

# Zingerone Mitigates Carrageenan-Induced Inflammation Through Antioxidant and Anti-inflammatory Activities

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**Abstract—** Inflammation is the body's response against various pathogens and has a critical role in numerous diseases. Zingerone (Zing), a bioactive substance derived from ginger root, has a variety of pharmacological properties, such as reducing inflammation, and antioxidant effects. We aimed to evaluate the beneficial effects of Zing in a carrageenan-induced inflammation model. Paw edema induced by carrageenan (100  $\mu$ l of 1%) was used to induce acute inflammation in rats. Different doses of Zing (10, 20, and 40 mg/kg) were administered intraperitoneally. Paw tissue levels of MDA, NO, CAT, SOD, GPx, GSH, COX-2, PGE<sub>2</sub>, TNF- $\alpha$ , and IL-1 $\beta$  were estimated. Our results showed that Zing, especially at the highest dose of 40 mg/kg, significantly reduced paw swelling in carrageenan-injected animals. Zing significantly increased paw enzymatic and nonenzymatic antioxidants except CAT. It also decreased paw levels of MDA, NO, COX-2, PGE<sub>2</sub>, TNF- $\alpha$ , and IL-1 $\beta$ . The results of this study show that Zing may provide an alternative for the clinical control of inflammation through antioxidant and anti-inflammatory activities.

**KEY WORDS:** zingerone; carrageenan; inflammation; antioxidant.

## INTRODUCTION

Inflammation is a clinical condition present in most pathologies including toxins, radiation, injury, and infection [8]. Moreover, inflammation is characterized by swelling, redness, heat, and pain. Furthermore, inflammation is one of the main causes of organ dysfunction which requires pharmacological intervention [18]. It is well-documented that inflammation is involved in the pathogenesis of many disorders such as aging and cardiovascular and neurological diseases [20]. Currently, steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used medications for treating inflammation. Besides

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their effectiveness for treatment of this disorder, these drugs have established serious adverse effects such as gastrointestinal ulcers, hepatotoxicity, bleeding, and renal disorders [11]. Therefore, searching for better anti-inflammatory agents with lower side effects is needed, and new biologically active compounds from plants are a promising strategy for discovering these agents [13].

Zingerone (Zing), a bioactive substance of ginger root, has a variety of pharmacological properties such as anticancer, antioxidant, anti-inflammatory, antimicrobial, and antiapoptotic effects [9, 27]. On the other hand, the antioxidant properties of Zing are mediated via increasing the antioxidant capacity and reducing the level of nitric oxide (NO) and lipid peroxidation [3, 35]. The anti-inflammatory effects of Zing have been established in several animal models including sepsis- and cisplatin-induced nephrotoxicity [4, 25]. Zing is a safe substance, and the lethal dose 50% (LD50) for rats is 2580 mg/kg [31].

Although the anti-inflammatory effects of Zing had been shown in different conditions, there is no available study investigating the anti-inflammatory properties of Zing in mitigating rat paw edema induced by carrageenan (Carr). The present investigation was aimed to document more detailed effects of Zing to protect rats from Carr-induced acute inflammation, which was established for evaluating anti-inflammatory agents.

## MATERIAL AND METHODS

### Drugs and Chemicals

Carr and Zing were obtained from Tocris Co. (Bristol, UK). Indomethacin (Ind) was kindly donated by Iran Daru Pharmaceutical Co. (Tehran, Iran). Carr was dissolved in 0.9% sodium chloride. Zing and Ind were dissolved in 10% DMSO.

### Animals

Adult male Wistar rats (200–220 g) were used in this study and were housed at standard conditions (23 ± 2 °C; 12 h/12 h light/dark cycle; 50% humidity). All procedures were conducted according to the Ethical Guideline for Research, Ahvaz University (Ethics code: IR.AJUMS.ABHC.REC.1397.002).

### Treatment

Forty-two rats were divided into 6 groups ( $n = 7$ ). The first group was i.p. injected with DMSO (10 ml/kg of 10%)

30 min prior sodium chloride (100 µl of 0.9%) injection i.pl. in the right paw of the rats. The second group was i.p. injected with DMSO 30 min prior Carr (100 µl of 1%) in the right paw. The third group was i.p. injected with Ind (5 mg/kg in a volume of 10 ml/kg) 30 min prior Carr injection. The fourth, fifth, and sixth groups were i.p. injected with Zing (10, 20, and 40 mg/kg in a volume of 10 ml/kg) 30 min prior Carr injection.

### Evaluation of Carr-Induced Paw Edema and Inflammation

Immediately before and at 0.5, 1, 2, 3, 4, and 5 h after carrageenan treatment, the volume (ml) of paw edema was quantified by measuring the volume of the paw using a plethysmometer (Ugo Basile Co., Italy).

The changes in edema volume (ml) were evaluated by the following formula:

$$\text{Changes of edema volume (ml)} = A - B$$

A and B are the volume (ml) of the right hind paw after (0.5, 1, 2, 3, 4, and 5 h) and before carrageenan administration, respectively.

The percent of inflammation was evaluated by the following formula:

$$\text{Percent of inflammation} = ((V2 - V1) / V1) * 100$$

V1 and V2 are the volume (ml) of the right hind paw before and 5 h after carrageenan administration, respectively.

### Sample Collection

Immediately after evaluating the paw edema, the animals were sacrificed, and the right hind paws were dissected. An ice-cold 0.1 M Tris-HCl buffer (1/10 w/v) was used for sample homogenization. Then, the samples were spun at 3500 rpm in a centrifuge for 10 min at 4 °C [17]. Measurement of oxidative stress and inflammatory indices were carried out on the supernatants, which was stored at –80 °C. The total protein concentration in the supernatant was estimated by the method of Bradford [6], using crystalline bovine serum albumin as standard.

### Assessment of Oxidative Stress Indices

Catalase (CAT) activity was measured by the method described by Aebi et al. [2]. Superoxide dismutase (SOD) activity was assessed by the method of Martin et al. [30]. The levels of glutathione (GSH) were evaluated by the method of Ellman et al. [14]. The activity of glutathione peroxidase (GPx) was determined by the commercial kit (Randox Labs, Crumlin, UK). The lipid peroxidation was assessed by the measurement of malondialdehyde (MDA) according to the method described by Buege et al. [7]. The levels of NO were evaluated using the Griess method [36].

### ELISA Measurements

The tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 1 $\beta$  (IL-1 $\beta$ ), cyclooxygenase-2 (COX-2), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) concentrations were evaluated in the paw homogenates. ELISA kit of Abcam Biochemical Co. (Cambridge, UK) for COX-2, ELISA kit of Crystal Day Biotech Co. (Shanghai, China) for PGE<sub>2</sub>, and ELISA kits of eBioscience International, Inc. (Camarillo, CA) for TNF- $\alpha$  and IL-1 $\beta$  were used.

### Statistical Analysis

Data were shown as mean  $\pm$  SD. Results were statistically compared using one-way analysis of variance (ANOVA) and Tukey post hoc test. A  $p$  value  $\leq$  0.05 was considered statistically significant.

## RESULTS

### Effects of Zing on Paw Edema and Inflammation

Our results showed that Ind reduced the paw edema of right hind paw in comparison with the Carr group at all evaluated times ( $p \leq 0.05$ ) (Fig. 1a). Pretreatment with Zing at the dose of 10 mg/kg reduced the paw edema of right hind paw 3 h after Carr injection as compared with the Carr group ( $p \leq 0.05$ ). Moreover, pretreatment with Zing at the doses of 20 and 40 mg/kg decreased the paw edema of right hind paw 2 h after Carr injection compared with the Carr group ( $p \leq 0.05$ ).

Also, data obtained from the Ind group revealed a significant reduction in the inflammation of right hind paw compared with the Carr group ( $p \leq 0.05$ ) (Fig. 1b). Pretreatment with Zing (20 and 40 mg/kg) 30 min before Carr caused a significant decrease in the inflammation of right hind paw compared with the Carr group ( $p \leq 0.05$ ).

### Effects of Zing on Paw Oxidative Stress Indices

The content of GSH and GPx activity was significantly reduced in the Carr group compared with the control group (all  $p \leq 0.05$ ) (Fig. 2 a and b). Also, the administration of Ind 30 min before i.pl. injection of Carr significantly elevated these indices in comparison with Carr group. Moreover, pretreatment with Zing at the dose of 40 mg/kg significantly increased the content of GSH and GPx activity in paw tissues as compared with the Carr group ( $p \leq 0.05$ ).

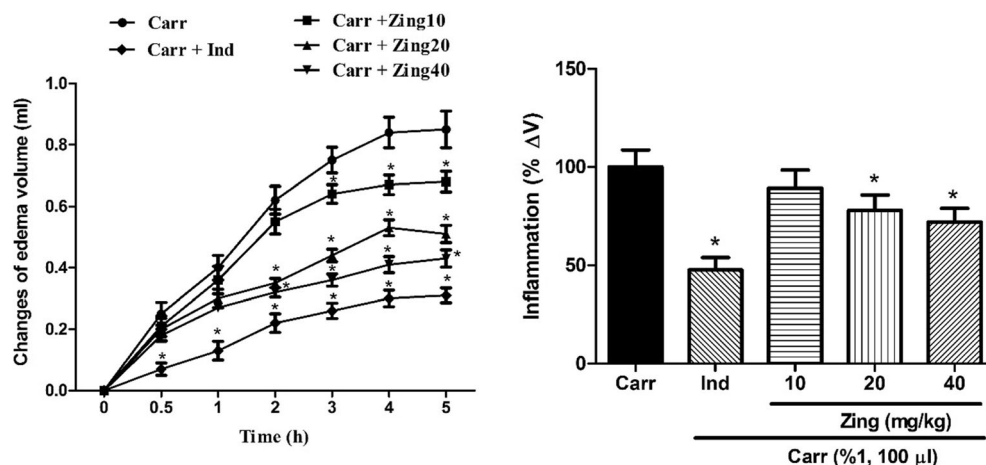
The SOD and CAT activities were significantly reduced in the Carr group compared with the control group (all  $p \leq 0.05$ ) (Fig. 3 a and b). Also, the administration of Ind 30 min before i.pl. injection of Carr significantly elevated these indices relative to Carr group. Moreover, pretreatment with Zing at doses of 20 and 40 mg/kg only significantly increased the SOD activity in paw tissues as compared with the Carr group (all  $p \leq 0.05$ ).

The MDA and NO levels were significantly increased in the Carr group in comparison with the control group (all  $p \leq 0.05$ ) (Fig. 4 a and b). Also, the administration of Ind 30 min before i.pl. injection of Carr significantly reduced these indices compared with the Carr group. Moreover, pretreatment with Zing at doses of 20 and 40 mg/kg significantly reduced the MDA level in paw tissues compared with the Carr group (all  $p \leq 0.05$ ). Furthermore, Zing at all doses (10, 20, and 40 mg/kg) significantly reduced the NO level as compared with the Carr group (all  $p \leq 0.05$ ).

### Effects of Zing on Paw Inflammatory Indices

The levels of COX-2 and PGE<sub>2</sub> were significantly increased in the Carr group compared with the control group (all  $p \leq 0.05$ ) (Fig. 5 a and b). Also, the administration of Ind 30 min before i.pl. injection of Carr significantly reduced these indices in comparison with the Carr group. Moreover, pretreatment with Zing at doses of 20 and 40 mg/kg significantly reduced the levels of COX-2 and PGE<sub>2</sub> in paw tissues compared with the Carr group ( $p \leq 0.05$ ).

The IL-1 $\beta$  and TNF- $\alpha$  levels were significantly elevated in the Carr group compared with the control group (all  $p \leq 0.05$ ) (Fig. 6 a and b). Also, the administration of Ind 30 min before i.pl. injection of Carr significantly reduced these indices compared with the Carr group. Moreover, pretreatment with Zing at doses of 20 and 40 mg/kg significantly reduced the level of IL-1 $\beta$  in paw tissues in comparison with the Carr group ( $p \leq 0.05$ ). Furthermore, Zing at all doses (10, 20, and 40 mg/kg) significantly reduced the TNF- $\alpha$  level compared with the Carr group (all  $p \leq 0.05$ ).



**Fig. 1.** Effect of zingerone (Zing; 10, 20, and 40 mg/kg; i.p.) and indomethacin (Ind; 5 mg/kg; i.p.) against carrageenan (Carr)-induced paw edema (a) and inflammation (b). Drugs were administrated 30 min before Carr injection. Asterisk indicates versus Carr-treated group ( $p \leq 0.05$ ). Data are expressed as mean  $\pm$  SD (one-way ANOVA followed by Tukey's test,  $n = 7$ ).

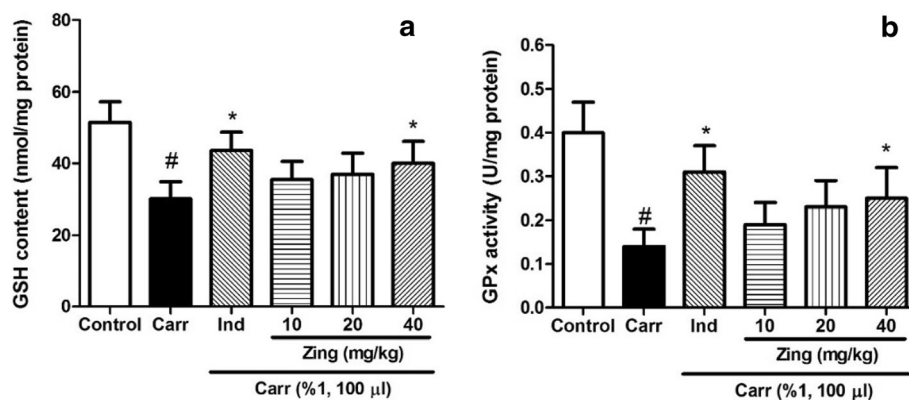
**DISCUSSION**

The protective role of Zing on Carr-induced acute inflammation in the rat paw was investigated in this study. The present study shows that Zing, especially at the highest dose of 40 mg/kg, is capable of mitigating Carr-induced rat paw edema in a dose-dependent manner. Upon oxidative stress and inflammatory measurement, we also noted that treatment with Zing resulted in a reduction of the inflammatory and oxidative reaction in the rat paw.

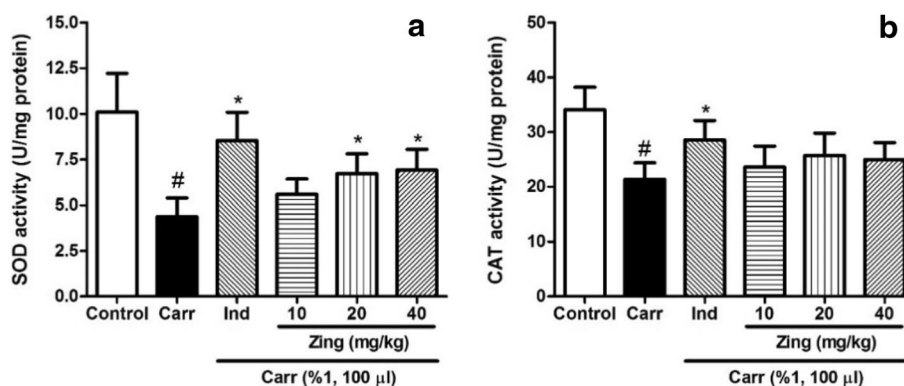
It has been described that Carr induced an inflammation with biphasic phenomenon (early and delayed phases). The early phase (0 to 1 h after injection) is dominantly associated with the serotonin, bradykinin, and histamine.

In this phase, the local blood flow and capillary permeability increased, resulting in edema initiation [34]. The delayed phase (after 1 h) is related with leukocyte migration and prostaglandins that considered the key elements for its maintenance. Inflammation induced by Carr has been applied as a useful model to identify the new anti-inflammatory agents [37].

In inflammatory conditions, over production of reactive oxygen species (ROS) is responsible for organ and cellular damages [16, 26]. On the other hand, high ROS causes the production of other oxidative stress markers such as MDA and NO [15, 21]. Moreover, inflammatory conditions reduced the level and/or activity of antioxidant defense mediators such as CAT, SOD, GPx, and GSH [5].



**Fig. 2.** Effect of zingerone (Zing; 10, 20, and 40 mg/kg; i.p.) and indomethacin (Ind; 5 mg/kg; i.p.) on GSH content (a) and GPx activity (b) of paw tissue. Drugs were administrated 30 min before Carr injection. Pound indicates versus control group ( $p \leq 0.05$ ). Asterisk sign indicates versus Carr-treated group ( $p \leq 0.05$ ). Data are expressed as mean  $\pm$  SD (one-way ANOVA followed by Tukey's test,  $n = 7$ ).

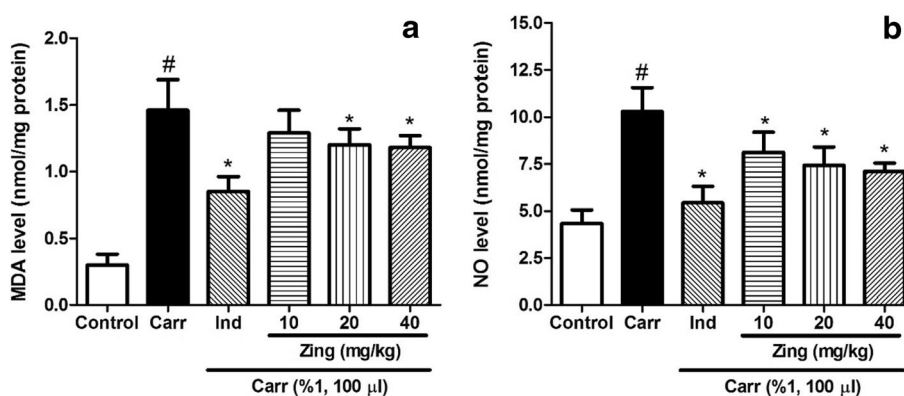


**Fig. 3.** Effect of zingerone (Zing; 10, 20, and 40 mg/kg; i.p.) and indomethacin (Ind; 5 mg/kg; i.p.) on SOD (a) and CAT (b) activities of paw tissue. Drugs were administrated 30 min before Carr injection. Pound indicates versus control group ( $p \leq 0.05$ ). Asterisk sign indicates versus Carr-treated group ( $p \leq 0.05$ ). Data are expressed as mean  $\pm$  SD (one-way ANOVA followed by Tukey's test,  $n = 7$ ).

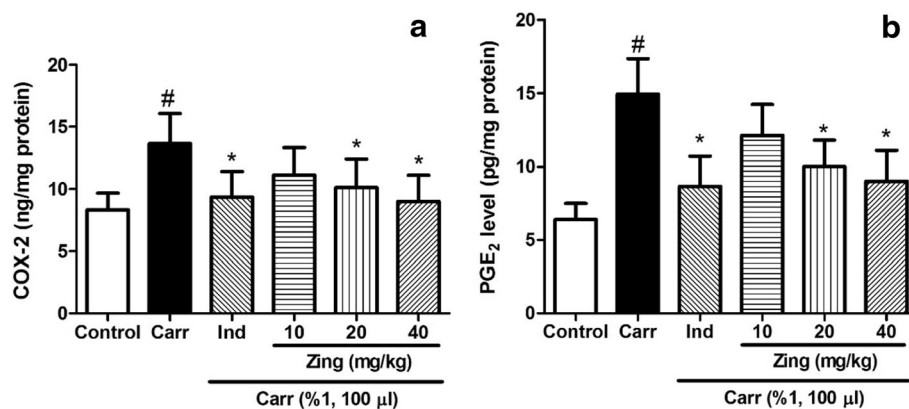
On the other hand, previous reports have shown that i.p. administration of Carr increases the level of MDA and NO and reduces the level and/or activity of these antioxidant parameters [13, 19, 37]. In agreement with these reports, we also found similar effects from i.p. administration of Carr. As potent NSAIDs, Ind attenuated all of these deleterious effects of Carr in paw tissue. Our results revealed that the treatment of animals with Zing (especially at dose of 40 mg/kg) before Carr significantly reduces the paw MDA and NO compared with the Carr group while causing a significant elevation in paw GSH, SOD, and GPx. Zing has potent direct and indirect antioxidative effects. Zing has scavenging activity against ROS via its phenolic group which reduces MDA and NO production [9]. Moreover, Zing indirectly reduces the oxidative stress via increasing the level and/or activity of antioxidant parameters [27]. It has been shown that Zing reduces the mouse testicular

damage induced by zinc oxide nanoparticles via reducing the levels of MDA and increasing SOD and CAT activities [32]. According to the reports of Soliman, Zing attenuated radiation oxidative stress in the heart tissue of rats [35]. In another study, Zing reduced the oxidative stress in the acute vancomycin-induced kidney damages [22]. They found that Zing acts through decreasing MDA level and elevating the activity of SOD, GPx, and CAT.

In the present study, we found a significant increase in the levels of inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , COX-2, and PGE<sub>2</sub> in the paw of Carr-administered animals. Ind and Zing (especially at the doses of 20 and 40 mg/kg) decreased the levels of inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , COX-2, and PGE<sub>2</sub> in the paw after the carrageenan injection. Cytokines such as TNF- $\alpha$  and IL-1 $\beta$  are regulatory mediators produced by a different kind of immune cells. It is well-established that these



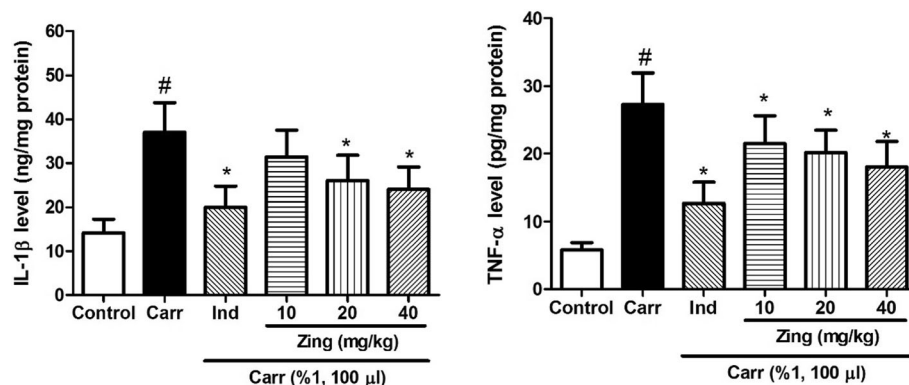
**Fig. 4.** Effect of zingerone (Zing; 10, 20, and 40 mg/kg; i.p.) and indomethacin (Ind; 5 mg/kg; i.p.) on MDA (a) and NO (b) levels of paw tissue. Drugs were administrated 30 min before Carr injection. Pound indicates versus control group ( $p \leq 0.05$ ). Asterisk sign indicates versus Carr-treated group ( $p \leq 0.05$ ). Data are expressed as mean  $\pm$  SD (one-way ANOVA followed by Tukey's test,  $n = 7$ ).



**Fig. 5.** Effect of zingerone (Zing; 10, 20, and 40 mg/kg; i.p.) and indomethacin (Ind; 5 mg/kg; i.p.) on COX-2 (a) and PGE<sub>2</sub> (b) levels of paw tissue. Drugs were administrated 30 min before Carr injection. Pound indicates versus control group ( $p \leq 0.05$ ). Asterisk sign indicates versus Carr-treated group ( $p \leq 0.05$ ). Data are expressed as mean  $\pm$  SD (one-way ANOVA followed by Tukey's test,  $n = 7$ ).

cytokines lead signs of inflammation and cellular injuries [10]. As a pro-inflammatory cytokine, TNF- $\alpha$  further regulates the production of other inflammatory mediators [29]. Moreover, TNF- $\alpha$  stimulates the expression of COX-2 and subsequently releases prostaglandins such as PGE<sub>2</sub> [1]. Prostaglandins are important contributors of inflammatory response. On the other hand, the COX-2/PGE<sub>2</sub> pathway has a critical role in different inflammatory disorders [33]. IL-1 $\beta$  is a prototypical pro-inflammatory cytokine that stimulates both local and systemic immune responses [38]. This cytokine induces the production of various enzymes, such as COX-2 and inducible nitric oxide synthase (iNOS), leading to the production of the inflammatory mediators PGE<sub>2</sub> and NO [19]. On the other hand, it has

been demonstrated that the NO production could influence the prostaglandin levels, so that the reduction of NO levels declines the expression of COX-2 and production of prostaglandin [12]. The results of present study are in agreement with the results of previous reports about anti-inflammatory effects of Zing in different inflammatory conditions. Kandemir et al. reported that Zing reduces the levels of COX-2, TNF- $\alpha$ , and IL-1 $\beta$  in nephrotoxicity induced by cisplatin [23]. Zing was able to attenuate the levels of the inflammatory markers TNF- $\alpha$ , IL-1 $\beta$ , COX-2, and iNOS in cisplatin-induced ovarian and uterine damage [24]. In another study, it was found that Zing reduces the expression of COX-2 and TNF- $\alpha$  in ethanol-induced hepatotoxicity [28]. Hence, it seems that the reduction of



**Fig. 6.** Effect of zingerone (Zing; 10, 20, and 40 mg/kg) and indomethacin (Ind; 5 mg/kg) on IL-1 $\beta$  (a) and TNF- $\alpha$  (b) levels of paw tissue. Drugs were administrated 30 min before Carr injection. Pound indicates versus control group ( $p \leq 0.05$ ). Asterisk sign indicates versus Carr-treated group ( $p \leq 0.05$ ). Data are expressed as mean  $\pm$  SD (one-way ANOVA followed by Tukey's test,  $n = 7$ ).

TNF- $\alpha$  and IL-1 $\beta$  concentration and COX-2 and PGE<sub>2</sub> levels in the paw can be responsible for the anti-inflammatory effects of Zing.

In summary, pretreatment with Zing before Carr injection has the following effects: (i) produces anti-inflammatory effect in an acute inflammatory model; (ii) produces an attenuated effect in TNF- $\alpha$ , IL-1 $\beta$ , COX-2, and PGE<sub>2</sub> levels; and (iii) produces a mitigated effect in activities of CAT, SOD, and GPx and level of GSH. This could provide future directions for studying the anti-inflammatory properties of Zing in inflammatory disorders.

### FUNDING INFORMATION

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### COMPLIANCE WITH ETHICAL STANDARDS

All procedures were conducted according to the Ethical Guideline for Research, Ahvaz University (Ethics code: IR.AJUMS.ABHC.REC.1397.002).

**Conflict of Interest.** The authors declare that they have no conflict of interest.

### REFERENCES

- Abdelazeem, A.H., S.A. Abdelatef, M.T. El-Saadi, H.A. Omar, S.I. Khan, C.R. McCurdy, and S.M. El-Moghazy. 2014. Novel pyrazolopyrimidine derivatives targeting COXs and iNOS enzymes; design, synthesis and biological evaluation as potential anti-inflammatory agents. *European Journal of Pharmaceutical Sciences* 62: 197–211. <https://doi.org/10.1016/j.ejps.2014.05.025>.
- Aebi, Hugo. 1984. Catalase in vitro. *Methods in Enzymology* 105: 121–126.
- Ahmad, B., M.U. Rehman, I. Amin, M.U.R. Mir, S.B. Ahmad, A. Farooq, S. Muzamil, I. Hussain, M. Masoodi, and B. Fatima. 2018. Zingerone (4-(4-hydroxy-3-methylphenyl) butan-2-one) protects against alloxan-induced diabetes via alleviation of oxidative stress and inflammation: probable role of NF- $\kappa$ B activation. *Saudi Pharmaceutical Journal* 26 (8): 1137–1145. <https://doi.org/10.1016/j.jsps.2018.07.001>.
- Alibakhshi, T., M.J. Khodayar, L. Khorsandi, M. Rashno, and L. Zeidooni. 2018. Protective effects of zingerone on oxidative stress and inflammation in cisplatin-induced rat nephrotoxicity. *Biomedicine & Pharmacotherapy* 105: 225–232. <https://doi.org/10.1016/j.biopha.2018.05.085>.
- Arulselvan, P., M.T. Fard, W.S. Tan, S. Gothai, S. Fakurazi, M.E. Norhaizan, and S.S. Kumar. 2016. Role of antioxidants and natural products in inflammation. *Oxidative Medicine and Cellular Longevity* 2016: 5276130–5276115. <https://doi.org/10.1155/2016/5276130>.
- Bradford, Marion M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72 (1): 248–254.
- Buege, John A., and S.D. Aust. 1978. Microsomal lipid peroxidation. *Methods in Enzymology* 52: 302–310.
- Chen, L., H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, and L. Zhao. 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 9 (6): 7204–7218. <https://doi.org/10.18632/oncotarget.23208>.
- Choi, J.G., S.Y. Kim, M. Jeong, and M.S. Oh. 2018. Pharmacotherapeutic potential of ginger and its compounds in age-related neurological disorders. *Pharmacology & Therapeutics* 182: 56–69. <https://doi.org/10.1016/j.pharmthera.2017.08.010>.
- Chou, T.C. 2003. Anti-inflammatory and analgesic effects of paeonol in carrageenan-evoked thermal hyperalgesia. *British Journal of Pharmacology* 139 (6): 1146–1152. <https://doi.org/10.1038/sj.bjp.0705360>.
- Conforti, Filomena, Silvio Sosa, Mariangela Marrelli, Federica Menichini, Giancarlo A. Statti, Dimitar Uzunov, Aurelia Tubaro, and Francesco Menichini. 2009. The protective ability of Mediterranean dietary plants against the oxidative damage: the role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. *Food Chemistry* 112 (3): 587–594.
- de Aquino, P.E., T.R. Magalhaes, L.A. Nicolau, L.K. Leal, N.C. de Aquino, S.M. Dos Santos, K.R. Neves, E.R. Silveira, and G.S. Viana. 2017. The anti-inflammatory effects of N-methyl-(2S,4R)-trans-4-hydroxy-l-proline from Syderoxylon obtusifolium are related to its inhibition of TNF-alpha and inflammatory enzymes. *Phytomedicine* 24: 14–23. <https://doi.org/10.1016/j.phymed.2016.11.010>.
- Dharmasiri, M.G., J.R. Jayakody, G. Galhena, S.S. Liyanage, and W.D. Ratnasooriya. 2003. Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. *Journal of Ethnopharmacology* 87 (2-3): 199–206. [https://doi.org/10.1016/s0378-8741\(03\)00159-4](https://doi.org/10.1016/s0378-8741(03)00159-4).
- Ellman, George L. 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* 82 (1): 70–77.
- Germolec, D.R., K.A. Shipkowski, R.P. Frawley, and E. Evans. 2018. Markers of Inflammation. *Methods in Molecular Biology* 1803: 57–79. [https://doi.org/10.1007/978-1-4939-8549-4\\_5](https://doi.org/10.1007/978-1-4939-8549-4_5).
- Ghaznavi, H., I. Fatemi, H. Kalantari, S.M.T. Hosseini Tabatabaie, M. Mehrabani, B. Gholamine, M. Kalantar, S. Mehrzadi, and M. Goudarzi. 2018. Ameliorative effects of gallic acid on gentamicin-induced nephrotoxicity in rats. *Journal of Asian Natural Products Research* 20 (12): 1182–1193. <https://doi.org/10.1080/10286020.2017.1384819>.
- Goudarzi, M., M.A. Mombeini, I. Fatemi, A. Aminzadeh, H. Kalantari, A. Nesari, H. Najafzadehvarzi, and S. Mehrzadi. 2019. Neuroprotective effects of Ellagic acid against acrylamide-induced neurotoxicity in rats. *Neurological Research* 41 (5): 419–428. <https://doi.org/10.1080/01616412.2019.1576319>.

18. Henschke, Nicholas, Steven J Kamper, and Chris G Maher. 2015. The epidemiology and economic consequences of pain. *Mayo Clinic Proceedings*: Elsevier.
19. Houshmand, G., M.T. Mansouri, B. Naghizadeh, A.A. Hemmati, and M. Hashemitarbar. 2016. Potentiation of indomethacin-induced anti-inflammatory response by pioglitazone in carrageenan-induced acute inflammation in rats: role of PPARgamma receptors. *International Immunopharmacology* 38: 434–442. <https://doi.org/10.1016/j.intimp.2016.06.027>.
20. Hunter, Philip. 2012. The inflammation theory of disease. *EMBO Reports* 13 (11): 968–970.
21. Javadi, Iraj, Mohammadreza Rashidi Nooshabadi, Mehdi Goudarzi, and Rahimeh Roudbari. 2015. Protective effects of celery (*Apium graveolens*) seed extract on bleomycin-induced pulmonary fibrosis in rats. *Journal of Babol University of Medical Sciences* 17 (1): 70–76.
22. Kandemir, F.M., S. Yildirim, S. Kucukler, C. Caglayan, A. Mahamadu, and M.B. Dortbudak. 2018. Therapeutic efficacy of zingerone against vancomycin-induced oxidative stress, inflammation, apoptosis and aquaporin 1 permeability in rat kidney. *Biomedicine & Pharmacotherapy* 105: 981–991. <https://doi.org/10.1016/j.biopha.2018.06.048>.
23. Kandemir, F.M., S. Yildirim, C. Caglayan, S. Kucukler, and G. Eser. 2019. Protective effects of zingerone on cisplatin-induced nephrotoxicity in female rats. *Environmental Science and Pollution Research International* 26 (22): 22562–22574. <https://doi.org/10.1007/s11356-019-05505-3>.
24. Kayguzozglu, E., C. Caglayan, F.M. Kandemir, S. Yildirim, S. Kucukler, M.A. Kilinc, and Y.S. Saglam. 2018. Zingerone ameliorates cisplatin-induced ovarian and uterine toxicity via suppression of sex hormone imbalances, oxidative stress, inflammation and apoptosis in female Wistar rats. *Biomedicine & Pharmacotherapy* 102: 517–530. <https://doi.org/10.1016/j.biopha.2018.03.119>.
25. Lee, B.S., C. Lee, S. Yang, S.K. Ku, and J.S. Bae. 2019. Renal protective effects of zingerone in a mouse model of sepsis. *BMB Reports* 52 (4): 271–276.
26. Li, S., Y. Wang, M. Zhao, J. Wu, and S. Peng. 2015. BPIC: a novel anti-tumor lead capable of inhibiting inflammation and scavenging free radicals. *Bioorganic & Medicinal Chemistry Letters* 25 (5): 1146–1150. <https://doi.org/10.1016/j.bmcl.2014.12.013>.
27. Mahomoodally, M.F., M.Z. Aumeeruddy, K.R.R. Rengasamy, S. Roshan, S. Hammad, J. Pandohee, X. Hu, and G. Zengin. 2019. Ginger and its active compounds in cancer therapy: from folk uses to nano-therapeutic applications. *Seminars in Cancer Biology*. <https://doi.org/10.1016/j.semcancer.2019.08.009>.
28. Mani, V., S. Arivalagan, A.I. Siddique, and N. Namasivayam. 2016. Antioxidant and anti-inflammatory role of zingerone in ethanol-induced hepatotoxicity. *Molecular and Cellular Biochemistry* 421 (1-2): 169–181. <https://doi.org/10.1007/s11010-016-2798-7>.
29. Mark, K.S., W.J. Trickler, and D.W. Miller. 2001. Tumor necrosis factor-alpha induces cyclooxygenase-2 expression and prostaglandin release in brain microvessel endothelial cells. *The Journal of Pharmacology and Experimental Therapeutics* 297 (3): 1051–1058.
30. Martin, Joseph P., Michael Dailey, and Elliott Sugarman. 1987. Negative and positive assays of superoxide dismutase based on hematoxylin autooxidation. *Archives of Biochemistry and Biophysics* 255 (2): 329–336.
31. Opdyke, D.L., and C. Letizia. 1982. Fragrance raw materials monographs. *Food and Chemical Toxicology* 20 (6): 637–852. [https://doi.org/10.1016/S0015-6264\(82\)80217-4](https://doi.org/10.1016/S0015-6264(82)80217-4).
32. Rafiee, Z., L. Khorsandi, and F. Nejad-Dehbashi. 2019. Protective effect of zingerone against mouse testicular damage induced by zinc oxide nanoparticles. *Environmental Science and Pollution Research International* 26 (25): 25814–25824. <https://doi.org/10.1007/s11356-019-05818-3>.
33. Seibert, K., Y. Zhang, K. Leahy, S. Hauser, J. Masferrer, W. Perkins, L. Lee, and P. Isakson. 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proceedings of the National Academy of Sciences of the United States of America* 91 (25): 12013–12017. <https://doi.org/10.1073/pnas.91.25.12013>.
34. Sengar, N., A. Joshi, S.K. Prasad, and S. Hemalatha. 2015. Anti-inflammatory, analgesic and anti-pyretic activities of standardized root extract of *Jasminum sambac*. *Journal of Ethnopharmacology* 160: 140–148. <https://doi.org/10.1016/j.jep.2014.11.039>.
35. Soliman, A.F., L.M. Anees, and D.M. Ibrahim. 2018. Cardioprotective effect of zingerone against oxidative stress, inflammation, and apoptosis induced by cisplatin or gamma radiation in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 391 (8): 819–832. <https://doi.org/10.1007/s00210-018-1506-4>.
36. Tracey, W. Ross, Joel Linden, Michael J. Peach, and Roger A. Johns. 1990. Comparison of spectrophotometric and biological assays for nitric oxide (NO) and endothelium-derived relaxing factor (EDRF): nonspecificity of the diazotization reaction for NO and failure to detect EDRF. *Journal of Pharmacology and Experimental Therapeutics* 252 (3): 922–928.
37. Vinegar, R., W. Schreiber, and R. Hugo. 1969. Biphasic development of carrageenin edema in rats. *The Journal of Pharmacology and Experimental Therapeutics* 166 (1): 96–103.
38. Yazdi, A.S., and K. Ghoreschi. 2016. The interleukin-1 family. *Advances in Experimental Medicine and Biology* 941: 21–29. [https://doi.org/10.1007/978-94-024-0921-5\\_2](https://doi.org/10.1007/978-94-024-0921-5_2).