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The Effect of Simultaneous Use of Opium and Ischemic Preconditioning on Ischemia/Reperfusion Injury in the Rat Liver

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Running title:

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Abstract

BACKGROUND: Ischemia preconditioning (IPC) is known as a protective procedure against the injury induced by ischemia/reperfusion (IR) injury. There is also evidence that the administration of opioids may have the same effects on the injury.

OBJECTIVES: The aim of this study was to investigate the ameliorative effects of simultaneous use of opium and IPC on lobar IR injury in the rat liver.

METHODS: Twenty-five adult male rats were randomly divided into 5 groups: 1) sham-operated, 2) IR, 3) IR+IPC, 4) opium+IPC+IR, and 5) naloxone+opium+IPC+IR. At the end of the reperfusion, blood and tissue samples were obtained to assay alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the blood as well as determining oxidative stress by measuring malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase (CAT) activities in the liver tissues.

RESULTS: The levels of ALT, AST and MDA were significantly increased in the IR group compared to the sham-operated group (P<0.05). However, the application of IPC and IPC+opium significantly decreased the release of these enzymes, while the simultaneous application of opium and IPC had a stronger restorative effect on the IR injury (P<0.05). The recovery effects induced by opium+IPC in terms of TAC, SOD and catalase were also higher than that of the IPC alone. However, the use of naloxone significantly inhibited the protective effects induced by the opium.

CONCLUSIONS: It can be concluded that the simultaneous use of opium and IPC is able to accelerate the protective effects of IPC on the IR injury.

Keywords: Ischemia preconditioning; Ischemia/reperfusion; Liver; Opium; Rat.

1. Introduction

Ischemia, the lack of blood flow to an organ, can cause malfunction of various organs in the body. Blood clots, vasoconstriction, embolism, tumors, congenital disorders, and surgery are among the factors that contribute to the blockage of vessels and the occurrence of ischemia. However, there is evidence that removing the blockage and restoring blood flow paradoxically causes more tissue damage known as reperfusion injury (Ye *et al*., 2020; Ghotbitabar *et al*., 2022; Nazari *et al*., 2024). Ischemia-reperfusion injury (IRI) in liver is a common clinical phenomenon reported in many conditions such as trauma and liver transplantation. After reperfusion, inflammatory cells, including neutrophils, appear through chemotaxis and release various pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and other local mediators (Wang *et al*., 2020). These cytokines induce the increased presence of other immune cells such as CD4+ T lymphocytes. Neutrophils also stimulate the release of reactive oxygen species (ROS), which can disrupt the balance between the oxidant and antioxidant systems in the body (Wu *et al*., 2018). An increase in circulating ROS levels causes oxidative stress, which has been implicated in the induction of several types of tissue damage (Soares *et al*., 2019). Various procedures, including ischemia preconditioning (IPC) as a surgical technique and the use of antioxidants as a pharmaceutical tool, are used to reduce the IRI induced by oxidative stress (Lin *et al*., 2019). Ischemic conditioning may enhance antioxidant effects and reduce inflammation caused by

ischemia-reperfusion in the liver. One study showed that the protective effect of post-conditioning against IRI may be due to a reduction in inflammation and oxidative stress (Afshar *et al.*, 2023).

IPC, as an important organ protection method, is applied by repeated short-term ischemia and reperfusion periods before long-term ischemia and reperfusion. The purpose of applying IPC is to provide organ tolerance to IR injury induced by oxidative stress (Zabala *et al.*, 2019). There is evidence that the application of IPC can have a protective effect in various organs such as the brain, heart, kidney, and liver (Annachhatre & Annachhatre, 2019). In a study conducted by Stokfisz *et al.* (2017), it was found that the application of IPC in any way to the liver under transplantation significantly reduced the IRI as shown by the reduction of liver enzymes, ROS, inflammation and apoptosis. It is also shown that IPC is able to inhibit inflammation by reducing various inflammatory cytokines, including TNF and interleukins, which leads to the prevention of oxidative stress by increasing the activity of antioxidants (Stokfisz *et al.*, 2017).

The use of appropriate exogenous antioxidants is another method of reducing IRI by preventing the production of ROS that occurs with the onset of reperfusion. There is evidence that administration of bucillamine in the early stages of reperfusion can significantly inhibit the severity of IR injury in the transplanted liver (Amersi *et al.*, 2002). Gross *et al.* (2009) observed that opioids may be useful as antioxidants and may also play a role in the protective mechanism of IPC. The role of the delta opioid receptor (δ-OR) in the protective effects of IPC has been reported by several investigators (Dragasis *et al.*, 2013). It has been shown that the use of naltrinol as an opioid antagonist for delta receptors, inhibited the effect of methadone and morphine to reduce the size of myocardial infarction induced by reperfusion (Gross *et al*., 2009). It has also been shown

that agonist stimulation of delta receptors may be associated with antioxidant effects (Yarahmadzehi *et al.*, 2020). Furthermore, Jian *et al.* (2019) found that different opioid compounds such as berberine, papaverine, morphine, and protopine have the potential to show antioxidant and anti-inflammatory effects. Therefore, we hypothesized here that the simultaneous use of opium and IPC can accelerate the protective effects against ischemia/reperfusion-induced injury in the rat liver.

2. Materials and Methods

Animals and experimental groups

Twenty-five adult male Wistar rats weighing 200-250 gr were obtained from the institutional animal facility. Animals were maintained under a 12-hour dark-light cycle in a temperature-controlled environment (24±2°C) with standard chow and water *ad libitum*, and all experiments were performed according to the standard procedures outlined in institutional guidelines. Rats were randomly divided into five experimental groups as follows:

1. Control (sham-operated) group: After anesthesia, laparotomy was performed and the liver was exposed, no ischemia was established, and after the abdomen was closed, the animal was left in normal condition for 180 minutes.
2. IR group: In this group, 60 minutes of ischemia and then 120 minutes of reperfusion were performed in the anesthetized rat.

3. IPC+IR group: The IPC was applied by a cycle of 10 minutes of ischemia followed by 10 minutes of reperfusion, and then the liver was exposed to 60 minutes of ischemia plus 120 minutes of reperfusion.
4. Opium+IPC+IR group: 15 min before IPC induction, 30 mg/kg opium was injected IP and then the procedure was performed as in group 3.
5. Naloxone+Opium+IPC+IR group: The procedure performed on the rat in this group was the same as in group 4 except that 15 minutes before opium injection, 3 mg/kg naloxone (Toliddarou Co, Iran) was injected IV into the rats.

Opium was obtained from the Police Anti-Drug Bureau and analyzed for purity and constituents using the GC mass spectrometry method. The obtained opium contained about 30% of alkaloids (morphine 15%, thebaine 4.4%, codeine 5.2%, and papaverine 3.7%) and the rest were non-alkaloid organic and inorganic substances with 15.5% water (moisture).

Surgical procedure for induction of IR and application of IPC

Before each experiment, the rat was fasted for approximately 15 h and then anesthetized with 80 mg/kg ketamine (Alfasan, Netherlands) plus 5 mg/kg xylazine (Alfasan, Netherlands) prepared as a cocktail and injected IP. After administration of 300 units of heparin to each rat through the femoral vein, laparotomy was performed. The method used to induce lobar IR was previously described by Arab *et al*. (2009). Briefly, the liver was exposed by cutting the ligaments attached to

the ventricular wall. To induce warm lobar ischemia, the portal vein, hepatic artery, and bile duct were clamped with a microvascular clamp for 60 minutes. After removal of the clamp, reperfusion was started and continued for 120 minutes. IPC was applied by a cycle of 10 minutes of ischemia followed by 10 minutes of reperfusion before performing prolonged (60 minutes) ischemia.

Sampling

At the end of the reperfusion period, blood was collected from the rats' hearts using a 5 ml disposable syringe. The blood samples were centrifuged at 2500 rpm for 10 minutes, and the serum was separated and then frozen at -20°C until analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST). After blood sampling, the rats were euthanized by injection of a large dose of thiopental, and tissue samples were immediately collected from the left and middle lobes of the liver. These samples were stored in a freezer at -70°C. The latter were used to measure the amount of malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase (CAT) activities.

Assay of enzyme release

An Alan Eppendorf (Germany) autoanalyzer system was used to measure the release of ALT and AST from hepatocytes. Serum samples were thawed and an autoanalyzer was used to measure ALT and AST using the Trucal-u calibrator. When all the necessary solutions for measuring the enzymes were prepared, the device was set to perform the analysis through the computer and then the serum levels of these enzymes were measured using the commercial kits of Byrex (Fars Co., Iran).

Oxidant/antioxidant assessment

The liver samples taken from the both left and median hepatic lobes were used to determine TAC, MDA, and the activity of the SOD and CAT in the liver. The level of MDA was used to estimate the amount of lipid peroxidation in the liver exposed to different treatments. The MDA level was measured according to the method developed by Placer *et al.* (1966). The working reagent solution was 20% trichloroacetic acid (TCA) (Merck, Germany) and 0.6% thiobarbituric acid (TBA) (Merck, Germany) in 0.25N hydrochloric acid (HCL) (Merck, Germany). The homogenized tissue sample was mixed with a working solution and placed in a boiling water bath for 30 minutes. After cooling, it was centrifuged at 5000 rpm for 5 minutes and then the absorbance of the supernatant was measured at 535 nm using a spectrophotometer (Biotech, USA). The activity of SOD was estimated by the method developed by Kono (1978), which is based on a color reaction and the ability of SOD to inhibit the formation of superoxide radicals. The TAC of tissue samples was measured according to the method developed by Benzie and Strain (1999). This method is based on the reduction of ferric TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) complex to ferro TPTZ under acidic conditions. The absorbance of the blue colored solution obtained from the reactions of the latter complex was measured spectrophotometrically (Biotech, USA) at a wavelength of 593 nm. The activity of catalase in the samples was measured by the method of Koroliuk *et al*. (1988). Tris-HCl buffer (0.05 mmol/L, pH 7.8, Merck, Germany) containing 10 mmol/L hydrogen peroxide was mixed with homogenized tissue samples. After 10 minutes, 4% ammonium molybdate was added to the solution to produce the color reaction. The optical absorbance of the colored mixture was measured at a wavelength of 410 nm using a spectrophotometer.

Statistical analysis

Biochemical assay data are expressed as the mean±SD of at least five experiments in each group. They were analyzed by ANOVA using the SPSS program, and the significance of differences between experimental groups was tested by Tukey's post hoc test. The *P* value less than 0.05 was considered statistically significant.

3. Results

The effects of IPC+opium on enzyme release

Significant differences were observed between the different experimental groups in terms of enzyme release (ALT, AST). As shown in the following Table, a significant increase in ALT and AST levels was observed in the ischemia/reperfusion group (118.67±17.68 and 108.13±12.33, respectively) compared with the control group (27.27±8.52 and 29.13±8.19, respectively) (P<0.005). However, the use of IPC was associated with a significant reduction (P<0.0001) in ALT and AST levels in the group receiving the treatment compared with the IR group (78.9±15.19 vs. 118.67±17.68 and 71.3±9.19 vs. 108.13±12.33, respectively). It was found that the simultaneous application of opium and IPC enhanced the protective effects of IPC, as shown by various assessment parameters, including changes in biochemical values. The levels of ALT and AST in the opium+IPC+IR group decreased significantly (P<0.01-0.001) from 78.9±15.19 to 49.04±13.21 and 71.3±9.19 to 49.67±12.32, respectively. No significant increases in the levels of ALT and AST were observed in the naloxone+opium+IPC+IR group compared to the opium+IPC+IR group.

Table. The values of AST and ALT (IU/L) in different experimental groups applied in the present study. These values are expressed as mean ± SD obtained from at least 5 experiments in each group.

Groups	AST (IU/L)	ALT (IU/L)
Control	29.13±8.19 [d]	27.27 ± 8.52 [d]
IR	108.13 ± 12.33 [a]	118.67 ± 17.68 [a]
IR + IPC	71.3 ± 9.19 [b]	78.9 ± 15.19 [b]
Opium + IPC + IR	49.67 ± 12.32 [c]	49.04 ± 13.21 [c]
Naloxone + Opium + IPC + IR	68.95 ± 6.92 [b]	76.11 ± 13.84 [b]

a, b, c, and d: Different superscripts in each column indicate significant differences ($P \leq 0.05$) between different groups.

Abbreviations: AST: aspartate aminotransferase; ALT: alanine aminotransferase; IR: Ischemia-reperfusion; IPC: Ischemia preconditioning.

The effects of IPC+opium on oxidative changes induced by IR

The results of the oxidative stress indices (MDA, SOD, CAT, TAC) assessment showed significant differences among the different experimental groups. As the Figure 1 shows the MDA level was significantly increased in the IR group compared to the sham-operated (control) group (71.63±4.31 vs. 34.33±4.25, $P<0.0001$). However, there were significant decrease ($P<0.0001$) in the activities of SOD (18.08±0.49 vs. 23.16±1.45) (Figure 2), CAT (31.51±9.24 vs. 109.1±8.08) (Figure 3), and TAC (46.49±1.77 vs. 88.49±5.04) (Figure 4) in the IR-treated group compared to

the control group. MDA levels in the IPC and IR groups were 48.98±0.61 and 71.63±4.3, respectively, showing a significant reduction (P<0.0001) in the treated group. The results showed that the activity of SOD, CAT and TAC were significantly (P<0.05-0.001) increased in the IPC group with the values of 20.77±0.11 vs. 18.08±0.49, 83.66±1.31 vs. 31.51±9.24 and 68.38±1.07 vs. 46.49±1.77, respectively. The level of MDA in the opium+IPC+IR group decreased significantly (P<0.01-0.001) from 48.98±0.61 to 38.6±0.28. The amount of CAT and TAC in the opium+IPC+IR group was also significantly increased compared to the IPC+IR group (P=0.01) with the values of 100.09±2.38 and 77.58±3.31, respectively. However, the application of naloxone, as opioid antagonist, inhabited the change induced in the values of some parameters. A significant increase in MDA level was observed in the naloxone+opium+IPC+IR group compared to the opium+IPC+IR group (60.04±1.33 vs. 38.6±0.28, P<0.001). A significant reduction (P=0.001) in the levels of SOD, CAT, and TAC was also observed in the naloxone-treated animals compared to the opium+IPC+IR group (19.54±0.3 vs. 22.22±0.57, 53.81±5.17 vs. 100.09±2.38, and 61.61±1.42 vs. 77.58±3.31, respectively). There was a significant increase in MDA level as a marker of lipid peroxidation in the rats exposed to 1 hour of ischemia followed by 2 hours of reperfusion compared to the group receiving opium and IPC. There was also a significant decrease (P<0.0001) in the activities of SOD, CAT, and TAC in the IR-treated group compared with the opium+IPC group.

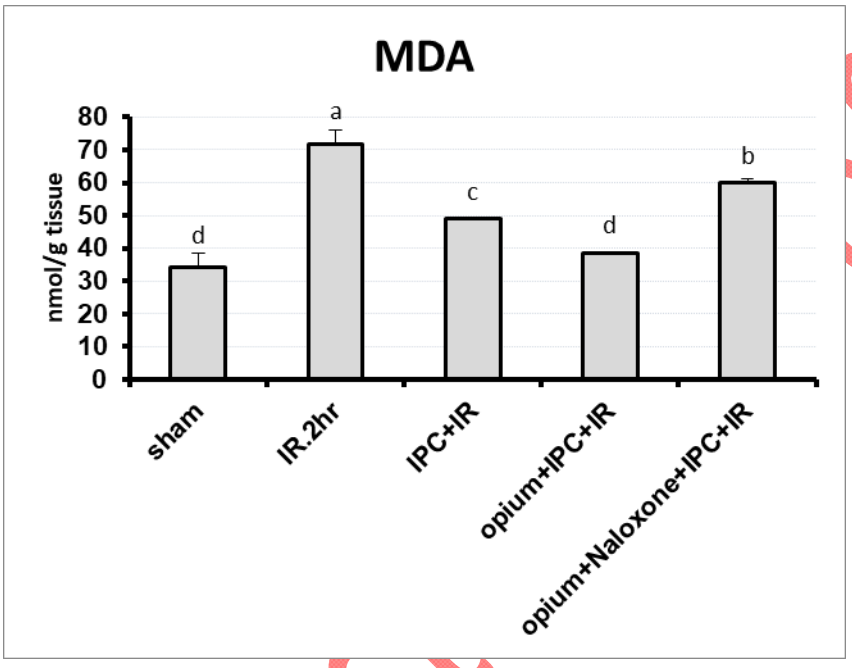

Figure 1. The levels of MDA (nmol/g) in different experimental groups. The data are expressed as mean ± SD obtained from at least 5 experiments in each group. Different superscripts in each column indicate significant differences ($P \leq 0.05$) between various groups.

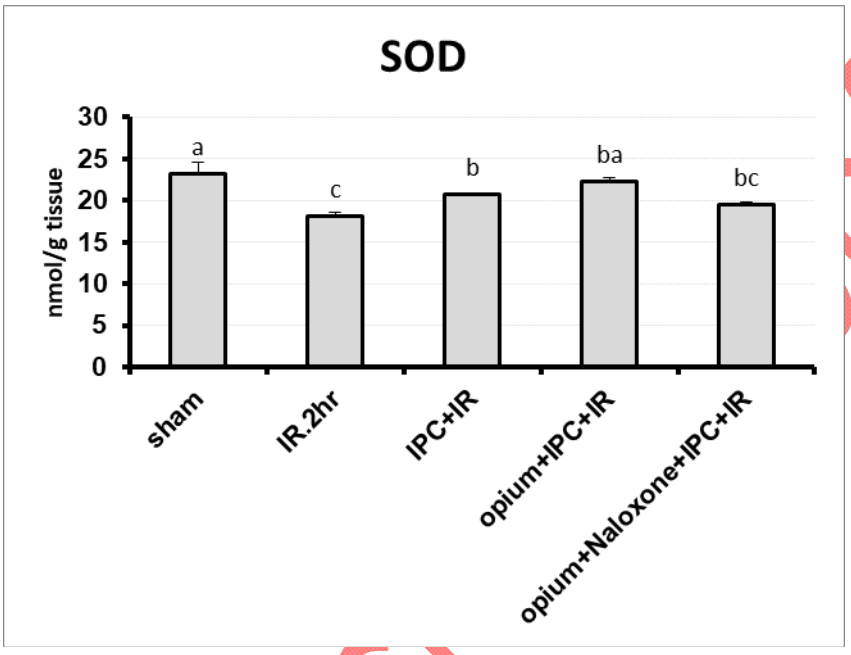

Figure 2. The values of SOD (nmol/g) in different experimental groups. The values are expressed as mean ± SD obtained from at least 5 experiments in each group. Different superscripts in each column indicate significant differences ($P \leq 0.05$) between various groups.

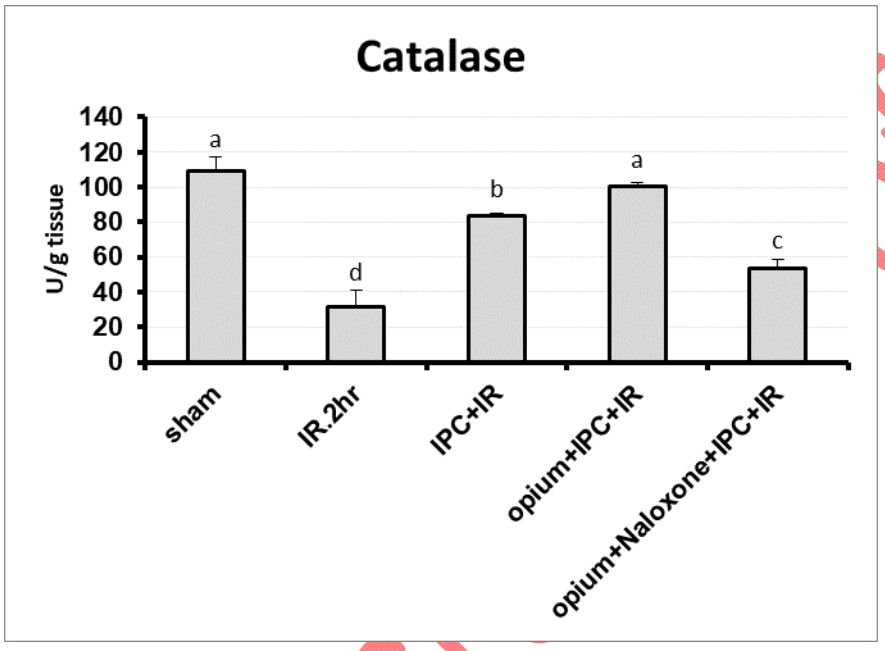

Figure 3. The values of Catalase (U/g) in different experimental groups. The data are expressed as mean ± SD obtained from at least 5 experiments in each group. Different superscripts in each column indicate significant differences ($P \leq 0.05$) between various groups.

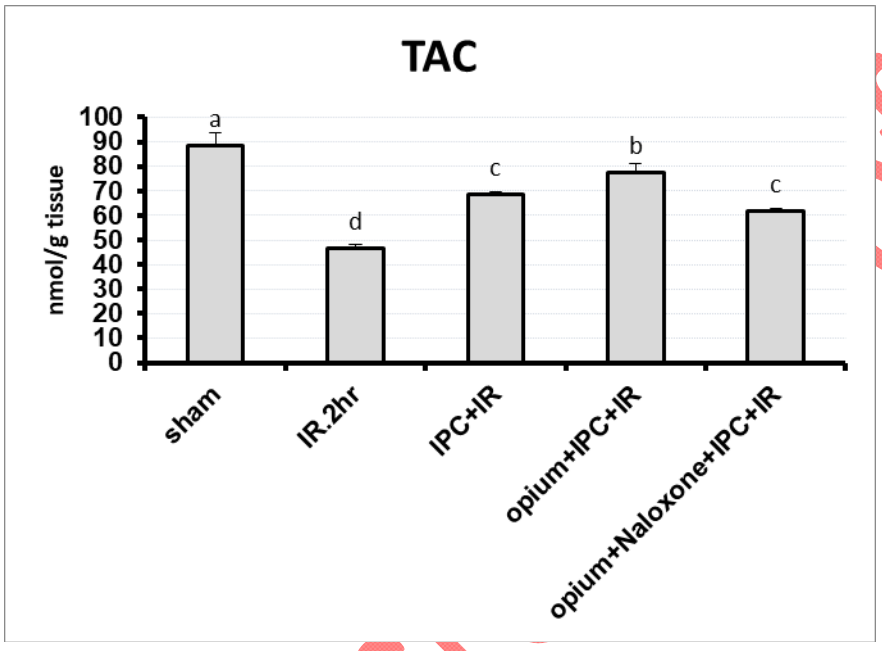

Figure 4. The values of TAC (nmol/g) in different experimental groups. The values are expressed as mean ± SD obtained from at least 5 experiments in each group. Different superscripts in each column indicate significant differences ($P \leq 0.05$) between various groups.

4. Discussion

The present study was designed to investigate the protective effects of the simultaneous use of opium and IPC on IR-induced injury. It was hypothesized that while the application of IPC is able to reduce IRI, the administration of opium before the application of a 10-minute ischemia and 10-minute reperfusion cycle, may significantly accelerate the protective effects of IPC. This suggests that opioids can be involved in the protective mechanism of the IPC method.

IRI in the liver is a pathophysiological phenomenon associated with surgery, liver transplantation, hemorrhage, hypovolemic shock, and partial hepatectomy. Studies show that this injury is the major cause of death in the surgery and liver transplantation through the production of various proinflammatory agents including cytokines, circulating chemokines, and ROS, which is leading to the damage of distant organs (Shen *et al*., 2020). Shen *et al.* (2020) showed that the liver exposed to IR is associated with increased liver enzymes and organ failure leading to liver dysfunction. They showed that the livers exposed to 1 hour of ischemia followed by 2 hours of reperfusion showed a significant increase in serum levels of ALT and AST, as well as tissue changes due to oxidative stress. The results of the present study are consistent with the results of many other studies. However, it was found that the simultaneous application of IPC and opium before ischemia was able to inhibit the increased levels of AST and ALT induced by IR in the liver. Kim *et al.* (2020) showed that the application of IPC by 10 min of hepatic ischemia followed by 15 min of reperfusion before 45 min of ischemia is able to inhibit the elevated levels of AST and ALT. Lin *et al.* (2019) also reported that the application of IPC before hepatic ischemia can improve the function of this organ by increasing the antioxidant activities (Lin *et al.*, 2019). The efficacy of opium and IPC in controlling oxidative stress has been investigated in various experimental models. The work of Shen *et al.* (2020) also shows that when the liver is exposed to IR, various indicators of oxidative stress, including MDA, are increased and the level of SOD and catalase activities are decreased, as it occurs in the present study (Soltanpour *et al.*, 2022). However, the amount of SOD, TAC, and catalase were significantly increased in the Opium+IPC group compared with the IR group. It can be concluded that the changes in the oxidative/antioxidant indices in the groups exposed to 1 hour of ischemia followed by 2 hours of

reperfusion is an indication of the occurrence of oxidative stress in the acute phase of the hepatic warm IR model. These results are consistent with the findings of other studies including Yarahmadzehi *et al.* (2020) and Li *et al.* (2014). The protective effects of opium may be due to the prevention of oxidative stress by increasing antioxidant activities. There is evidence that various opioid compounds including papaverine, morphine, and protopine are able to exert antioxidant and anti-inflammatory effects (Jian *et al*., 2019). Studies show that opioids can play an important role in reducing IR-induced injury in various organs, including the liver (Wang *et al.*, 2012). Morphine has been shown to have promising effects in patients with myocardial infarction by exerting an anti-inflammatory effect (Dorsch *et al.*, 2016). There is evidence that fentanyl was also able to reduce infarct size and increase cardiac contractile function. In addition, remifentanil, an ultrashort-acting opioid, has shown similar cardioprotective effects to fentanyl (Annachhatre & Annachhatre, 2019). Yarahmadzehi *et al.* (2020) reported that opium was effective in reducing ischemia-induced brain injury by increasing antioxidant and anti-inflammatory activities, ultimately leading to the neuroprotective effect.

Recent studies suggest that endogenous opioids are involved in the protective effects of IPC in the treated organs. Husain *et al.* (2009) reported that activation of opioid receptors is required to induce the protective effects of IPC in the retina and showed that the use of a broad-spectrum opioid agonist such as morphine can reduce retinal ischemic injury (Husain *et al.*, 2009). In the present study, we found that the administration of opium before the application of IPC increased the level of antioxidant activities in the liver tissue exposed to ischemia/reperfusion injury. Yarahmadzehi *et al.* (2020) reported that the protective effects of various opium alkaloids (morphine, papaverine, protopine, and berberine) against postischemic brain cell death may be

exerted through the anti-inflammatory mechanism. It has been shown that the therapeutic effects of berberine as a natural compound against diabetes mellitus and insulin resistance may be partially contributed to its antioxidant and anti-inflammatory activities (Li *et al.*, 2014). It has also been shown that the neuroprotective effects of papaverine are exerted through anti-inflammatory and antioxidant properties (Solmaz *et al.*, 2022). Finally, there is evidence that morphine induces both oxidant and antioxidant activities by producing ROS and increasing SOD and glutathione reductase in peripheral blood cells (Zhang *et al.*, 2004).

5. Conclusion

In the present study, it was shown that the simultaneous application of opium and IPC can accelerate the reduction of hepatic enzymes and oxidative stress induced by IR in rat liver as well as improving the antioxidant activities. These accelerating effects of opium are probably due to the antioxidant role of morphine and other alkaloids present in the opium. So, the results of the present study showed that the simultaneous use of opium and IPC can be more effective in the reducing ischemia-reperfusion injury. It can be concluded that the simultaneous use of opium and IPC may open a new perspective in IPC mechanisms of action and may be a promising method to protect the liver against the IR injury in the future.

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References

Afshar, N. G. P., Arab, H. A., Vatannejad, A., Ashabi, G., & Golabchifar, A. A. (2023). The Antioxidant and Anti-Inflammatory Effects of Ischemia Post-Conditioning on the Injury Induced by Ischemia/Reperfusion in the Rat Liver. *Journal of Veterinary Research/Majallah-i Taḥqīqāt-i Dāmpizishkī University*, *78*(3). DOI: 10.22059/JVR.2022.348794.3302

Amersi, F., Nelson, S. K., Shen, X. D., Kato, H., Melinek, J., Kupiec-Weglinski, J. W., Horwitz, L. D., Busuttil, R. W., & Horwitz, M. A. (2002). Bucillamine, a thiol antioxidant, prevents transplantation-associated reperfusion injury. *Proceedings of the National Academy of Sciences*, *99*(13), 8915-8920. DOI: https://doi.org/10.1073/pnas.132026099

Annachhatre, A. S., & Annachhatre, S. R. (2019). Preconditioning in cardiac anesthesia…… where are we? *Annals of Cardiac Anaesthesia*, *22*(4), 412. DOI: 10.4103/aca.ACA_116_18

Arab, H. A., Sasani, F., Rafiee, M. H., Fatemi, A., & Javaheri, A. (2009). Histological and biochemical alterations in early-stage lobar ischemia-reperfusion in rat liver. *World journal of gastroenterology: WJG*, *15*(16), 1951. DOI: 10.3748/wjg.15.1951

Benzie, I. F., & Strain, J. (1999). [2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods in enzymology* (Vol. 299, pp. 15-27). Elsevier. DOI: https://doi.org/10.1016/S0076-6879(99)99005-5

Dorsch, M., Behmenburg, F., Raible, M., Blase, D., Grievink, H., Hollmann, M. W., Heinen, A., & Huhn, R. (2016). Morphine-induced preconditioning: involvement of protein kinase A and mitochondrial permeability transition pore. *PLoS One*, *11*(3), e0151025. DOI: https://doi.org/10.1371/journal.pone.0151025

Dragasis, S., Bassiakou, E., Iacovidou, N., Papadimitriou, L., Steen, P. A., Gulati, A., & Xanthos, T. (2013). The role of opioid receptor agonists in ischemic preconditioning.

European Journal of Pharmacology, *720*(1-3), 401-408. DOI: https://doi.org/10.1016/j.ejphar.2013.10.001

Ghotbitabar, Z., Asghari, A., Hassanpour, S., & Jahandideh, A. (2022). Effects of Quebracho Tannin Extract on Testicular Ischemia-/Reperfusion. *Iranian Journal of Veterinary Medicine*, *16*(4). DOI: 10.22059/IJVM.2022.333335.1005206

Gross, E. R., Hsu, A. K., & Gross, G. J. (2009). Acute methadone treatment reduces myocardial infarct size via the δ-opioid receptor in rats during reperfusion. *Anesthesia and analgesia*, *109*(5), 1395. DOI: 10.1213/ANE.0b013e3181b92201

Husain, S., Potter, D. E., & Crosson, C. E. (2009). Opioid receptor-activation: retina protected from ischemic injury. *Investigative ophthalmology & visual science*, *50*(8), 3853-3859. DOI: https://doi.org/10.1167/iovs.08-2907

Jian, Z., Liu, R., Zhu, X., Smerin, D., Zhong, Y., Gu, L., Fang, W., & Xiong, X. (2019). The involvement and therapy target of immune cells after ischemic stroke. *Frontiers in immunology*, *10*, 2167. DOI: https://doi.org/10.3389/fimmu.2019.02167

Kono, Y. (1978). Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Archives of biochemistry and biophysics*, *186*(1), 189-195. DOI: https://doi.org/10.1016/0003-9861(78)90479-4

Koroliuk, M., Ivanova, L., Maĭorova, I., & Tokarev, V. (1988). A method of determining catalase activity. *Laboratornoe delo*(1), 16-19. PMID: 2451064

Li, Z., Geng, Y.-N., Jiang, J.-D., & Kong, W.-J. (2014). Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. *Evidence-based complementary and alternative medicine*, *2014*. DOI: https://doi.org/10.1155/2014/289264

Lin, J., Huang, H., Yang, S., Duan, J., Xu, W., & Zeng, Z. (2019). Protective effects of ischemic preconditioning protocols on ischemia-reperfusion injury in rat liver. *Journal of Investigative Surgery*. DOI: https://doi.org/10.1080/08941939.2018.1556753

Nazari, M., Kheradmand, A., Alirezaei, M., & Raisi, A. (2024). Protective Effects of Ghrelin Following Experimentally Induced Ischemia-Reperfusion in the Rat Ovary. *Iranian Journal of Veterinary Medicine*. DOI:10.22059/IJVM.2024.370891.1005503

Placer, Z. A., Cushman, L. L., & Johnson, B. C. (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical biochemistry*, *16*(2), 359-364. DOI: https://doi.org/10.1016/0003-2697(66)90167-9

Shen, Y., Shen, X., Cheng, Y., & Liu, Y. (2020). Myricitrin pretreatment ameliorates mouse liver ischemia reperfusion injury. *International immunopharmacology*, *89*, 107005. DOI: https://doi.org/10.1016/j.intimp.2020.107005

Soares, R. O., Losada, D. M., Jordani, M. C., Évora, P., & Castro-e-Silva, O. (2019). Ischemia/reperfusion injury revisited: an overview of the latest pharmacological strategies. *International journal of molecular sciences*, *20*(20), 5034. DOI: https://doi.org/10.3390/ijms20205034

Solmaz, V., Kaya, M., Uslu, F. B., Atasoy, O., & Erbaş, O. (2022). Papaverine has therapeutic potential for sepsis-induced neuropathy in rats, possibly via the modulation of HMGB1-RAGE axis and its antioxidant prosperities. *Journal of Investigative Surgery*, *35*(1), 7-13. DOI: 10.1080/08941939.2020.1809751

Soltanpour, M., Khojasteh, S. M. B., Hamidian, G., Nahali, S., & Morovvati, H. (2022). Evaluation of the effect of vanadium-zinc complex on the changes in histological and stereological structure, functional enzymes, and oxidative stress indices of liver tissue in adult male rats with type 2 diabetes. DOI: 10.22059/jvr.2022.325647.3167

Stokfisz, K., Ledakowicz-Polak, A., Zagorski, M., & Zielinska, M. (2017). Ischaemic preconditioning–Current knowledge and potential future applications after 30 years of experience. *Advances in medical sciences*, *62*(2), 307-316. DOI: https://doi.org/10.1016/j.advms.2016.11.006

Wang, Y., Liu, J., Qu, Y., Fan, Y., & Yu, W. (2020). Intrathecal morphine pretreatment ameliorated hepatic ischemia-reperfusion injury by reducing inflammation through

peripheral and spinal opioid activation in rats. DOI: https://doi.org/10.21203/rs.2.21894/v1

Wang, Y., Wong, G., Man, K., & Irwin, M. (2012). Pretreatment with intrathecal or intravenous morphine attenuates hepatic ischaemia–reperfusion injury in normal and cirrhotic rat liver. *British journal of anaesthesia*, *109*(4), 529-539. DOI: https://doi.org/10.1093/bja/aes209

Wu, H.-H., Huang, C.-C., Chang, C.-P., Lin, M.-T., Niu, K.-C., & Tian, Y.-F. (2018). Heat shock protein 70 (HSP70) reduces hepatic inflammatory and oxidative damage in a rat model of liver ischemia/reperfusion injury with hyperbaric oxygen preconditioning. *Medical science monitor: international medical journal of experimental and clinical research*, *24*, 8096. DOI: 10.12659/MSM.911641

Yarahmadzehi, S., Fanaei, H., Mirshekar, M. A., & Atashpanjeh, A. R. (2020). Opium consumption exerts protective effect against cerebral ischemia through reducing inflammation and enhancing antioxidant defense in male rats. *Neurology, Psychiatry and Brain Research*, *37*, 15-20. DOI: https://doi.org/10.1016/j.npbr.2020.05.005

Ye, L., He, S., Mao, X., Zhang, Y., Cai, Y., & Li, S. (2020). Effect of hepatic macrophage polarization and apoptosis on liver ischemia and reperfusion injury during liver transplantation. *Frontiers in immunology*, *11*, 1193. DOI: https://doi.org/10.3389/fimmu.2020.01193

Zabala, V., Boylan, J. M., Thevenot, P., Frank, A., Senthoor, D., Iyengar, V., Kim, H., Cohen, A., Gruppuso, P. A., & Sanders, J. A. (2019). Transcriptional changes during hepatic ischemia-reperfusion in the rat. *PLoS One*, *14*(12), e0227038. DOI: https://doi.org/10.1371/journal.pone.0227038

Zhang, Y. T., Zheng, Q. S., Pan, J., & Zheng, R. L. (2004). Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. *Basic & clinical pharmacology & toxicology*, *95*(2), 53-58. DOI: https://doi.org/10.1111/j.1742-7843.2004.950202.x

تأثیر مصرف همزمان اوپیوم و پیش‌شرطی‌سازی برآسیب ناشی از ایسکمی/ریپرفیوژن در کبد موش صحرایی

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چکیده:

زمینه مطالعه: پیش شرطی سازی ایسکمیک (IPC) به عنوان یک روش محافظتی در برابر آسیب ناشی از ایسکمی/ریپرفیوژن (IR) شناخته می‌شود. همچنین شواهدی وجود دارد که نشان می دهد تجویز اوپیوئیدها ممکن است اثرات مشابهی بر آسیب داشته باشند.

هدف: هدف از این مطالعه بررسی اثرات بهبودی مصرف همزمان اوپیوم به همراه IPC بر آسیب ناشی از IR در کبد موش صحرایی بود.

روش کار: 25 سر موش صحرایی نر بالغ به طور تصادفی به 5 گروه تقسیم شدند: گروه اول: Sham، گروه دوم:IR، گروه سوم: IPC+IR، گروه چهارم: Opium+IPC+IR، گروه پنجم: Naloxone+Opium+IPC+IR. در پایان ریپرفیوژن، نمونه های خون

برای سنجش آلانین آمینوترانسفراز (ALT) و آسپارتات آمینوترانسفراز (AST) و همچنین نمونه‌های بافتی برای تعیین استرس اکسیداتیو با اندازه گیری مالون دی آلدئید (MDA)، ظرفیت آنتی اکسیدانی تام (TAC)، فعالیت سوپراکسید دیسموتاز (SOD) و کاتالاز (CAT) از بافت کبد گرفته شد.

نتایج: سطوح AST،ALT و MDA در گروه IR نسبت به گروه Sham به طور معنی‌داری افزایش یافت ($P<0.05$). استفاده همزمان از IPC و اوپیوم به طور قابل توجهی باعث کاهش آزادسازی این آنزیم‌ها شد که اثر ترمیمی قوی‌تری بر آسیب IR داشت ($P<0.05$). اثرات بهبودی ناشی از مصرف همزمان اوپیوم در کنارIPC از نظر TAC، SOD و کاتالاز نیز بیشتر از گروه IPC به تنهایی بود. با این حال، استفاده از نالوکسان به طور قابل توجهی اثرات محافظتی ناشی از اوپیوم را مهار کرد.

نتیجه‌گیری نهایی: می‌توان نتیجه گرفت که استفاده همزمان از اوپیوم در کنارIPC می‌تواند اثرات محافظتی IPC را در برابر آسیب IR تسریع کند.

کلیدواژه: پیش‌شرطی‌سازی ایسکمیک، ایسکمی/ریپرفیوژن، کبد، اوپیوم، موش صحرایی