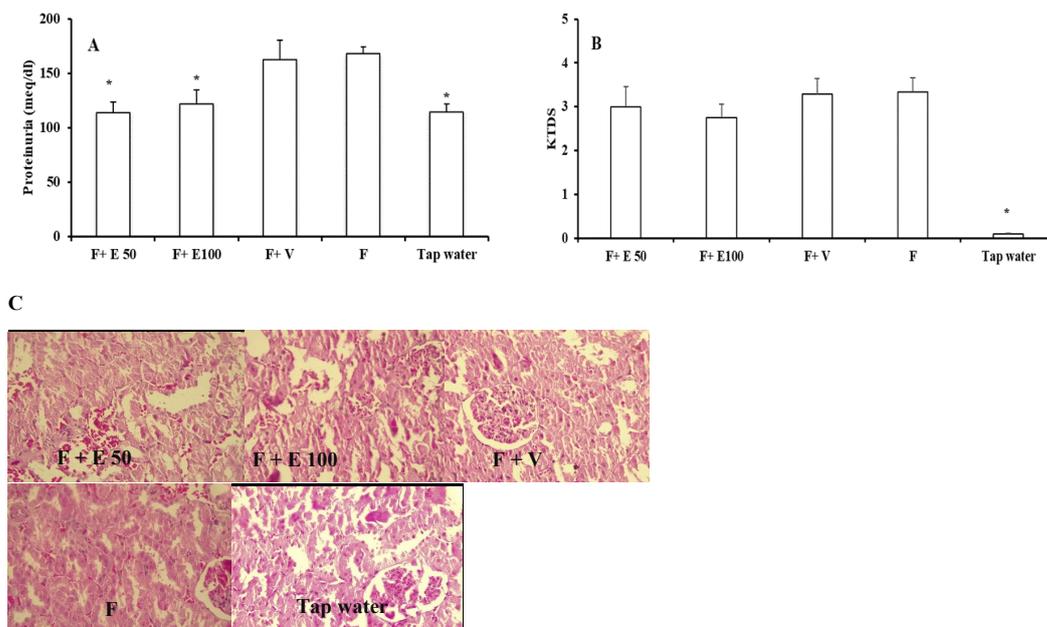
In this regard, eugenol by its hydroxyphenolic group eliminates electrons from free radicals and prevents oxidation of  $Fe^{2+}$  by  $H_2O_2$ . Consequently, it reduces radical  $\dot{O}H$  production, which begins with lipid peroxidation. There are also reasons that eugenol reduces cytokines by suppressing cyclooxygenase II and inhibits cell proliferation (1).

Garud et al reported the effects of eugenol on diabetic nephropathy. They showed that eugenol at dosages of 5

and 10 mg/kg for 28 days reduced the gene-expression of transforming growth factor beta 1 ( $TGF-\beta 1$ ) level and improved tissue damage in diabetic rats (22).

### Conclusion

Our results also indicate improved BUN, Cr and decrease in lipid peroxidation, although eugenol doses used in this study did not reduce proteinuria and KTDS. This finding may be related to the applied doses or their duration.



**Figure 3.** Evaluation of urine protein (A), KTDS, kidney tissue damage score (B) and pathology images (magnification  $\times 400$ ) of kidney tissue (C). The groups received, tap water, fructose (F), Fructose+ eugenol 50 mg/kg (F+E 50), Fructose+ eugenol 100 mg/kg (F+ E100) and Fructose+ almond oil (F+V). \* Significant differences compared to fructose and fructose+ almond oil group ( $P<0.05$ ).

### Authors' contribution

TS and FK planned, guided, oversaw and evaluated and analyzed the study and ready the first draft of document. HL, FK, AN, MJ, MS and TS partaken in the running of the study and gathered the data. TS and FK shared in the inscription and correcting of the article. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

### Conflicts of interest

The authors declare that they have no competing interests.

### Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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