

Effect of Co-administration Rotenone and Crocin on Biochemical and Histopathological Changes in Rat's Kidney

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ABSTRACT

Background: Rotenone is one of the most widely used pesticides in agriculture and may affect public health through the food chain. A possible mechanism of rotenone toxicity is oxidative stress. This study, the supportive effect of crocin (a substance derived from saffron) as an antioxidant on the renal toxicity of rotenone in an experimental animal model was investigated. **Methods:** First, male rats were divided into 4 groups (6 in each group) and received saline, crocin, rotenone and rotenone-crocin combination intraperitoneally for 10 days, respectively. Blood and kidney tissue samples were then isolated and biochemical factors including creatinine, nitrogen blood urea (BUN), sodium, potassium, Malondialdehyde and catalase were measured. The kidneys were examined histopathologically with a light microscope. **Results:** The results showed that rotenone caused damage to kidney tissue, which was observed in the form of vacuole degeneration, hyperemia and influx of inflammatory cells.

Crocin has also been shown to reduce the severity of rotenone-induced kidney damage. Also results showed that biochemical changes were not statistically significant between the groups. **Conclusions:** the crocin has protective influence on histopathological changes in rat's kidney following rotenone administration.

Key words: Crocin, Rotenone, Renal histopathology, Rats, Biochemical factors.

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INTRODUCTION

Rotenone is a natural insecticide with high fat solubility and can easily cross the blood-brain barrier. This toxin inhibits complex 1 in the electron transport chain in the mitochondria.¹ Some researchers showed that dopaminergic neurons in the brain are sensitive to this toxin and prolonged exposure of mice to rotenone could damage dopaminergic neurons and impairs their movement.² Jiang *et al.* showed that rotenone can cause kidney toxicity. This compound causes histopathological changes in renal tissue including edema, tubular dilatation, glomerular damage and glomerular epithelium atrophy.³ Rotenone has also been shown to alter oxidative stress factors and antioxidant defence enzymes in the kidney.⁴ Crocin is one of the most important active components of saffron and the colour of saffron is due to the presence of this compound. Previous studies have shown that saffron extract has many properties such as antimicrobial, anticonvulsant, anti-inflammatory, anti-tumor, analgesic and antioxidant properties⁵ and has also been shown to be effective in preventing colorectal cancer.⁶ Crocin reduces colon inflammation by acting on the NF- κ B signalling pathway.⁷ Crocin has a protective role in diabetes and reduction of renal complications and reduces serum levels of glucose, urea and creatinine in diabetic rats.⁸ According to a study by Hosseinzadeh *et al.* (2005), aqueous saffron extract improves oxidative damage due to ischemia and renal blood flow restoration in rats.⁹ Crocin acts as an antioxidant by removing free radicals and protects cells and tissues against oxidative stresses.¹⁰ Salahshour *et al.* (2016) reported that crocin can prevent morphine-induced liver toxicity as an antioxidant agent.¹¹ Therefore, in this study, the supportive effect of crocin as an antioxidant on the renal toxicity of rotenone in an experimental animal model was investigated.

MATERIALS AND METHODS

The study protocol, designed according to the National Guidelines for Laboratory Animal Welfare and the European Economic Community Guidelines for the care and use of laboratory animals (EEC Directive of 1986; 86/609/EEC), was approved by the Ethics Committee of Babol University of Medical Sciences (approval number: IR. MUBABOL. REC. 1397.015). The smallest possible number of animals was recruited and the depth of anaesthesia was constantly monitored during the surgery to prevent any response to painful stimuli. Euthanasia was conducted through deep anaesthesia with Ketamine-Xylazine solution. The animals were kept at a constant temperature of 25°C, humidity of 55% at 8:00 – 20:00 h light and 20:00 – 8:00 h dark cycle. The animals were fed standard chow until time of sacrifice.

Male Wistar rats (200-250 g) were obtained from Babol University of Medical Sciences and the randomly divided into 4 groups, each group consisting of 6 rats. Group 1 received saline, group 2 received crocin, group 3 received rotenone and group 4 received crocin and rotenone intraperitoneally (IP) for 10 days. The doses of crocin and rotenone were 50 mg/kg and 2 mg/kg, respectively.

One day after the last injections and in accordance with the ethics of working with animals, the rats were first anesthetized with Ketamine-Xylazine and blood was drawn from their hearts. A longitudinal incision was then made in the linea alba and their kidneys were isolated and homogenized in phosphate buffer. Part of rat kidney in each group is placed in 10% formalin to prepare pathological sections. For this purpose, the fixed kidney tissues were embedded in paraffin and 5- μ m-thick sections were obtained using a microtome. Sections were subjected to staining with H&E and pathological changes in kidney tissue were

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examined using a light microscope. In microscopic studies, pathological lesions in kidney tissue were identified and graded. Tissue damage such as vacuolation, necrosis, hyperemia and etc. were recorded in each slide in each group. The severity of pathologically observed lesions was scored. Determination of malondialdehyde concentration in serum and organ homogenates was performed using the method of thiobarbituric acid which measures MDA-reactive products as described by Todorova *et al.*¹² Catalase activity was determined using spectrophotometric method designed by the Koroliuk *et al.*¹³ Briefly, 10 μ L of sample was incubated with 100 μ mol/mL of H₂O₂ in 0.05 mmol/L Tris-HCl buffer, pH = 7 for 10 min. The reaction was terminated using rapidly adding 50 μ L of 4% ammonium molybdate. Yellow complex of ammonium molybdate and H₂O₂ was measured at 410 nm. Catalase activity was defined as the amount of enzyme required to decompose 1 μ mol H₂O₂ per min.

The analysis of serum BUN and serum creatinine (Cr), sodium and potassium levels was estimated by using diagnostic kits (ParsAzmoon kit, IRI) and an automatic analyser (Abbott, model Alcyon 300, USA).

RESULTS

Biochemical parameters

Figure 1 showed that the lowest amount of serum BUN was in the group receiving rotenone which is significantly different from the control group ($P < 0.05$) and the group receiving rotenone+crocin ($P < 0.01$).

Figure 2 showed that creatinine concentration was not different significantly in the control and other groups. Also, it was observed that the amount of sodium and potassium elements

in the control, crocin, rotenone and crocin + rotenone groups were not significantly different ($p > 0.05$).

Comparison of mean malondialdehyde concentration (MDA) in the kidney homogenized tissue and serum showed that there are no significant difference between control group and other groups. Also, the same results were observed for kidney homogenized tissue and serum catalase enzyme activity (Figure 3).

Histopathological analysis

Histopathological examination of renal tissue showed that in the control group, the kidney structure is normal and glomeruli, proximal and distal tubules are normal shape and normal cells (Figure 4 Part A). With the use of crocin in the second group, no changes were observed in kidney tissue and a structure similar to the control group was seen (Figure 4 Part B). While injecting rotenone in the third group, tissue damages such as hyperemia, vacuolar degeneration, inflammatory cell infiltration were observed in the kidneys (Figure 4 Part C). In the fourth group, which received crocin and rotenone at the same time, the severity of rotenone-induced tissue damage was lower and no infiltration of inflammatory cells was observed (Figure 4 Part D). The scour of histopathological changes is presented in Table 1.

Table 1: Pathological lesions observed in different groups.

Groups	Hyperemia	Vacuolar degeneration	Inflammatory cell infiltration
Control	--	--	--
Crocin	--	--	--
Rotenone	++	+++	+
Crocin+Rotenone	+	++	--

DISCUSSION

In the present study, the effect of crocin on changes in renal-related biochemical factors as well as histopathological changes in rat kidneys following intraperitoneal administration of rotenone and crocin (alone and in combination) were investigated. The serum biochemical analysis showed that there were no significant changes in creatinine, sodium and potassium and no significant changes were observed in the group receiving the rotenone along with the group receiving crocin. The reason for this can be various, one of which is the dose (amount and duration of toxin consumption) as well as genetic differences in mice. A study conducted by Jiang *et al.* (2017) stated that renal toxicity of rotenone is dose-dependent. While in histopathological examinations of kidney tissue, it was observed that rotenone can cause hyperemia, vacuolar degeneration and invasion of inflammatory cells.³ This indicates that the amount of toxin or its purity could change the tissue but could not change the function of the tissue, leading to biochemical changes.

Our results showed that by using crocin, the severity of tissue changes in the kidneys was reduced and at least no influx of inflammatory cells was observed which indicating the protective effect of crocin against rotenone-induced damage in the kidney tissue. Since rotenone could not cause functional damage to the kidneys, no changes were observed in the levels of oxidative stress factors, including Malondialdehyde concentration and catalase enzyme activity.

Jiang *et al.* (2017), suggested that rotenone alters oxidative stress and induced damage in the kidney tissues(3). In this study, rotenone at doses of 1, 2 and 4 mg/kg was administered orally to mice for 28 days, while in our study, rotenone at a dose of 2 mg/kg was administered to mice intraperitoneally for 10 days. In the Jiang *et al.* experiment, rotenone at doses of 2 and 4 mg/kg significantly reduced the weight of mice and also increased creatinine and BUN and excreted protein and phosphate in the urine. Also were reported some histopathological changes, such as vacuolar degeneration and the influx of inflammatory cells in the kidney tissues following treatment with rotenone (3), which is consistent with the results of our studies. They also reported that rotenone can cause changes in antioxidant defence factors, including a significant reduction in superoxide dismutase and glutathione peroxidase at doses of 2 and 4 mg/kg (3), while in our study, the dose of 2 mg/kg could not have any

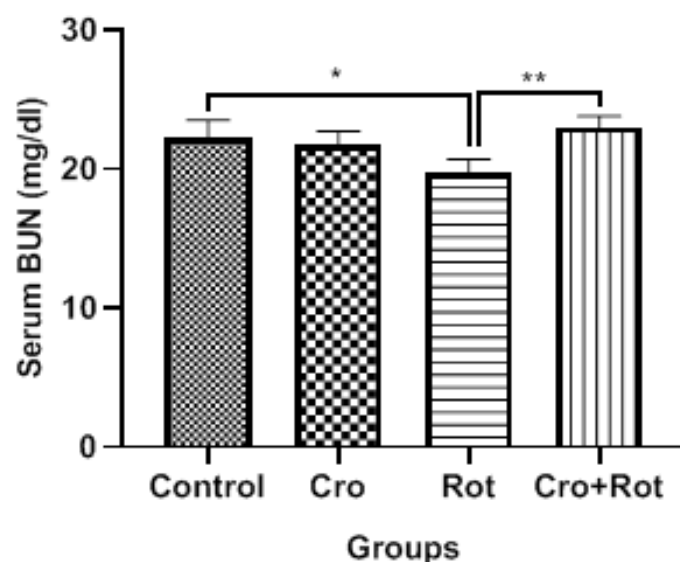


Figure 1: Results of serum BUN in the control, crocin, rotenone and crocin + rotenone groups. As shown the lowest amount of serum BUN was observed in the group receiving rotenone.

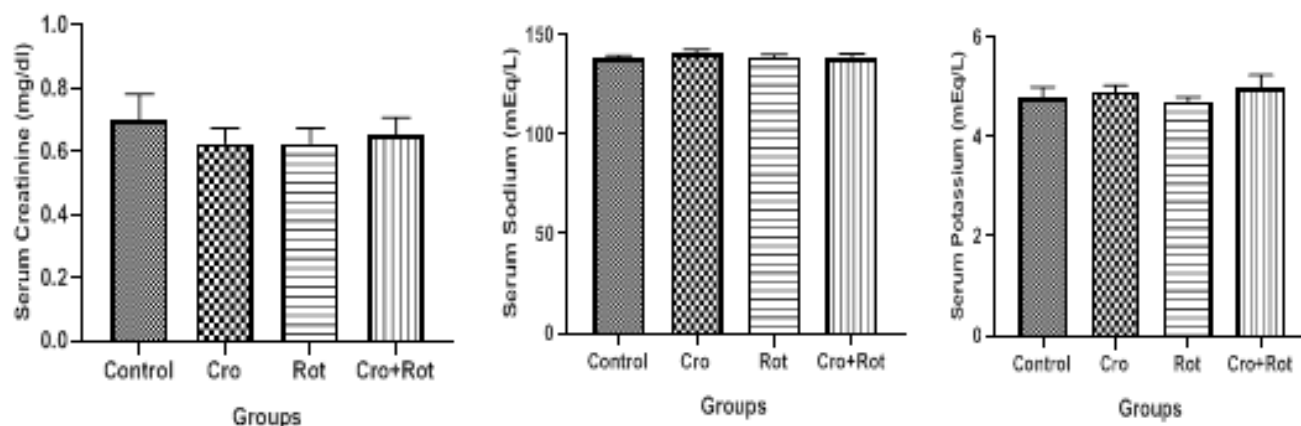


Figure 2: Serum creatinine, sodium and potassium in the control, crocin, rotenone and crocin+rotenone groups. No significant differences were observed in the control and other groups.

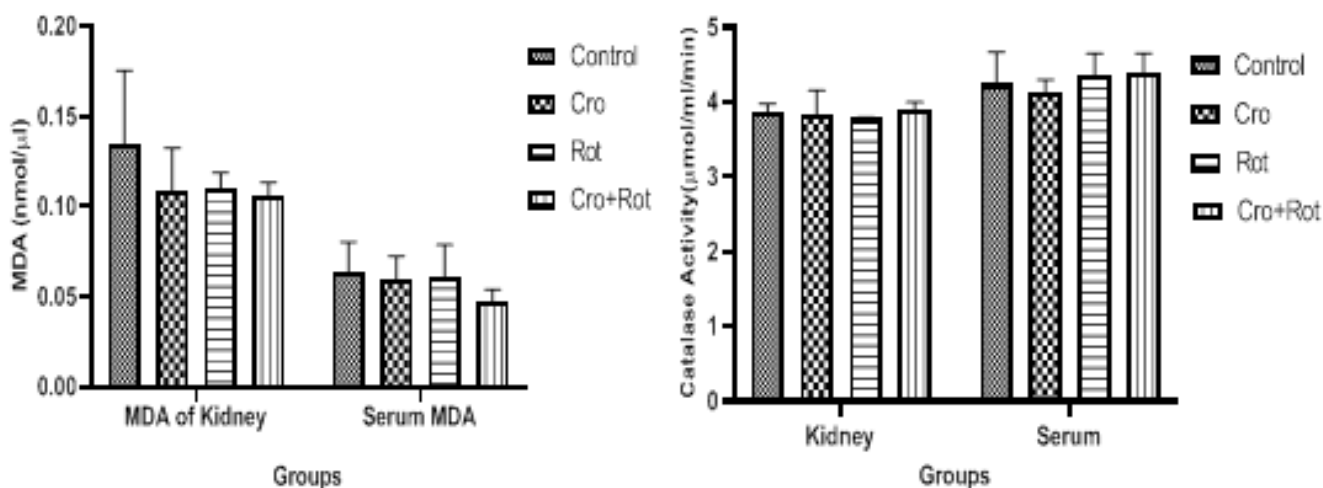


Figure 3: MDA concentration and catalase activity in the kidney homogenized tissue and serum samples. Results showed that there were no significant difference between control and other groups.

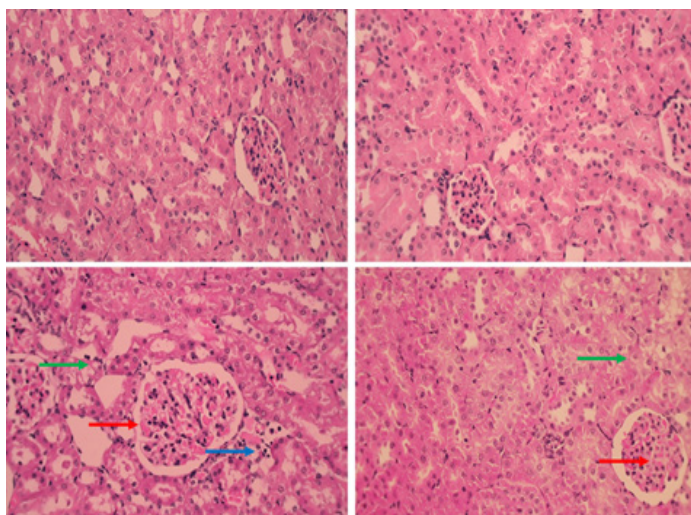


Figure 4: A: Control group, normal tissue, B: Crocin receiving group, normal tissue, C: Rotenone receiving group, hyperemia, vacuolar degeneration, inflammatory cell infiltration, D: Crocin and rotenone receiving group, hyperemia, vacuolar degeneration. H&E staining. 40X.

effect on antioxidant defence factors or oxidative stress. It is thought that the duration of toxin used in our study (10 days), was probably insufficient as compared to the Jiang *et al.* study (28 days).

In the Akinmoladun *et al.* (2018) study, treatment of rats with rotenone at a dose of 1.5 mg/kg for 10 days caused liver tissue damage.¹⁴ Tripathi *et al.* (2019) studied the oxidative stress induced by rotenone. They found that a significant alleviation in the levels of rotenone-induced pro-oxidant and anti-oxidant markers following exposure of metformin. They suggest that metformin supplementation shows a protective role in against rotenone-induced redox imbalance and cytotoxicity in rat erythrocytes.¹⁵ Rotenone is a potent mitochondrial toxin that blocks complex-1 in the mitochondrial electron chain. It also inhibits oxidative reductase, ubiquinone and NADH, leading to the accumulation of large amounts of free radicals and reactive oxygen species and causing oxidative damage.¹⁶ Because of high metabolic status and many active enzymes in the Kidney tissue and also consuming a lot of oxygen, this tissue can be exposed to rotenone-induced damage.¹⁷ Radad *et al.* (2013) conducted a study for the short-term effects of rotenone on neural and non-neural tissues and they reported that subcutaneously administration of rotenone at a dose of 10 mg/kg for 7 days and also at a dose of 3 mg/kg for 30 days can cause damage to neural and non-neural tissues;

Including kidney tissue and also can cause interstitial haemorrhage. The researchers concluded that rotenone could damage tissues by damaging blood vessels.¹⁸ Abdel-Salam *et al.* (2019) subcutaneously administered rotenone 1.5 mg/kg three times a week for two weeks. They showed that rotenone can damage kidney tissue which is consistent with our result.⁴ Crown *et al.* (2019) investigated the protective effect of the 3 flavonoids catechin, quercetin and taxifolin on rotenone-induced renal impairment in rats. They showed that rotenone increased oxidative stress and impaired renal function. While these flavonoids reduced the effects of rotenone. Crown *et al.* also stated that rotenone caused changes in Malondialdehyde, carbonyl protein glutathione, FRAP score and activity of glutathione transferase, superoxide dismutase, xanthine oxidase, lactate and dehydrogenase enzymes.¹⁹ In our study, crocin was able to reduce the severity of rotenone-induced tissue damage in the kidneys. Crocin is a plant carotenoid with antioxidant properties that can counteract the mechanism of toxicity of rotenone, which damages mitochondria and increases oxidative stress. This important carotenoid has a protective effect against some plant and metal toxins for various tissues. Some of these studies mentioned the protective effect of crocin in kidney tissues. For example, Bandegi *et al.* (2014) investigated the effect of crocin and saffron extract on kidney protection in mice under chronic stress and showed that these two compounds can prevent kidney damage with antioxidant properties.²⁰ Jalili *et al.* (2020) investigated the protective effect of crocin on methotrexate induced-renal toxicity. They found that crocin methotrexate caused oxidative stress that damaged the kidneys and crocin with antioxidant effect reduced this complication.²¹ Naghizadeh *et al.* (2010) investigated the protective effect of crocin against cisplatin-induced toxicity in rats by administering high doses of crocin (100, 200 and 400 mg/kg) for 4 consecutive days. They observed that crocin could significantly reduce MDA and increase glutathione peroxidase levels and reduced cisplatin toxicity to the kidneys.^{19,22} Lai *et al.* (2020) reported the combination crocin and quercetin can more significantly improve renal fibrosis in high-fat diet- and streptozotocin-induced type 2 diabetes.²³ Ajami *et al.* (2010) evaluated the protective effect of saffron against gentamicin-induced renal toxicity in rats. Saffron was able to neutralize the effects of gentamicin on the kidney so that the amount of creatinine and BUN was reduced and also the amount of MDA and tissue damage was reduced significantly.²⁴ Yarjani *et al.* (2017) showed that administration of 100 mg/kg crocin intraperitoneally for 7 days reduced the toxic effects of gentamicin in renal tissue. This protective effect of crocin is related to its antioxidant properties, which prevent the production of reactive oxygen species, prevent DNA breakage and ultimately prevent cell necrosis.²⁵ Some studies have shown that crocin also has anti-inflammatory properties, as Nam *et al.* (2010) showed that crocin prevents the release of TNF- α and IL- β cytokines and the activation of NF-K β protein. Therefore, the pharmacological effects of crocin are important from both antioxidant and anti-inflammatory aspects.²⁶

CONCLUSION

In the present study, rotenone was administered intraperitoneally at a dose of 2 mg/kg for 10 days at the tissue level and was able to damage the kidneys, although this damage did not occur biochemically. While crocin consumption was able to reduce the pathological effects of rotenone on the kidneys.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

ANOVA: One-way variance analysis; **BUN:** nitrogen blood urea; **i.p:** Intraperitoneally; **LSD:** Least statistical differences; **S.E.M:** Standard error of the mean.

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