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NATURAL DRUGS

THE EFFECT OF CARVACROL ON TRAMADOL-INDUCED OXIDATIVE RENAL INJURY IN RATS: BIOCHEMICAL AND HISTOPATHOLOGICAL EVALUATION

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Abstract: Long-term treatment with tramadol is reported to be toxic to the kidneys. Research shows that tramadol reduces antioxidants and increases oxidants produced in renal tissue. We aimed to investigate the carvacrol effect on tramadol-induced renal injury in rats. The animals were divided into four groups: healthy (HG), individual tramadol (TR), individual carvacrol (CR), and tramadol + carvacrol (TC). Malondialdehyde (MDA), total glutathione (tGSH), glutathione peroxidase (GPO), superoxide dismutase (SOD), total oxidant status (TOS), total antioxidant status (TAS), blood urea nitrogen (BUN), and creatinine (Cr) were measured. Renal tissue was examined histopathologically. MDA, TOS, BUN, and Cr were significantly elevated in group TR and identified as low in group TC compared to group TR but higher than in the HG group. The tGSH, SOD, GPO, and TAS levels were low in group TR and higher in group TC than in group TR but lower than in the HG group. Histological examination of the TR group revealed diffuse necrosis and focal polymorphonuclear leukocytes (PMNLs) in the tubules. In the TC group, tubular atrophy and necrosis were minimal, and PMNL was rare. We observed that tramadol increases oxidants, decreases antioxidants, increases BUN and Cr, and examined whether the toxic effect on renal tissue regressed with carvacrol.

Keywords: antioxidant, carvacrol, nephrotoxicity, oxidant, pain, tramadol.

Tramadol is an analgesic hydrochloride synthetic codeine analog with a chemical formula (1RS, 2RS)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride (1). It is a weak analgesic and has both opioid and nonopioid mechanisms. The analgesic exerts its effects by inhibiting the reuptake of norepinephrine and serotonin, accompanied by stimulation of mu-opioid receptors (2). It can also produce an antagonistic effect on the NMDA receptor and an agonistic effect on the GABA receptor (3). The analgesic efficacy of tramadol is achieved by the active metabolite O-desmethyl-tramadol (M1) of its primary molecule. The μ-receptor affinity of the M1 metabolite is 200-fold higher than that of the primary molecule (2). Tramadol has analgesic efficacy comparable to potent opioids like morphine(4). The combination with metamizole has been used for many years, especially in abdominal procedures (5). Consistent with the accepted efficacy of tramadol in treating pain, several cases of toxicity resulting in death have been reported (6). These toxic effects occur when tramadol is taken in an overdose or over a prolonged period (7). The literature reports that the metabolite of tramadol causes renal and other organ toxicity (8). Experimental studies show that repeated administration of tramadol decreases enzymatic and nonenzymatic antioxidants such as glutathione (GSH), glutathione reductase (GSHRd), glutathione peroxidase (GPO), glutathione S-transferase (GST), catalyst (CAT), and superoxide dismutase (SOD) and increases malondialdehyde (MDA) (9). Haytham A. et al. reported that tramadol-induced renal injury was due to increased oxidant levels and decreased antioxidant levels and found that it further caused

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an increase in BUN and creatinine (10). These findings suggest that oxidative stress is an essential factor in the pathogenesis of tramadol nephrotoxicity.

Carvacrol, whose action against the nephrotoxicity of tramadol we will test, is a monomeric phenol found in thyme and the fat of some plants (11). It is responsible for thyme's biological activity and possesses potent antioxidant, antibacterial, anticancer, antimutagenic, antigenotoxic, analgesic, antispasmodic, anti-inflammatory, antiparasitic, antiplatelet, insecticidal, and hepatoprotective properties (12). The preventive effect of carvacrol is apparent in elevated MDA levels and reduced antioxidant levels, which is an oxidative parameter (13). Samarghandian. S et al. emphasized that carvacrol obviates increases in MDA, GSH, SOD, GPx, GR, and CAT levels in the brain, liver, and kidney tissues (14). In another study, carvacrol showed beneficial effects in improving the quality of broiler meat and preventing or delaying its oxidation (15). These results suggest that carvacrol may effectively prevent tramadol-induced oxidative kidney damage.

Therefore, our study aims to test the effect of carvacrol on tramadol-induced oxidative kidney damage in rats.

EXPERIMENTAL

Animals and experimental design

The female albino Wistar rats were used in our study. Twenty-four rats weighing between 265 and 278 grams were used for the experiment. Before the experiment, animals were housed and fed at average room temperature (22°C) for 12 hours in the light and 12 hours in the dark. The animals were kept in the laboratory, where the experiment was performed for one week to acclimate to the environment. The experiment was conducted in compliance with the Arrive 2.0 Guidelines.

Chemicals

Thiopental Sodium, IE Ulagay, Turkey.

Carvacrol, Sigma Chemical Co, USA.

Tramadol, Abdi Ibrahim Pharmaceuticals industry, Turkey.

Experimental groups

The experimental animals were divided into four groups: healthy (HG), tramadol (TR) alone, carvacrol (CR) alone, and tramadol + carvacrol (TC).

Conduct of the experiment

Carvacrol was injected intraperitoneally (i.p.) at a dose of 50 mg/kg to the CR (n-6) and TC (n-6) groups. For the TR (n-6) and HG (n-6) groups, the same volume of distilled water i.p. was injected. One hour after administering carvacrol and distilled water, the TC and TR groups received an oral injection of tramadol at a dosage of 50 mg/kg. This procedure was repeated twice daily for two weeks. Following this period, the animals were killed with high-dose thiopental sodium (50 mg/kg), and the renal tissues were removed. MDA, tGSH, GPO, SOD, TOS, and TAS were measured. Renal tissue was also examined histopathologically. BUN and creatinine were analyzed in blood samples taken from the tail veins.

BIOCHEMICAL ANALYZES

Preparation of Samples

Weighed 0.2 grams of each tissue removed. To determine MDA, tissue was placed into a 1.15% potassium chloride solution. To measure tGSH, it was in phosphate buffer with pH = 7.5, and all solutions were made up to 2 mL and homogenized in an icy environment. Then, centrifugation was performed at 10,000 rpm for 15 min at +4°C. The supernatant portion was used as an analytical sample.

MDA quantification

It is based on the principle of measuring the absorbance of the pink complex formed spectrophotometrically by thiobarbituric acid (TBA) and MDA at a high temperature (95°C) at a wavelength of 532 cm (16).

According to Sedlak J and Lindsay RH, quantification of tGSH activity was performed by the spectrophotometric method at a wavelength of 412 nm (17).

Assignment of GPO activity

Glutathione peroxidase activity was measured after the homogenization of the tissue. The supernatant was used for GPO measurement, and the mixture was incubated after adding monopotassium phosphate, EDTA, GSH, beta-NADPH, sodium azide, and GRx. After adding hydrogen peroxide (H2O2), the chronometer was turned on, and absorbance was measured at 340 nm every 15 seconds for 5 minutes according to the method described (18).

Determination of the activity of SOD

Cayman SOD Measurement Kit (Part No: 706002);

It is based on the principle of using a tetrazolium salt to detect superoxide radicals formed by hypoxanthine and xanthine oxidase. One unit of SOD is defined as the enzyme required to dismutate 50%

of the superoxide radicals. When measuring SOD, all three types of SOD (Cu/Zn- SOD, Mn-SOD, Fe-SOD) were measured. This method provides a simple, repeatable, and rapid measurement for determining SOD activity in tissue homogenates.

Measurement of TOS and TAS

The concentrations of TOS and TAS in tissue homogenates were determined using a novel automated measurement method and commercially available kits (Rel Assay Diagnostics, Turkey), both developed by Erel (19, 20).

Bun and creatinine measurement

Quantitative determination of serum BUN and creatinine was performed according to the manufacturer's instructions using a spectrophotometric method on a Roche brand Cobas 8000 autoanalyzer.

Histopathological procedure

Kidney tissues were collected at the necropsy of rats and identified in a 10% neutral formalin solution. Tissues were embedded into paraffin blocks after routine alcohol-xylol dehydration procedures. The 5 μ sections were stained with hematoxylin-eosin and examined for hemorrhage, degeneration, and mononuclear cell infiltration, which were graded as absent (-), mild (+), moderate (++), and severe (+++).

Statistical analysis

The experimental results are expressed as the mean ± standard error of the mean (x ±SEM), and the normality of the distribution for continuous variables was confirmed using the Shapiro–Wilk test. Significant differences between groups were determined using one-way ANOVA. Tukey's test was then performed as a post hoc test. All statistical analyses were performed using the statistical program "SPSS for Windows, version 22.0" and p values < 0.05 were considered significant.

Ethics statement

The protocols and procedures were approved by the Local Animal Experimentation Ethics Committee (Date: October 28, 2021, Meeting number: 2021/02)

RESULTS

When the biochemical results in the study groups were compared, it was found that the MDA levels in the TR group were higher than those in the HG group ($P < 0.001$). MDA levels in the CR group were similar to those in the HG group. In the TC group, MDA values decreased compared to those in the TR group ($P < 0.001$) but were higher than those in the HG group ($P < 0.001$). When the tGSH values were checked, the value in the TR group was lower than that in the HG group ($P < 0.001$). The tGSH values in the CR group were similar to those in the HG group ($p = 0.447$). In the TC group, tGSH levels were found to increase ($P < 0.001$) in the TR group but were lower than those in the HG group ($P < 0.001$). The levels of SOD and GPO were significantly higher in the HG group than in the TR group ($P < 0.001$). There were no statistically significant differences in the values of SOD and GPO between the HG and CR groups. While the values of tGSH, GPO, and SOD were significantly higher in the TC group than in the TR group, the MDA values were decreased ($P < 0.001$). There was no significant difference between the HG and CR groups when the values of GPO and SOD were compared ($p > 0.05$) (Figure 1). While the TOS values were significantly higher in the TR group than in all other groups, it

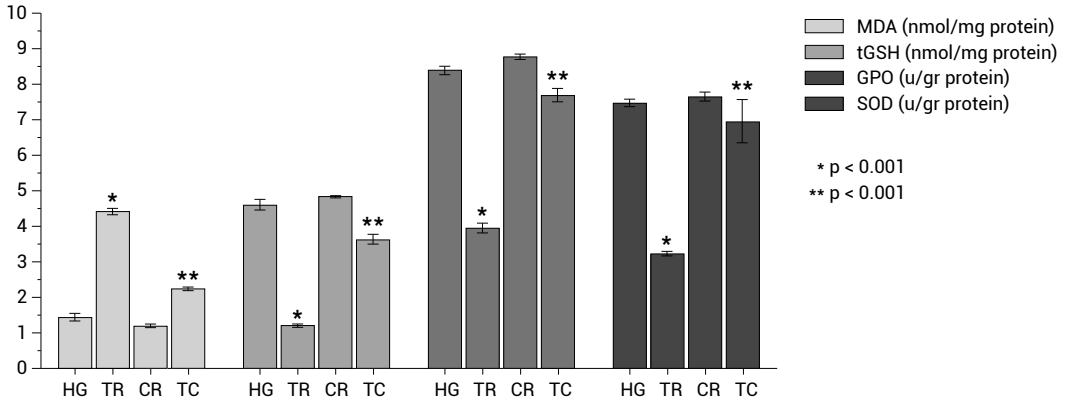


Figure 1. The amounts of MDA, tGSH, GPO, and SOD levels in the kidney tissue in experimental groups. Bars are mean ±SEM. *P < 0.001 according to HG and CR groups, **P < 0.001 according to TR group. MDA: malondialdehyde; tGSH: total glutathione; GPO: glutathione peroxidase; SOD: superoxide dismutase; HG: healthy group; TR: tramadol group; CR: carvacrol group; TC: tramadol + carvacrol group. n = 6.

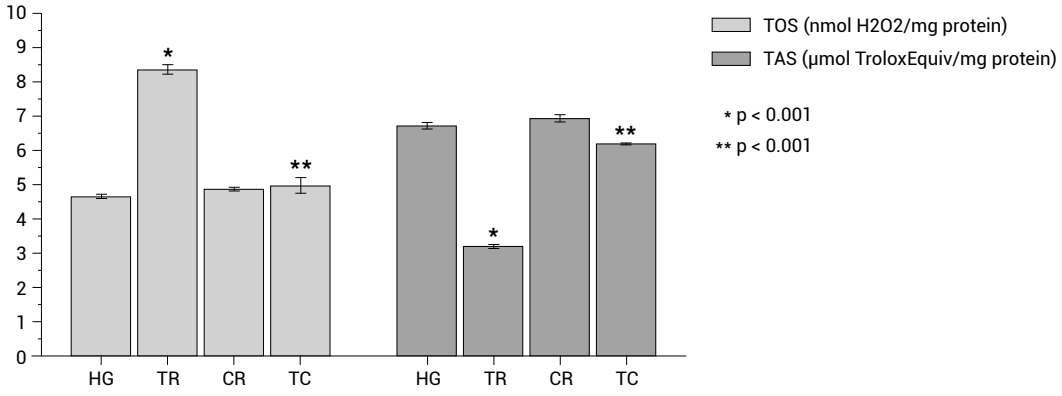


Figure 2. The amounts of TOS and TAS levels in the kidney tissue in experimental groups. Bars are mean ±SEM.*P < 0.001 according to HG and CR groups, **P < 0.001 according to TR group. TOS: total oxidant status; TAS: total antioxidant status; HG: healthy group; TR: tramadol group; CR: carvacrol group; TC: tramadol + carvacrol group. n = 6.

was also found that the TAS values were significantly lower than those in all other groups (P < 0.001). Although the TC group had higher TOS values than the HG group, the difference was not significant (p = 0.396). The TAS values were significantly lower in the TC group than in the HG group (P < 0.001) (Figure 2). BUN and creatinine (Cr) values were high in the HG group in the TR group (P < 0.001). BUN and Cr levels were significantly lower in the TC group (P < 0.001) than in the TR group, but no significant difference was found between the TC and HG groups (P < 0.05) (Table 1, Figures. 3-4).

Histopathological results

On histopathologic examination, the glomerular and tubular structures of the control group appeared normal (Figure 5a). The tubular and glomerular structures of the carvacrol-treated group were histologically normal and looked equivalent to those of the healthy group (Figure 5b). The TR group showed diffuse necrosis and focal polymorphonuclear leukocytes (PMNLs) in the tubules (Figure 5c). Dilation of the glomerular Bowman's capsule and damage to the arterial wall was also observed (Figure 5d).

Table 1. Biochemical findings according to the groups.

	Groups				p-values				Pairwise comparisons			
	HG	TR	CR	TC	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4		
	(1)	(2)	(3)	(4)								
MDA (nmol/mg protein)	1.4 ± 0.1	4.4 ± 0,9	1.2 ± 0.0	2.2 ± 0.0	< 0.001	0.119	< 0.001	< 0.001	< 0.001	< 0.001		
tGSH (nmol/mg protein)	4.6 ± 0.2	1.2 ± 0.0	4.8 ± 0.0	3.6 ± 0.1	< 0.001	0.447	< 0.001	< 0.001	< 0.001	< 0.001		
GPO (u/gr protein)	8.4 ± 0.1	4.0 ± 0.1	8.8 ± 0.8	7.7 ± 0.2	< 0.001	0.219	< 0.05	< 0.001	< 0.001	< 0.001		
SOD (u/gr protein)	7.5 ± 0.1	3.2 ± 0.1	7.7 ± 0.1	6.5 ± 0.1	< 0.001	0.664	< 0.001	< 0.001	< 0.001	< 0.001		
TOS (nmol H_2O_2/mg protein)	4.7 ± 0.1	8.4 ± 0.1	4.9 ± 0.1	5.0 ± 0.2	< 0.001	0.705	0,396	< 0,001	< 0.001	0.951		
TAS (µmolTroloxEquiv/mg protein)	6.7 ± 0.1	3.2 ± 0.1	6.9 ± 0.1	6.2 ± 0.0	< 0.001	0.206	< 0.001	< 0.001	< 0.001	< 0.001		
Creatinine (mg/dL)	1.1 ± 0.0	2.0 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	< 0.001	0.997	0.486	< 0.001	< 0.001	0.375		
BUN (mg/dL)	49.3 ± 2.0	164.5 ± 3.4	52.5 ± 2.9	59.0 ± 1.5	< 0.001	0.815	0.063	< 0.001	< 0.001	0.299		

* ANOVA test was performed with Tukey test as post-hoc. Results were presented as mean ±standard error of the mean (x ±SEM).
HG: healthy group; TR: tramadol group; CR: carvacrol group; TC: tramadol + carvacrol group.

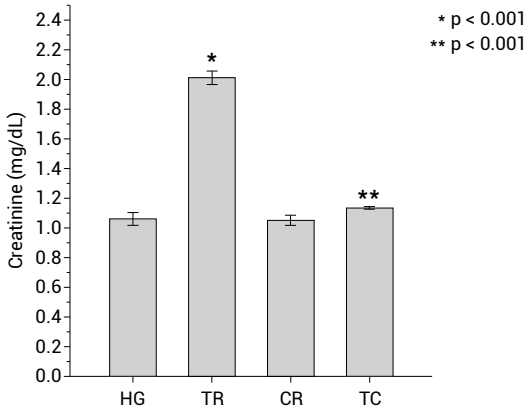


Figure 3. The amounts of creatinine levels in the blood serum at experimental groups. Bars are mean ±SEM. *P < 0.001 according to HG and CR groups, **P < 0.001 according to TR group. HG: healthy group; TR: tramadol group; CR: carvacrol group; TC: tramadol + carvacrol group. n = 6.

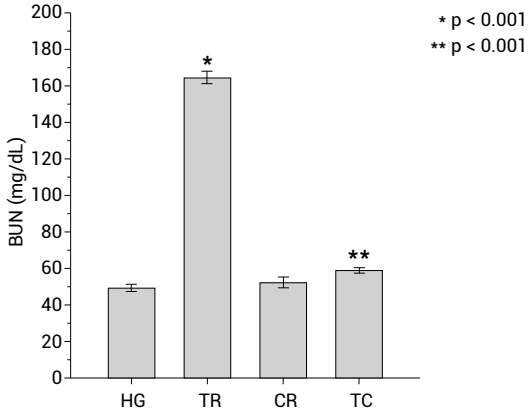


Figure 4. The amounts of BUN levels in the blood serum in experimental groups. Bars are mean ±SEM. *P < 0.001 according to HG and CR groups, **P < 0.001 according to TR group. HG: healthy group; TR: tramadol group; CR: carvacrol group; TC: tramadol + carvacrol group. n = 6.

The TC group showed minimal atrophy and minimally focal necrosis of the tubules, normal glomeruli, and rare PMNL (Figure 5e).

DISCUSSION AND CONCLUSION

The cytochrome P450 (CYP) system metabolizes tramadol and induces oxidative stress in various organs. Long-term use of tramadol may cause toxic effects. Neurotoxic effects are caused by oxidative stress, inflammation, and apoptosis (21). Given the great importance of the liver and kidney in the metabolism and excretion of tramadol, these organs are particularly vulnerable to toxicological damage. In a study examining the effects of tramadol on the kidneys, an increase in renal 8-OHdG and MDA levels, a significant decrease in GSH, CAT, and SOD activities, and a dose-dependent increase in urea, creatinine, and renal function parameters were observed. Tramadol has also been reported to impair renal function through DNA damage and oxidative stress generation with nephrotoxic effects on the concentration (10). An experimental study by Elkhateeb et al. investigated tramadol-induced hepatotoxicity and nephrotoxicity. They found a significant increase in hepatic and renal MDA levels and

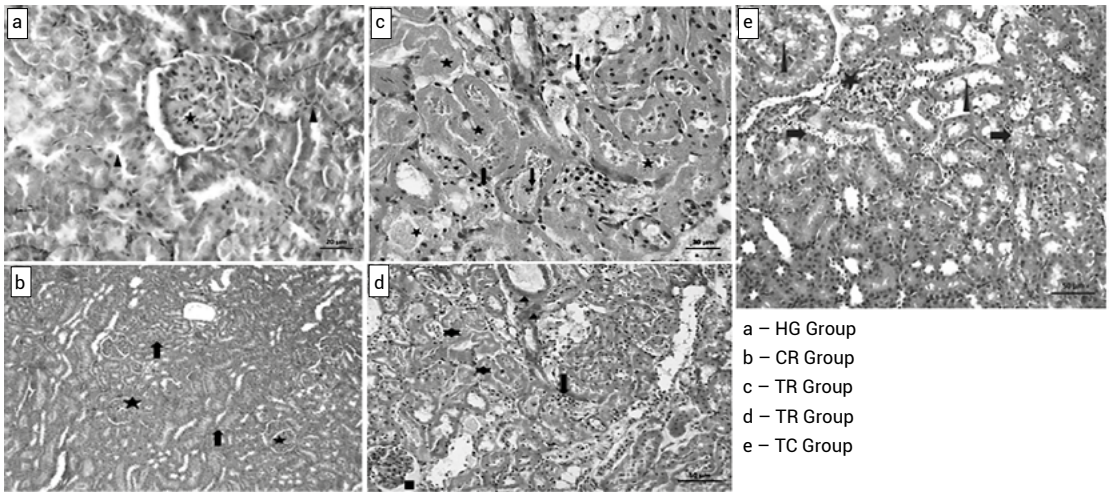


Figure 5a. Hematoxylin–Eosin staining in kidney tissue in healthy control group HG; ➔: epithelium, ⇉: gastric glands, ★: glomerule, ×40.
Figure 5b. Hematoxylin–Eosin staining in kidney tissue in CR group; ➔: Tubular necrosis, ⮝: minimal tubular atrophy ★: Glomerul ×20.
Figure 5c. Hematoxylin–Eosin staining in kidney tissue in TR group; ➔: polymorphonuclear cell infiltration, ★: Tubular necrosis, ×40.
Figure 5d. Hematoxylin–Eosin staining in kidney tissue in TR group; ➔: polymorphonuclear cell infiltration □: Glomerul bowman dilatation, ⮝: Arterial wall damage ★: Tubular necrosis, ×20.
Figure 5e. Hematoxylin–eosin staining in kidney tissue in TC group; ➔: minimally focal necrotic tubule, ⮝: minimal tubular atrophy ★: Glomerul ×20.

a decrease in GPO levels. The induction of oxidative stress by tramadol was administered at similar doses and times as in our study. They have seen atrophic glomeruli in kidney sections with collapsed clusters, large Bowman cavities, degenerated tubules, hemorrhage, and mononuclear cell infiltration (22). In another study, tramadol was also found to induce oxidative stress, reduce antioxidants in renal tissue, and cause renal damage (9). Barbosa et al. demonstrated the toxicological effects of tramadol in vivo. It histopathologically showed inflammation in renal tissue and sinusoidal dilatation with cell damage, microstates, vacuolization, cell infiltrates, and cell degeneration. They concluded that even a single effective analgesic dose could cause hepatotoxicity and nephrotoxicity (23). We administered 50 mg/kg tramadol daily for two weeks in our study. In blood biochemistry, BUN and Cr levels showed that renal function was increased in the tramadol group. In the biochemical analysis of renal tissue, we found a significant increase in the levels of oxidants MDA and TOS in the TR group compared to the HG group. However, we observed a significant decrease in the antioxidant parameters tGSH, GPO, SOD, and TAS in the TR group. Our histopathological study also hypothesizes that inflammation in the TR group resulted in cell degeneration, tubular necrosis, Bowman's capsule expansion, and PMNL infiltration. These histological and biochemical data indicate that tramadol can increase oxidative stress and inflammation in renal tissue and cause histopathologically detectable damage.

We used carvacrol with the hypothesis that it can prevent this damage after histopathological and biochemical detection of tramadol-induced kidney damage. Although carvacrol is a food additive due to its antibacterial properties, it has been shown to have potent antioxidant, anti-inflammatory, and neuroprotective effects. A monoterpene phenol is the main component of essential oils extracted from Lamiaceae plants, such as thyme, bergamot, and mint. It has antimicrobial, antioxidant, anticancer, anti-inflammatory, and anticholinesterase effects (24). While few studies have investigated the protective effects of carvacrol on the kidneys, anti-inflammatory, and antioxidant effects, specifically, are used to prevent organ damage. Çelik. et al. reported that whereas carvacrol decreased TOS, MDA, IL-1β, and TNF-α levels, increased TAS levels decreased the pro-inflammatory response (25).

Another experimental study on rats with colon cancer where chronic inflammation was a risk factor found that carvacrol significantly increased the levels of antioxidant enzymes such as SOD, CAT, and GSH and decreased lipid peroxide levels (LPO), myeloperoxidase, and nitric oxide (NO). However, they found that proinflammatory mediators, including inducible nitric oxide synthase (iNOS) and interleukin-1 beta (IL-1), were decreased in rats, suggesting that carvacrol could be a potential dietary antioxidant and anti-inflammatory agent when combined (26).

In our study, the renal function, oxidant, and antioxidant parameter levels of the CR group were similar to those of the HG group. Histologically, no damage was seen in renal tissues when used alone. When tramadol was used together with carvacrol, we observed in our study that BUN, Cr, MDA, and TOS levels were decreased in the TC group, while tGSH, GPO SOD, and TAS levels increased at similar levels to the HG group. Histopathological evaluation showed tubular atrophy and necrosis regressed, as did glomerular expansion and PMNL infiltration.

As a result, we biochemically and histopathologically demonstrated that tramadol caused an increase in oxidant substances and decreased antioxidant biomolecules, inducing oxidative damage and affecting renal function parameters such as BUN and Cr. Moreover, the toxic effect in the kidney tissue regressed with added antioxidant and anti-inflammatory carvacrol.

Tramadol is an opioid analgesic frequently used to treat acute and chronic pain. Although the efficacy of the analgesic is satisfactory, its toxic effects, especially on the kidney, should not be ignored. This damage can be reversed by using carvacrol, which has antioxidant and anti-inflammatory effects. The results of this experimental study suggest that carvacrol may be helpful in the clinic for preventing renal toxicity associated with tramadol.

Conflicts of interest

The authors declare no conflict of interest.

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