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Research Article
Comparative Evaluation of Ovarian Ischemia-Reperfusion Induced Distant Organ Damage in Rats

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Abstract
Background and Objective: Ischemia-reperfusion (I/R) injury is seen in many conditions, including ovarian torsion/detorsion. Ovarian ischemia is an emergency that occurs as a result of torsion of the ovaries. This study aimed to investigate the extent to which brain, heart, lung, kidney and liver tissues were affected by ovarian ischemia-reperfusion damage in albino Wistar-type female rats and to evaluate them by comparison. **Materials and Methods:** Animals were divided into two groups: The group to be applied sham operation (SG) and the group to be applied the ischemia-reperfusion procedure to the ovaries (OIR). As 2 hrs of ischemia and 6 hrs of reperfusion were conducted in the right ovaries of OIR group rats. After reperfusion, the ovarian, brain, heart, lung, kidney and liver tissues of the animals sacrificed were removed. The severity of tissue damage was assessed by the degree of significance of the increase in oxidative and proinflammatory parameters and the decrease in antioxidants. **Results:** The organs obtained from the OIR group in which MDA and IL-6 levels were highest, while tGSH, SOD and CAT levels were the lowest, were ovary>kidney>lung>heart, respectively. The levels of these parameters in the brain and liver tissues were found to be almost the same as in the SG group. Current findings showed that ovarian ischemia-reperfusion damage did not affect brain and liver tissues, but the heart was mildly affected, the lung was moderately and kidney tissue was severely affected. **Conclusion:** Current experimental results showed that it may be useful to consider the possibility of damage to organs such as the heart, lungs and kidneys before and after the ovarian ischemia-reperfusion procedure and take the necessary precautions.

Key words: Heart, kidney, lung, ovarian ischemia-reperfusion, oxidative and inflammatory damage

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ischemia, as is known, is the event that the tissue is deprived of oxygen due to the decrease or complete cessation of the blood flow to the tissue[1,2]. Reperfusion is the re-blooding of ischaemic tissue[3]. Ischemia-reperfusion (I/R) damage is observed in conditions such as organ resection and transplantation, various types of shock and ovarian torsion/detorsion[4]. Ovarian ischemia is an emergency condition resulting from torsion of the ovaries[5]. Ovarian torsion occurs as a result of a complete or partial sprain of the ovaries around the ligaments that support them[6]. Ovarian torsion is observed in pathological conditions such as benign ovarian cysts, teratomas, congenital long ovarian ligaments, excessive laxity of the pelvic ligaments and a small uterus[7]. Ovarian torsion is a pathological case that can be observed in women at any age and requires urgent surgical intervention[8]. If torsioned ovaries are not intervened promptly, it causes necrosis of the ovaries and concludes in ovariectomy[9]. For this reason, for the protection of the ovaries, re-blooding (reperfusion) of the torsioned ovary by detorsion is provided[10,11]. However, the provision of reperfusion by detorsion causes more severe damage than the damage caused by ischemia[11,12]. This event takes place when O_2, which is presented to the tissue with a large amount of blood during reperfusion, induces hypoxanthine metabolism. This reaction causes the overproduction of reactive oxygen species (ROS)[3]. As such, local inflammation and ROS production are further increased during reperfusion and lead to more severe damage[13]. Furthermore, it has been reported that the severity of I/R damage increases in direct proportion to the increase in inflammatory response and oxidant production[3]. Studies have shown that I/R event causes damage to primary and distant organs with the same severity[14]. In a previous study, it was reported that the I/R procedure increases the oxidant and proinflammatory cytokines such as malondialdehyde (MDA), Tumor Necrosis Factor-Alpha (TNF-α) and Interleukin-One Beta (IL-1β) on an equal footing in primary (ovary) and distant organ (kidney) tissue[15]. This information signs that the procedure of detorsion applied to torsioned ovaries in the clinic may lead to damage to distant organs. It is not known in the literature whether the procedure of I/R applied to ovaries leads to damage in many organs outside the ovaries or not. Additionally, studies comparing the severity of damage in distant organs such as the brain, heart, lung, kidney and liver caused by the procedure of I/R applied to ovaries have not been found in the literature. Therefore, current study aims to investigate whether the brain, heart, lung, kidney and liver tissues were affected or not by ovarian I/R damage in rats and to evaluate by comparing.

MATERIALS AND METHODS

Study area: The present study was carried out in Erzincan Binali Yıldırım University Animal Experiments Laboratory between 5 and 19 January, 2023 in two weeks.

Animals: A total of 16 albino Wistar-type female rats with a weight ranging from 275-287 g were used in the experiment. All of the experimental animals were obtained from Erzincan Binali Yıldırım University Experimental Animals Application and Research Center. The animals were kept for one week in the laboratory room (22°C) where the experiment was going to be conducted, with 12 hrs of bright and 12 hrs of dark so that they could adapt to the environment before the experiment. During this period, the rats were fed with normal animal feed and tap water. The experiments were conducted by the Turkey Regulation of Animal Research Ethics. In addition, this study was carried out in accordance with the principles of the Declaration of Helsinki. The protocols and procedures were approved by the Local Animal Experimentation Ethics Committee of Erzincan Binali Yildirim University (Meeting Date: 29 December, 2022, Meeting No: 2022/12, Decision No: 62).

Chemical agents: The ketamine used in the experiment was obtained from Pfizer Ilaçları Ltd. Sti. (Turkey).

Experimental groups: The experimental animals were divided into two groups: Sham operation to the ovaries (SG) and the groups in which the I/R procedure will be applied to the ovaries (OIR).

Surgical procedures

Experimental procedure: Surgical interventions were conducted under sterile conditions, in an appropriate laboratory environment, with intraperitoneal (IP) ketamine (60 mg kg^{-1}) anesthesia. After the ketamine injection, the animals were expected to be immobilized in the supine position and surgical intervention was started[16]. During anesthesia, the ovaries of the SG and OIR rat groups were reached by a 2-2.5 cm long vertical incision applied to the lower part of the abdomen. Then, ischemia was created for 2 hrs by applying a vascular clip to the lower part of the right ovary of the OIR group rats. The abdominal region was closed by suturing with surgical thread without ischemia to the ovaries of the SG group. At the end of this period, the vessel clip was removed and reperfusion was provided for 6 hrs. After reperfusion, the ovaries, brain, heart, lungs, kidneys and liver tissues of the animals sacrificed with high-dose ketamine (120 mg kg^{-1}) anesthesia were removed. The MDA, Total Glutathione (tGSH), superoxide dismutase (SOD), catalase (CAT) and IL-6 levels were measured in these removed tissues. In addition, blood samples were obtained from the tail veins of rats to determine troponin I (TpI), Creatine Kinase-MB (CK-MB), BUN, Creatinine, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels. The results determined from the SG and OIR groups were evaluated by comparison to other groups. The severity of tissue damage was assessed by the degree of significance of the increase in oxidative and proinflammatory parameters and the decrease in antioxidants.

Biochemical analysis

Determination of MDA, tGSH, SOD, CAT and protein: A commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit designed for experimental animals was used to measure MDA, tGSH and SOD in tissue samples (MDA: Catalog No. 10009055, tGSH: Catalog No. 703002, SOD: Catalog No. 706002, Cayman Chemical Co., Michigan, USA). The CAT determination was performed according to the method proposed by Goth[17]. Protein determination was determined spectrophotometrically at 595 nm according to the Bradford method[18].

Tissue IL-6 analysis: All samples were weighed before being cut, rapidly frozen with liquid nitrogen and homogenized with a pestle and mortar, samples were kept between 2-8°C after melting. A solution of PBS (pH 7.4), 1/10 (w/v), was added, vortex for 10 sec, centrifuged for 20 min at 10000×g and the supernatants were carefully collected. Interleukin-6 (IL-6, pg mL^{-1}) levels were measured by using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Catalog No. SEA079Ra, Wuhan USCN Business Co. Ltd., Wuhan, Hubei, China) according to kit instructions.

Determination of serum TpI and CK-MB: The TpI levels in plasma obtained from blood samples received from the tail veins of animals were measured in the VIDAS Troponin I Ultra kit (Marcy-I Etoile/France) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. Furthermore, CK-MB determination in the plasma collected from animals was photometrically measured in the Roche/Hitachi cobas c 701 systems.

Measurement of serum BUN: Quantitative determination of serum urea levels was studied by the spectrophotometric method on a Roche brand cobas 8000 autoanalyzer. It was calculated with the formula:

$$\text{BUN} = \text{UREA}\times 0.48$$

Urea hydrolysis into ammonium and carbonate ions is the basis of the kinetic test with urease and glutamate dehydrogenase:

$$\text{Urea}+2\ H_2O\rightarrow\text{(Urease) } 2\ NH_4^{+}+CO_3^{2-}$$

In the second reaction, when glutamate and Dehydrogenase (GLDH) and coenzyme Nicotinamide Adenine Dinucleotide (NAD)+hydrogen is present in a 2-oxoglutarate medium, it reacts with ammonium to form L-glutamate. Two moles of NADH are oxidized to NAD$^+$ for every mole of urea hydrolyzed in this reaction.

$$NH_4^{+}+\text{2-oxoglutarate}+\text{NADH}\xrightarrow{\text{(GLDH)}}\text{L-glutamate}+NAD^{+}+H_2O$$

The rate of decrease in NADH concentration is directly proportional to the urea concentration in the sample and the measurement was made at a wavelength of 340 nm.

Measurement of serum creatinine: The Roche brand cobas 8000 autoanalyzer was used to measure serum creatinine quantitatively. The colorimetric test was performed according to the Jaffe method. In the alkaline solution, creatinine and picrate form a complex that causes a yellow-orange color change. This formation was detected at a wavelength of 505 nm. The direct proportion between the amount of creatinine in the sample and the rate of dye formation was determined. Bilirubin interference was minimized via "rate-blanking" in this test. To correct for the nonspecific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, serum or plasma results were corrected with -26 μmol L^{-1} (-0.3 mg dL^{-1}).

$$\text{Creatinine}+\text{picric acid}\rightarrow\text{(Alkaline pH) yellow-orange complex}$$

Serum ALT and AST analysis: Venous blood samples were placed in tubes that did not contain anticoagulants. Centrifuged serum after clotting was stored at -80°C until assayed. The AST and ALT activities, which are liver function tests, were determined spectrophotometrically by Cobas 8000 autoanalyzer (Roche Diagnostics GmBH, Mannheim, Germany) and commercial kits (Roche Diagnostics).

Statistical analysis: The results obtained from experiments were expressed as Mean Value±Standard Error of the Mean (Mean±SEM). The statistical analyses were performed with the IBM SPSS Statistics program for Windows (IBM Corp., Version 22.0, released 2013, Armonk, New York, USA). The normality of the distribution for continuous variables in the biochemical test results was checked by the Shapiro-Wilk Test. Levene's Test was performed to determine whether the homogeneity of variances was achieved. The significance of differences between the groups was determined using a T-test except for SOD levels in cerebrum tissue and IL-6 levels in lung tissue. Since there is no normal distribution for SOD levels in the cerebrum tissue and IL-6 levels in lung tissue, the Kruskal-Wallis test, which is one of the non-parametric tests, was used. The Mann-Whitney U-test was used for these two parameters to determine the group that created the difference. The probability value of $p<0.05$ was regarded to indicate statistical significance.

RESULTS

MDA analysis results of the ovary, brain, heart, lung, kidney and liver tissue: Figure 1 shows the amount of MDA in the ovarian tissue of the OIR group, which underwent the I/R procedure, was found to be higher than in the ovarian tissue of the SG group, which underwent a sham operation. The difference in MDA amount in the ovarian tissues of the SG and OIR groups was statistically significant ($p<0.001$, Table 1 and 2). However, the amount of MDA in the brain tissue of the OIR group was almost the same as that of the SG group and the difference in MDA amounts was statistically insignificant ($p = 0.964$). In the heart tissue of the OIR group, the amount of MDA was statistically significantly higher than that of the SG group ($p = 0.004$). In addition, while the amount of MDA in the lung tissue increased significantly compared to the SG group ($p = 0.001$), it increased more significantly in the kidney tissue ($p<0.001$). The amount of MDA in the liver tissue of the OIR group was not found to be statistically different from that of the SG group ($p = 0.249$).

tGSH analysis results of the ovary, brain, heart, lung, kidney and liver tissues: The ovarian tissue tGSH levels of the OIR group were significantly lower than in the SG group. The difference in tGSH levels in the ovarian tissue of the OIR and SG groups was calculated as significant ($p<0.001$, Fig. 2, Table 1 and 2). However, no significant decrease in tGSH amount was observed in the brain tissue of the OIR group. Statistical analysis showed that the difference in tGSH amount between the OIR and SG groups in brain tissue was insignificant ($p = 0.137$). However, a significant decrease ($p = 0.011$) in tGSH amount was observed in the heart tissue of the OIR group compared to SG. The tGSH amount also significantly decreased ($p = 0.001$) in the lung tissue of the OIR group compared to SG. The organ tissue where the tGSH amount decreased most significantly ($p<0.001$) compared to SG was the kidneys. The amount of tGSH in the liver tissue of the OIR group was not found to be statistically different from that of the SG group ($p = 0.067$).

SOD analysis results of the ovary, brain, heart, lung, kidney and liver tissues: Figure 3 and Table 1-2 shows that, the I/R process applied to the ovaries has led to a decrease in SOD activity in the ovary tissue. The difference between the SOD activities in the ovarian tissue of the OIR and SG groups was significant ($p<0.001$). However, the SOD activities in the brain tissue of the OIR and SG groups were found to be similar to each other and the difference between them was evaluated as insignificant ($p = 0.699$). In the heart tissue of the OIR group, SOD activity was detected to be significantly lower than that of the SG group ($p = 0.014$). While SOD activity in the lung tissue of the OIR group decreased significantly ($p = 0.001$) compared to the SG, it decreased more significantly ($p<0.001$) in the kidney tissue. In addition, the difference between SOD activities in the liver tissue of the OIR and SG groups was calculated to be insignificant ($p = 0.555$).

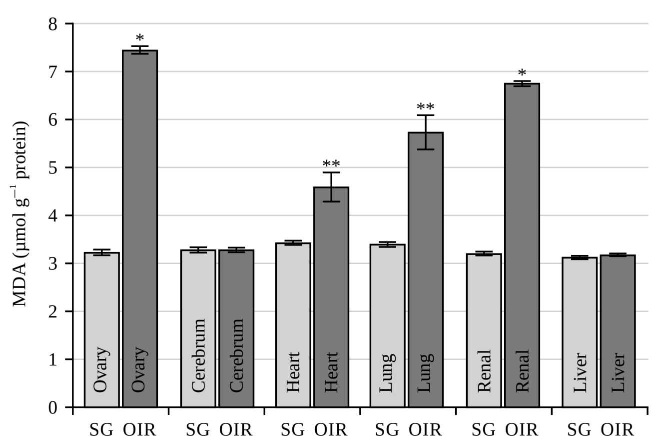

Fig. 1: MDA levels in the tissues of experimental groups

MDA: Malondialdehyde, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error), *Means $p<0.001$ according to the SG, **Means $p<0.05$ according to the SG and $n = 6$

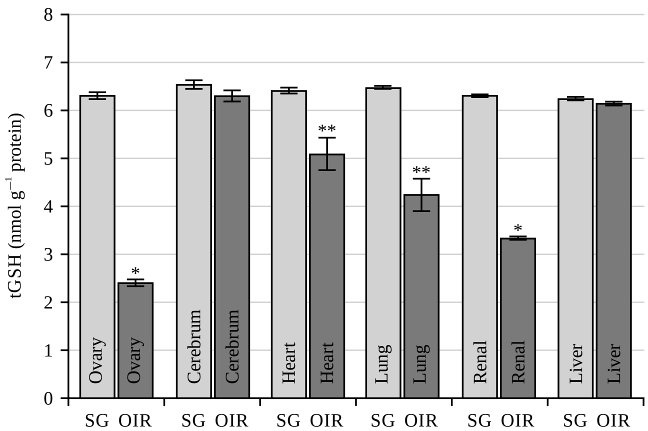

Fig. 2: tGSH levels in the tissues of experimental groups

tGSH: Total glutathione, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error), *Means p<0.001 according to the SG, **Means p<0.05 according to the SG and n = 6

Table 1: Mean and standard error values of the experimental groups in terms of tissue biochemical test results

Tissue	Groups	MDA	tGSH	SOD	CAT	IL- 6
Ovary	SG	3.24±0.06	6.32±0.07	8.78±0.07	7.57±0.07	2.29±0.03
	OIR	7.46±0.08	2.41±0.07	3.47±0.04	3.19±0.03	5.99±0.06
Cerebrum	SG	3.29±0.05	6.54±0.09	8.34±0.32	7.49±0.06	2.10±0.03
	OIR	3.29±0.05	6.31±0.12	8.63±0.07	7.43±0.02	2.17±0.02
Heart	SG	3.44±0.04	6.42±0.06	8.44±0.04	7.82±0.03	2.23±0.03
	OIR	4.60±0.31	5.10±0.34	7.25±0.32	6.30±0.34	3.54±0.34
Lung	SG	3.40±0.05	6.49±0.03	8.57±0.04	7.53±0.02	2.25±0.03
	OIR	5.74±0.36	4.25±0.34	6.20±0.33	5.15±0.37	4.59±0.34
Renal	SG	3.22±0.04	6.31±0.03	8.58±0.07	7.55±0.07	2.55±0.07
	OIR	6.76±0.05	3.34±0.04	4.74±0.05	4.29±0.03	5.79±0.03
Liver	SG	3.13±0.03	6.25±0.03	8.73±0.06	7.60±0.06	2.12±0.03
	OIR	3.18±0.03	6.15±0.04	8.69±0.04	7.56±0.03	2.20±0.04

MDA: Malondialdehyde, tGSH: Total glutathione, SOD: Superoxide dismutase, CAT: Catalase, IL-6: Interleukin six, SG: Sham operation group and OIR: ovarian ischemia-reperfusion group

Table 2: p-values of the experimental groups in terms of the tissue biochemical test results

	MDA	tGSH	SOD	CAT	IL-6
Tissue	SG vs OIR	SG vs OIR	SG vs OIR	SG vs OIR	SG vs OIR
Ovary	<0.001	<0.001	<0.001	<0.001	<0.001
Cerebrum	0.964	0.137	0.699*	0.383	0.106
Heart	0.004	0.011	0.014	0.006	0.012
Lung	0.001	0.001	0.001	0.001	0.002*
Renal	<0.001	<0.001	<0.001	<0.001	<0.001
Liver	0.249	0.067	0.555	0.577	0.140

MDA: Malondialdehyde, tGSH: Total glutathione, SOD: Superoxide dismutase, CAT: Catalase, IL-6: Interleukin six, SG: Sham operation group, OIR: Ovarian ischemia-reperfusion group, *Statistical evaluation was done with Kruskal-Wallis Test, After that, the Mann-Whitney U test was used as *post hoc*, Other statistical evaluations were done by using a T-test

CAT analysis results of the ovary, brain, heart, lung, kidney and liver tissues: Figure 4 showed the I/R procedure caused a decrease in CAT activities in the ovary tissue. The difference in CAT activity between the OIR and SG groups in ovary tissue was found to be significant (p<0.001, Table 1 and 2). However, CAT activity did not decrease in the brain tissue of the OIR group and the difference in CAT activities between the OIR and SG groups in brain tissue was found to be insignificant (p = 0.383). The statistical data revealed the fact that CAT activity in the heart tissue of the OIR group was significantly lower than that of the SG group (p = 0.006). While CAT activity decreased significantly in the lung tissue of the OIR group (p = 0.001), it was also found to decrease more significantly in the kidney tissue (p<0.001). In the liver tissue of the OIR group, the CAT activity was observed to be similar to that of the SG group and no statistically significant difference was detected (p = 0.577).

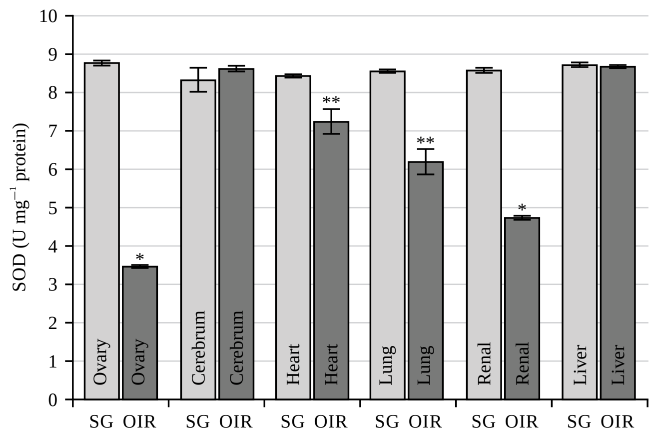

Fig. 3: SOD levels in the tissues of experimental groups

SOD: Superoxide dismutase, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error), *Means p<0.001 according to the SG, **Means p<0.05 according to the SG and n = 6

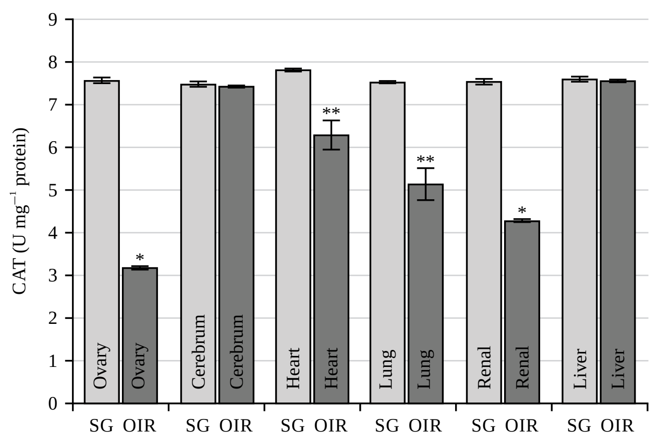

Fig. 4: CAT levels in the tissues of experimental groups

CAT: Catalase, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error), *Means p<0.001 according to the SG, **Means p<0.05 according to the SG and n = 6

IL-6 analysis results of the ovary, brain, heart, lung, kidney and liver tissues: In Fig. 5, it is seen that, the I/R procedure significantly increases the amount of IL-6 in the ovarian tissue. The difference between the amounts of IL-6 in the ovarian tissues of the SG and OIR groups was statistically significant (p<0.001, Table 1 and 2). It is understood that the brain tissue is not affected by the ovarian I/R procedure. The statistical analysis results also showed that the levels of IL-6 were similar in the brain tissues of the OIR and SG groups and that the difference between them was insignificant (p = 0.106). However, while the amount of IL-6 in the heart tissue of the OIR group was significantly higher (p = 0.012) compared to the SG group, it increased even more significantly (p = 0.002) in the lung tissue. The highest amount of IL-6 was found in the kidney tissue. The difference between the amount of IL-6 in the kidney tissue of the OIR and SG groups was detected to be the most significant (p<0.001). In the liver tissue of the OIR group, the IL-6 activity was found to be similar to that of the SG group and no statistically significant difference was detected (p = 0.140).

Blood serum TpI, CK-MB, BUN, creatinine, ALT and AST analysis results: As can be seen from Fig. 6-8, blood serum TpI (p<0.001), CK-MB (p<0.001), BUN (p<0.001) and creatinine (p<0.001) levels of the OIR group that underwent I/R procedure to the ovaries increased significantly compared to the SG group, while ALT (p = 0.900) and AST (p = 0.968) levels were not significantly different (Table 3).

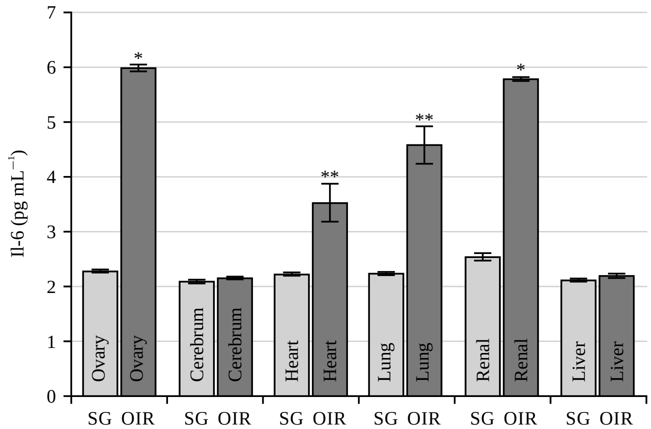

Fig. 5: IL-6 levels in the tissues of experimental groups

IL-6: Interleukin six, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error), *Means $p<0.001$ according to the SG, **Means $p<0.05$ according to the SG and n = 6

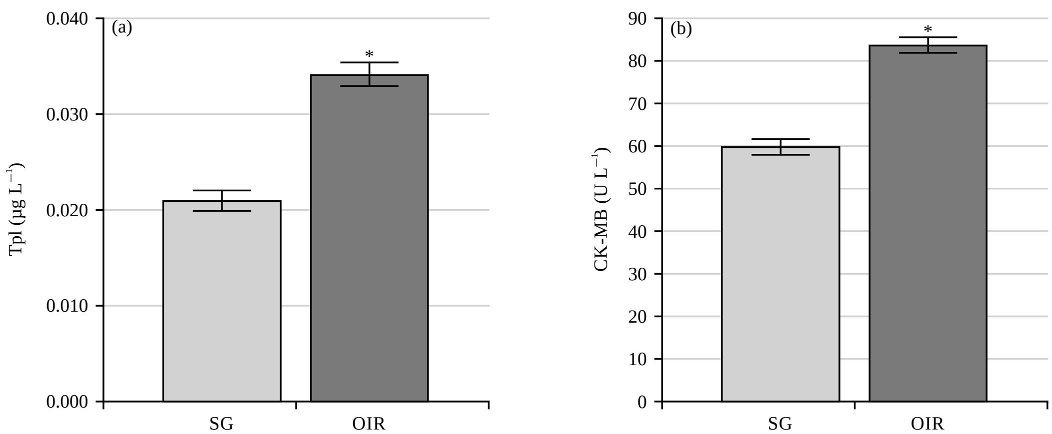

Fig. 6(a-b): TpI and CK-MB levels in the blood serum of experimental groups

TpI: Troponin I, CK-MB: Creatine kinase-MB, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error), *Means $p<0.001$ according to the SG and n = 6

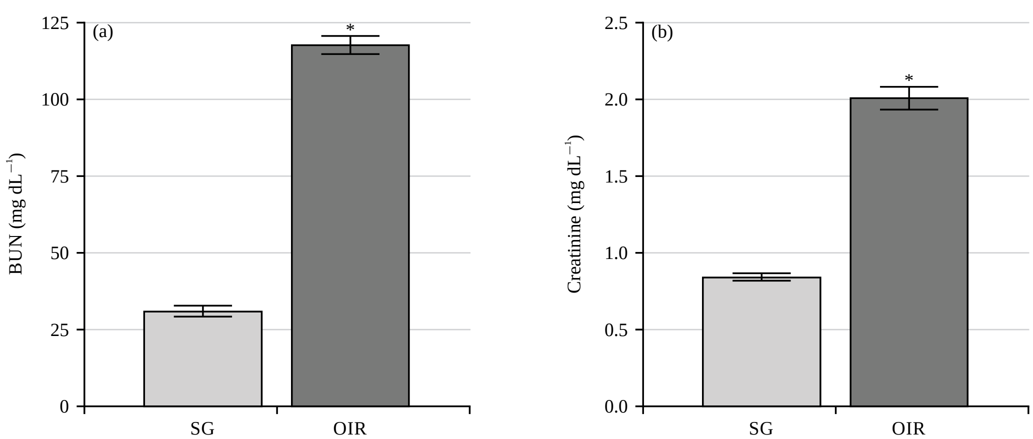

Fig. 7(a-b): BUN and creatinine levels in the blood serum of experimental groups

BUN: Blood urea nitrogen, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error), *Means $p<0.001$ according to the SG and n = 6

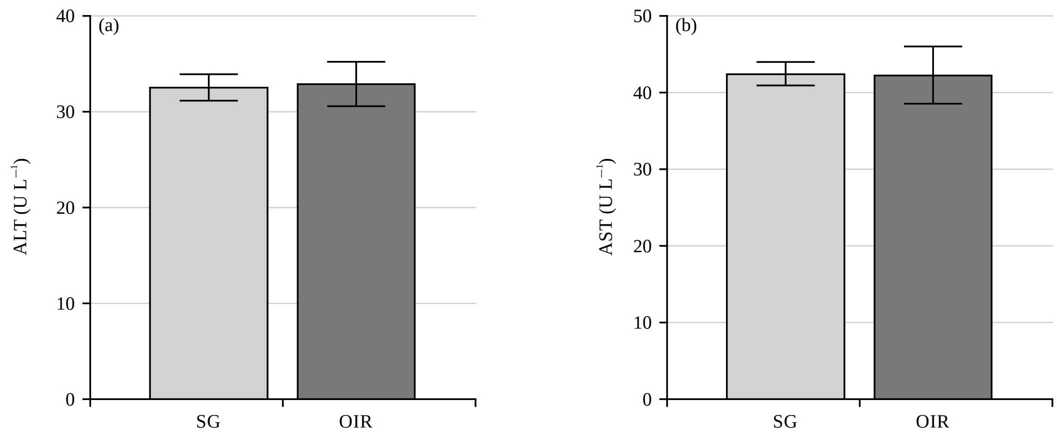

Fig. 8: ALT and AST levels in the blood serum of experimental groups

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, SG: Sham-operation group, OIR: Ovarian ischemia-reperfusion group, Bars are Mean±SEM (standard error) and n = 6

Table 3: Mean, standard error and p-values of the experimental groups in terms of blood serum biochemical test results

Groups	TpI	CK-MB	BUN	Creatinine	ALT	AST
SG	0.021±0.00	59.90±1.82	30.97±1.78	0.84±0.02	32.48±1.39	42.33±1.52
OIR	0.034±0.00	83.80±1.85	117.50±2.92	2.00±0.07	32.83±2.33	42.17±3.72
SG vs OIR	<0.001	<0.001	<0.001	<0.001	0.900	0.968

TpI: Troponin I, CK-MB: Creatine kinase-MB, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, SG: Sham operation group, OIR: Ovarian ischemia-reperfusion group and statistical evaluations were done by using a T-test

DISCUSSION

Although the damage caused by the procedure of I/R applied to the primary tissue in distant organs usually occurs by similar mechanisms, there is no clear information about which organ is most affected. In this study, the extent to which brain, heart, lung, kidney and liver tissue are affected by ovarian I/R damage in rats was investigated and evaluated by comparison. It has been shown that I/R events associated with thrombolytic therapy, organ transplantation, coronary angioplasty and aortic cross-clamp cause local and systemic damage[19,20]. Current experimental results revealed that MDA levels were significantly higher in the I/R applied ovarian tissue compared to the sham operation group, while tGSH, SOD and CAT levels were low. As mentioned above, ROS production increases during reperfusion[3]. Superoxide anion ($O_2\cdot^-$), hydroxyl radicals (•OH) and hydrogen peroxide (H_2O_2) are the most examined types of ROSs today[21]. These ROSs, known as reperfusion mediators, oxidize cell membrane lipids, providing the origination of toxic products such as MDA from lipids[22]. The MDA itself, which occurs as a result of LPO, is also toxic and can cause further destruction[23]. However, in living tissues, the excessive production of ROSs is prevented by Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and other endogenous antioxidant defense systems[24]. As it is known, GSHs scavenge ROSs[25], while SOD converts $O_2\cdot^-$ into H_2O_2 and the H_2O_2 molecule is hydrolyzed into harmless molecules by CAT[26]. Therefore, the oxidant/antioxidant system in living organs and tissues is in balance. The deterioration of this balance and the increase of oxidants is defined as oxidative stress[27]. The information obtained from the literature signs that the oxidant/antioxidant balance in the ovary tissue where we applied I/R shifted in favor of oxidants and oxidative stress developed. In addition, the level of IL-6 in the ovary tissue where we applied I/R was found to be significantly higher than in the sham operation group. It is known that IL-6 has an important role in the pathogenesis of inflammation[28]. The current study findings coincide with studies that report that the severity of I/R damage is directly proportional to the increase in the inflammatory response and oxidant production[3]. In addition, studies showing a link between ROS and IL-6 production support experimental results[29,30].

Studies have revealed that I/R causes damage not only to primary tissues and organs but also to distant organs with the same severity[14]. Inflammation is held responsible for the pathogenesis of I/R-related multiple distant organ damage[19]. It is advocated that inflammation observed in distant organs also is caused by the excessive production of ROSs in the primary tissue[31]. In the past and current literature, it has been shown that brain tissue is severely affected by kidney, liver and heart I/R damage[32-34]. However, there was no information that the brain tissue was affected by the ovarian I/R event. In this study, it was also observed that the oxidant, antioxidant and proinflammatory cytokine (IL-6) levels in the brain tissue of the rats to which we applied I/R to the ovaries were almost at the same level as the sham group.

Current experimental results revealed the fact that the I/R procedure applied to ovarian tissue significantly increased MDA and IL-6 levels in heart tissue. As mentioned above, the severity of I/R damage is directly proportional to the inflammatory response and increased oxidant production[3]. Current findings revealed the severity of oxidative stress and inflammation in heart tissue. It also overlaps with data reporting that I/R causes damage not only to primary tissues and organs but also to distant organs[14]. It was also reported in the studies of Delibas *et al.*[35] that the I/R procedure applied to the ovaries also causes oxidative damage in the heart. Similarly, the I/R process directly increases the production of oxidant and proinflammatory cytokines in the heart tissue and suppresses the production of antioxidants[36]. Other parameters that support that the heart is affected by ovarian I/R damage have been TpI and CK-MB. In this study, serum TpI and CK-MB levels were found to be high compared to the sham operation group. Serum TpI and CK-MB levels are important markers of myocardial ischemia and hypoxia-related damage[37]. The increase in these cardiac markers has been associated with an increase in oxidants and proinflammatory cytokines[38].

Oxidant and pro-inflammatory cytokine levels were found to be high in the lung tissue of animals that underwent the I/R process to ovaries compared to the sham group. Tanyeli *et al.*[39] reported that ovary torsion/detorsion increases oxidative and inflammatory markers both in the ovaries and lungs. The pulmonary system is known to be affected by distant organ I/R damage[40]. In current study, while the levels of oxidant and pro-inflammatory cytokines were higher in lung tissue than in heart tissue, the levels of antioxidants were found lower. This indicates that the lungs are more affected by the ovarian I/R event than the heart. Mura *et al.*[41] also explained in a study that the lung was the organ that was most severely damaged by intestinal I/R damage.

Also, in this study, the I/R procedure applied to ovaries caused oxidant and proinflammatory parameters to increase more in the renal tissue of animals than in the brain, heart and lungs. Current findings revealed the fact that the kidneys are seriously affected by the ovarian I/R event. There is information in the literature reporting that oxidative and inflammatory damage of kidneys develops secondary to ovarian I/R damage[15]. As it is known, kidney damage causes kidney dysfunction. Biochemical parameters such as BUN and creatinine are used to determine renal dysfunction[42]. Current experimental results showed that ovarian I/R damage increases serum BUN and creatinine levels. The BUN and creatinine levels increase in renal failure due to acute kidney damage. In addition, long-term kidney damage increases the risk of cardiovascular complications and death[43]. In the literature, it is emphasized that kidney I/R damage is associated with an increase in oxidant, pro-inflammatory cytokine, BUN and creatinine[44]. This information obtained from the literature shows that I/R procedure applied to the ovaries causes significant damage to the kidneys.

Again, in this study, there was no significant increase in the levels of the oxidant, pro-inflammatory cytokines, ALT and AST in the liver tissue of animals that underwent I/R on ovaries. Current biochemical findings sign that liver tissue is not affected by ovarian I/R damage. There is not found information in the literature that the liver is affected by ovarian I/R damage. However, it has been reported that the heart, lungs and kidneys are damaged by the intestinal I/R event, while the liver is not affected[41]. Just because the liver is not affected by ovarian I/R damage does not mean that it also will not be affected by I/R damage that occurs in other organs. Grams and Rabb[45] reported that the liver and other organs were affected by acute kidney damage.

CONCLUSION

Present study experimental results revealed that the I/R procedure can cause different degrees of damage not only in primary tissue but also in distant organs. Brain and liver tissues were unaffected by ovarian I/R damage, the heart was mildly affected, the lung moderately and the kidney was severely affected. These findings allowed us to determine the degree and extent of particular distant organ damage caused by ovarian I/R. Although the clinical translation of ovarian I/R-related distant organ damage is not clearly understood for now, special attention may be required to kidney and lung damage after ovarian torsion and related conditions. Current study recommends that the research of each organ histopathologically is done to investigate in more detail whether distant organs are affected by ovary I/R damage.

SIGNIFICANCE STATEMENT

Ovarian torsion is a pathological condition that can be seen in women of all ages. The present study discussed whether ovarian I/R may cause severe damage to distant organs as well as ovarian necrosis. Our experimental results revealed that I/R damage to the ovaries did not cause any damage to the brain and liver, but caused mild damage to the heart tissue, moderate damage to the lung tissue and severe damage to the kidney tissues. In this respect, it would be beneficial to consider distant organ damage after ovarian ischemia.

REFERENCES

1. Majno, G. and I. Joris, 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. Am. J. Pathol., 146: 3-15.
2. Zhang, Z., X. Ma, G. Yang and L. Zhang, 2022. Cardioprotective effects of glatiramer against ischemia-reperfusion injury in coronary artery ligation model in rats through activation of AKT-GSK-3β-TNF-α-Nrf2 signalling pathway. Int. J. Pharmacol., 18: 79-86.
3. Zimmerman, B.J. and D.N. Granger, 1992. Reperfusion injury. Surg. Clin. North Am., 72: 65-83.
4. Ingec, M., U. Isaoglu, M. Yilmaz, M. Calik and B. Polat *et al*., 2011. Prevention of ischemia-reperfusion injury in rat ovarian tissue with the on-off method. J. Physiol. Pharmacol., 62: 575-582.
5. Kirmizi, D.A., E. Baser, A. Okan, M. Kara, E.S. Yalvac and Z. Doganyigit, 2021. The effect of a natural molecule in ovary ischemia reperfusion damage: Does lycopene protect ovary? Exp. Anim., 70: 37-44.
6. Oelsner, G. and D. Shashar, 2006. Adnexal torsion. Clin. Obstet. Gynecol., 49: 459-463.
7. ACOG, 2019. Adnexal torsion in adolescents. Obstet. Gynecol., 134: e56-e63.
8. Beaunoyer, M., J. Chapdelaine, S. Bouchard and A. Ouimet, 2004. Asynchronous bilateral ovarian torsion. J. Pediatric Surg., 39: 746-749.
9. Poonai, N., C. Poonai, R. Lim and T. Lynch, 2013. Pediatric ovarian torsion: Case series and review of the literature. Can. J. Surg., 56: 103-108.
10. Çelik, A., O. Ergün, H. Aldemir, C. Özcan, G. Özok, A. Erdener and E. Balýk, 2005. Long-term results of conservative management of adnexal torsion in children. J. Pediat. Surg., 40: 704-708.
11. Ince, S., M. Ozer, B.G. Kad, M. Kuzucu and B. Gundogdu *et al*., 2021. Biochemical and histopathological evaluation of sunitinib effect on ovarian injuries by ischemia-reperfusion in rats. Int. J. Pharmacol., 17: 65-72.
12. Kadıoğlu, B.G., B. Gündoğdu, N. Kurt, H. Süleyman and Z. Süleyman, 2020. Effect of desloratadine on oxidative and inflammatory ovarian ischemia-reperfusion injury in female rats: Biochemical and histopathological evaluation. Anal. Quant. Cytol. Histol., 42: 61-68.
13. Wu, M.Y., G.T. Yiang, W.T. Liao, A.P.Y. Tsai and Y.L. Cheng *et al*., 2018. Current mechanistic concepts in ischemia and reperfusion injury. Cell Physiol. Biochem., 46: 1650-1667.
14. Carden, D.L. and D.N. Granger, 2000. Pathophysiology of ischaemia-reperfusion injury. J. Pathol., 190: 255-266.
15. Karaca, İ., İ.B. Delibaş, Ö.E. Yapça, M. İngeç and S. Kumbasar *et al*., 2017. Oxidative renal damage in rats subjected to ovarian ischemia reperfusion and protective effect of tianeptine. Latin Am. J. Pharm., 36: 2339-2347.
16. Demiryilmaz, I., M.I. Turan, A. Kisaoglu, M. Gulapoglu, I. Yilmaz and H. Suleyman, 2014. Protective effect of nimesulide against hepatic ischemia/reperfusion injury in rats: Effects on oxidant/antioxidants, DNA mutation and COX-1/COX-2 levels. Pharmacol. Rep., 66: 647-652.
17. Góth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta, 196: 143-151.
18. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
19. Cowled, P. and R. Fitridge, 2020. Pathophysiology of Reperfusion Injury. In: Mechanisms of Vascular Disease: A Textbook for Vascular Specialists, Fitridge, R. (Ed.), Springer, Cham, Switzerland, ISBN: 978-3-030-43683-4, pp: 415-440.
20. Collard, C.D. and S. Gelman, 2001. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology, 94: 1133-1138.
21. Suleyman, H. and A. Ozcicek, 2020. Molecular mechanism of ischemia reperfusion injury. Arch. Basic Clin. Res., 2: 25-27.
22. Yapca, O.E., B. Borekci and H. Suleyman, 2013. Ischemia-reperfusion damage. Eurasian J. Med., 45: 126-127.
23. Ayala, A., M.F. Muñoz and S. Argüelles, 2014. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longevity, Vol. 2014. 10.1155/2014/360438.
24. Urso, M.L. and P.M. Clarkson, 2003. Oxidative stress, exercise, and antioxidant supplementation. Toxicology, 189: 41-54.
25. Liu, T., L. Sun, Y. Zhang, Y. Wang and J. Zheng, 2022. Imbalanced GSH/ROS and sequential cell death. J. Biochem. Mol. Toxicol., Vol. 36. 10.1002/jbt.22942.
26. Buettner, G.R., C.F. Ng, M. Wang, V.G.J. Rodgers and F.Q. Schafer, 2006. A new paradigm: Manganese superoxide dismutase influences the production of H_2O_2 in cells and thereby their biological state. Adv. Free Radical Biol. Med., 41: 1338-1350.
27. Kisaoglu, A., B. Borekci, O.E. Yapca, H. Bilen and H. Suleyman, 2013. Tissue damage and oxidant/antioxidant balance. Eurasian J. Med., 45: 47-49.
28. Tanaka, T., M. Narazaki, K. Masuda and T. Kishimoto, 2016. Regulation of IL-6 in Immunity and Diseases. In: Regulation of Cytokine Gene Expression in Immunity and Diseases, Ma, X. (Ed.), Springer, Netherlands, ISBN: 978-94-024-0921-5, pp: 79-88.
29. Kumari, N., B.S. Dwarakanath, A. Das and A.N. Bhatt, 2016. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumor Biol., 37: 11553-11572.
30. Han, F., S. Li, Y. Yang and Z. Bai, 2021. Interleukin-6 promotes ferroptosis in bronchial epithelial cells by inducing reactive oxygen species-dependent lipid peroxidation and disrupting iron homeostasis. Bioengineered, 12: 5279-5288.

31. Halladin, N.L., 2015. Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries. Dan. Med. J., Vol. 62.
32. Kovalčíková, A., M. Gyurászová, D. Vavrincová-Yaghi, P. Vavrinec and Ľ. Tóthová *et al*., 2018. Oxidative stress in the brain caused by acute kidney injury. Metab. Brain Dis., 33: 961-967.
33. Zhu, L., H. Zhou, F. Xu, H. Yang and P. Li *et al*., 2021. Hepatic ischemia-reperfusion impairs blood-brain barrier partly due to release of arginase from injured liver. Front. Pharmacol., Vol. 12. 10.3389/fphar.2021.724471.
34. Surinkaew, P., N. Apaijai, P. Sawaddiruk, T. Jaiwongkam, S. Kerdphoo, N. Chattipakorn and S.C. Chattipakorn, 2020. Mitochondrial fusion promoter alleviates brain damage in rats with cardiac ischemia/reperfusion injury. J. Alzheimers Dis., 77: 993-1003.
35. Delibas, I.B., I. Karaca, O.E. Yapca, M. Ingec and S. Kumbasar *et al*., 2018. The effect of nizatidine on ovarian ischaemia/reperfusion injury in rats. Indian J. Anim. Res., 52: 1488-1493.
36. Gouda, E. and F. Babiker, 2022. Gum Arabic protects the rat heart from ischemia/reperfusion injury through anti-inflammatory and antioxidant pathways. Sci. Rep., Vol. 12. 10.1038/s41598-022-22097-0.
37. Cordwell, S.J., A.V.G. Edwards, K.A. Liddy, L. Moshkanbaryans and N. Solis *et al*., 2012. Release of tissue-specific proteins into coronary perfusate as a model for biomarker discovery in myocardial ischemia/reperfusion injury. J. Proteome Res., 11: 2114-2126.
38. Mahmoud, K.M. and A.S. Ammar, 2011. Effect of N-acetylcysteine on cardiac injury and oxidative stress after abdominal aortic aneurysm repair: A randomized controlled trial. Acta Anaesthesiologica Scand., 55: 1015-1021.
39. Tanyeli, A., F.N.E. Akdemir, E. Eraslan, M.C. Güler, T. Nacar, S. Çomakli and I. Gülçin, 2022. The role of p-coumaric acid on reproductive and remote organ damages created by adnexal torsion/detorsion: Biochemical and immunohistochemical a study. Clin. Exp. Health Sci., 12: 1005-1012.
40. Ferrari, R.S. and C.F. Andrade, 2015. Oxidative stress and lung ischemia-reperfusion injury. Oxid. Med. Cell. Longevity, Vol. 2015. 10.1155/2015/590987.
41. Mura, M., C.F. Andrade, B. Han, R. Seth and Y. Zhang *et al*., 2007. Intestinal ischemia-reperfusion-induced acute lung injury and oncotic cell death in multiple organs. Shock, 28: 227-238.
42. Peng, R., K. Liu, W. Li, Y. Yuan and R. Niu *et al*., 2021. Blood urea nitrogen, blood urea nitrogen to creatinine ratio and incident stroke: The Dongfeng-Tongji cohort. Atherosclerosis, 333: 1-8.
43. Kang, H.G., H.K. Lee, K.B. Cho and S.I. Park, 2021. A review of natural products for prevention of acute kidney injury. Medicina, Vol. 57. 10.3390/medicina57111266.
44. Erdem, K.T.O., Z. Bedir, U. Kuyrukluyildiz, H.G. Tas and Z. Suleyman *et al*., 2022. Effect of tocilizumab on ischemia-reperfusion-induced oxido-inflammatory renal damage and dysfunction in rats. Exp. Anim., 71: 491-499.
45. Grams, M.E. and H. Rabb, 2012. The distant organ effects of acute kidney injury. Kidney Int., 81: 942-948.