

# Protective Effect of Safranal against Gentamicin-Induced Nephrotoxicity in Rat

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## Abstract

Gentamicin is an important aminoglycoside antibiotic. However its use is limited to serious and life threatening gram negative infections, because of its high nephrotoxicity potential in patients. There are reports that safranal, the active ingredient of saffron with antioxidant properties, exerts protective effect against ischemic injuries occurred by certain nephrotoxins including gentamicin. Therefore, in the present study, we examined the protective effect of safranal against gentamicin-induced nephrotoxicity in rat. After acclimatization, animals were randomly divided to three groups (8 rats /each group). On day one, each animal was placed separately in a metabolic cage for collecting 24-hour urine samples. On day two, after collecting urine samples for measuring glucose and protein, the rats in group 1 received saline 1 ml/kg for 6 days, those in group 2 received gentamicin 80 mg/kg/day for 6 days, and the remaining rats in group 3 received safranal 0.5 ml/kg followed by gentamicin 80 mg/kg/day for 6 days. Injections were intraperitoneally. All the animals were euthanized 24 hours after the last dose. Blood samples were collected by cardiac puncture, and concentration of blood urea, creatinine, and urinary glucose and protein, as the indicators of nephrotoxicity were measured. Our results showed that in group 2, concentration of blood urea nitrogen  $p < 0.01$ , creatinine  $p < 0.05$ , urinary glucose  $p < 0.001$ , and protein  $p < 0.01$  were significantly increased compared with the control and safranal-treated groups. There was no significant difference between the control and safranal-treated groups. Safranal exerts protective effects against gentamicin-induced nephrotoxicity in rat.

**Iran J Med Sci 2009; 34(4): 285-288.**

**Keywords** • Safranal • gentamicin • nephrotoxicity

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Received: 28 December 2008

Revised: 8 March 2009

Accepted: 23 April 2009

## Introduction

**G**entamicin is an important aminoglycoside antibiotic. Its use is limited to serious and life threatening gram negative infections because of its high nephrotoxicity potential by inhibiting the protein synthesis in renal cells in 13-30% of treated patients. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure.<sup>1</sup>

*Crocus sativus* L. (Iridaceae), commonly known as saffron is used in folk medicine for various purposes such as an aphrodisiac, antispasmodic, and expectorant.<sup>2</sup> Modern pharmacological studies have demonstrated that saffron extracts exert antitumor,<sup>3-5</sup> free radical scavenger, hypolipemic,<sup>6</sup> and anticonvulsant activities,<sup>7</sup> as well as improving learning and memory,<sup>6,8</sup>

Phytochemical studies on *Crocus sativus* have shown the presence of constituents such as crocin, crocetin, safranal, and picrocrocin.<sup>9-11</sup> Among constituents of saffron extract, crocetin is mainly responsible for these pharmacological activities.<sup>6</sup>

In traditional medicine, the stigma of this plant is used as an antiedematogenic remedy.<sup>2</sup> In addition some studies indicated that saffron has antioxidant activity.<sup>12-19</sup> An investigation showed that aqueous extract of saffron could inhibit cisplatin, cyclophosphamide, mitomycin-C, and urethane induced alterations in lipid peroxidation.<sup>16</sup> Also our laboratory findings showed that safranal was able to protect kidney against hexachlorobutadiene-induced nephrotoxicity and ischemia/reperfusion injury in rat.<sup>17,18</sup> Therefore, it was decided that validation of the protective effect of safranal against gentamicin-induced nephrotoxicity also be investigated.

### Materials and Methods

Gentamicin was purchased from (Daru-Pakhsh Pharmaceutical Company, Tehran, Iran) and safranal was obtained from (Fluka Chemie, Switzerland).

In the present study, Wistar albino rats weighing 150-200g of either sex, provided by Animal Breeding Unit, Department of Pharmacology, School of Medicine, Mashhad, Iran, were used. The animals were kept under light/dark cycle with 20 °C and 50% humidity for 12 hours. After acclimatization, the animals were divided randomly into three groups (8 rats / each group), and placed in metabolic cages separately for collecting 24-hour urine samples.

After collecting the first urine samples, the rats in group 1 received saline 1 ml/kg for 6 days, those in group 2 received gentamicin 80mg/kg/day for 6 days,<sup>19</sup> and the remaining rats in group 3 received safranal 0.5ml/kg<sup>17</sup> plus gentamicin 80 mg/kg/day after 1 hour for 6 days. Urine samples were collected daily. All injections were intraperitoneal.

Twenty-four hours after the last injection, urine samples were collected for measuring glucose and protein, using enzymatic method glucose oxidase that catalyses the oxidation of glucose to gluconic acid, and turbidimetry (trichloroacetic acid),<sup>20</sup> respectively. Then the

animals were euthanized under ether anesthesia. Blood samples were collected by cardiac puncture for measuring urea and creatinine as an indicator of kidney damage, using urease (a nickel-metallo enzyme that catalyzes the degradation of urea to ammonia and carbon dioxide),<sup>21</sup> and Jaffé (the combined use of creatinine, amidohydrolase, and alkaline sodium picrate) methods, respectively.<sup>22</sup>

### Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). The differences among treated groups were analyzed by one-way ANOVA followed by Tukey test. P<0.05 was considered statistically significant.

### Results

Concentrations of urea, creatinine, glucose, and protein, as indicators of kidney damage, are shown in table-1. Concentration of urea in gentamicin-treated group was significantly higher than the control and safranal-treated groups (P<0.001). Concentration of creatinine, in gentamicin-treated group was also higher (P<0.05) than the control and safranal-treated groups. Concentration of urinary glucose in gentamicin-treated group was significantly higher than the control (P<0.001) and safranal-treated groups (P<0.01). And concentration of urinary protein in gentamicin-treated group was significantly higher than the control (P<0.01) and safranal-treated groups (P<0.05). There was no significant difference between the control and safranal-treated groups.

### Discussion

Our findings indicated that safranal, the active constituent of *Crocus sativus* L. is able to protect kidneys against gentamicin-induced nephrotoxicity in rats. Concentration of blood urea, creatinine, and urinary glucose and protein were used as indicators of damage to kidney.

It has been shown that aqueous extract of saffron inhibits oxidative stress caused by cisplatin, cyclophosphamide, and mitomycin-C.<sup>16</sup> Saffron also increases the reduced intracellular glutathione and enzymes including glutathione reductase and glutathione-S-transferase.<sup>23</sup>

**Table 1:** Concentration of blood urea, creatinine, and urinary glucose and protein in different animal groups in the last day of study

Animal group	Blood urea (mg/dl)	Blood creatinine (mg/dl)	Urinary glucose (mg/dl)	Urinary protein (mg/dl)
Control	33.75 ± 1.6***	0.53 ± 0.02*	7 ± 0.71***	1.68 ± 0.09**
Gentamicin	85 ± 7.6	1.95 ± 0.49	15.75 ± 0.85	3.83 ± 0.17
Safranal + gentamicin	30.95 ± 3.7***	0.59 ± 0.08*	9.88 ± 0.89**	2.45 ± 0.35*

\* P<0.05 \*\*P<0.01 \*\*\*P<0.001 compare with gentamicin-treated group

There are other investigations on pharmacological effect of safranal including the effect on extracellular hippocampal levels of glutamate and aspartate,<sup>24</sup> inhibition of pentylentetrazol-induced seizures,<sup>25</sup> and radical scavenging activity.<sup>26</sup> These studies indicated that safranal was a potent antioxidant and able to protect body organs against certain toxic materials.

In conclusion, findings of the present study show that safranal is a protective agent against gentamicin-induced nephrotoxicity in rat. However, the exact protective mechanism(s) of safranal is unknown and need to be more investigated.

### Acknowledgement

This study was financially supported by the Vice-Chancellor for Research, Mashhad University of Medical Sciences, Mashhad, Iran.

**Conflict of Interest:** None declared

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