Therapeutic potential of L-arginine in a rat model of ovarian ischemia-reperfusion injury

Environmental and Experimental Biology

UNIVERSITY OF LATVIA

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Abstract

Ischaemia-reperfusion (I/R) injury is a serious problem subsequent to reperfusion treatment for ovarian torsion. The role of nitric oxide (NO) in ovarian I/R injury is debatable. The main focus of this study was to explore the protective role of L-arginine, a potent NO precursor, on ovarian I/R injury. Female Wistar rats were divided into three groups (n = 5). In the control group, only laparotomy was performed. In the I/R group, ischaemia and reperfusion were performed and no drug was given. In the I/R + arginine group, ischaemia was followed by reperfusion and 200 mg kg^{-1} L-arginine was injected 5 min before reperfusion. Concentration of malondialdehyde, NO and reduced glutathione, as well as activity of superoxide dismutase and catalase were analyzed. Hematoxylin and eosin-stained slides were microscopically examined for histological evaluation of the ovaries. Superoxide dismutase and catalase activity along with concentration of reduced glutathione and NO were significantly lower in the I/R group in comparison to the control group. Malondialdehyde concentration was significantly higher in the I/R group than in control group. These results were reversed with supplementation of L-arginine. Light microscopic examination revealed severe vascular congestion, edema, haemorrhage, and follicular degeneration in the ovary tissue. The extent of ovarian damage was much higher in the I/R group than in the I/R + L-arginine group. Treatment with L-arginine seems to have an ameliorating effect against oxidative stress in I/R injury in rat ovary. It considerably reduced the altered histological changes in the ovaries. Thus, it can be speculated that L-arginine might play a pivotal role as a potent therapeutic agent against ovarian torsion.

Key words: antioxidant enzymes, ischaemia–reperfusion, L-arginine, ovarian torsion, oxidative stress.
Abbreviations: CAT, catalase; DTNB, 5, 5 -dithiobis-2-nitrobenzoic acid; GSH, reduced glutathione; I/R, ischaemia reperfusion; MDA, malondialdehyde; NBT, nitroblue tetrazolium; ROS, reactive oxygen species; SO, sham operated; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid.

Introduction

Ovarian torsion, also termed adnexal torsion, is a sporadic gynaecologic emergency plight, which has a global prevalence of 2.7% in women (Hibbard 1985). Ovarian torsion refers to the bending of the ovary and fallopian tube around the broad ligament. Women of reproductive age are affected in most of the cases reported, although it might affect women of all ages (Becker et al. 2009). However the symptoms lack specificity and hence, the diagnosis and treatment is often delayed. This clinical condition can slowly lead to permanent tissue damage, as arterial, venous and lymphatic tissue damage for prolonged period can result in reduced blood flow to tissue; hence early diagnosis and treatment are necessary to restore the normal functioning of the affected ovary and that of fertility (Gasser et al. 2016). The main objective of this ischaemia treatment is

not only to successfully bring about the blood circulation, but also to re-establish tissue reperfusion. After ischaemia, when the blood flow and reperfusion are retained, a new physio-pathological process termed 'reperfusion injury' is experienced, and this results in severe tissue damage (Kalogeris et al. 2012). Previous reports have suggested that the damage caused by reperfusion is more severe than that of ischaemia alone (Kaleli et al. 2003; Calis et al. 2015). The total injury that the tissue suffers from is the bulk of that caused by ischaemia and reperfusion (Sussman, Bulkley 1990; Rangan, Bulkley 1993; Das, Maulik 1994). Consequently, there are high chances of success in treatments that would prevent the reperfusion injury.

Earlier reports have indicated ischaemia followed by reperfusion leads to increased production of reactive oxygen species (ROS) in various tissues including brain, heart and muscle (Yoshida et al. 1982), which further results

in ROS mediated injury to the cell membrane (Fridovich 1983). Reperfusion mediated oxidative stress is due to the imbalance between the production of reactive oxygen species and antioxidants (Sinning et al. 2017). Tissue injury is incurred following ischaemia during the reperfusion period when the hypoxic tissue experiences reoxygenetion, which further gives rise to reactive oxygen species. Among others, superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide are three major forms of ROS that have physiological significance. Cells are equipped with a variety of antioxidants (both enzymatic and non enzymatic) that serve to counterbalance the effect of ROS (Birben et al. 2012). Superoxide dismutase (SOD) and catalase (CAT) fall under the enzymatic defence machinery (Sayin et al. 2011). These enzymes usually release the ROS load of the cell and thereby protect the tissue against oxidative damage. The level of reactive oxygen species further increases if the cells are not equipped with enough antioxidant enzyme activity (Poljsak et al. 2013). To regain the normal functioning of ischaemic tissue and to restore future fertility, prevention of reperfusion injury of the ovarian tissue should garner the main focus. In past, several other antioxidants with free radical scavenging activity agents have been used to protect the tissues against ischaemia-reperfusion (I/R) injury (Cosar et al. 2007). Thus, if the influence of these deleterious effects of ROS could be compensated, ischaemia-reperfusion injury can be reduced.

Vasoconstriction, or microcirculatory disturbance, has been considered one of major reasons of organ injury related to I/R injury (Wang 2009). Nitric oxide has diverse protective effects on cells during I/R injury. NO is a gaseous free radical that is said to exert protective effects on cells undergoing ischemic-reperfusion injury by inhibiting oxidative stress and cytokine production (Phillips et al. 2009). Previous reports have demonstrated that reduced nitric oxide synthase activity caused a remarkable reduction in NO level during hepatic I/R injury, which ultimately led to liver damage (Köken, İnal 1999). Reports have also suggested that pre-treatment with nitric oxide ahead of reperfusion-followed ischaemia mitigated the repercussion of I/R injury on cardiac muscle (Bolli 2001), which is consistent with earlier findings. Collectively, these observations suggest that the evolution of new restorative approaches to ameliorate I/R injury may be assisted by a proper perception of the defence mechanisms of the organs undergoing ischemic abuse. As NO availability has been proved to be beneficial in different organs subjected to ischemic insult, supplementation with L-arginine, a precursor of NO synthase might be propitious in ameliorating ovarian damage by improving microcirculation. NO is an enigmatic substance as it has a protective function due to its vasodilatory and scavenging property, and can also exert many detrimental effects by interacting with superoxide radicals (Nita, Grzybowski 2016). Previous reports demonstrated the effectiveness of arginine in I/R injury in liver (Lucas et al. 2015), kidney

(Hubert et al. 2002), skeletal (Huk et al. 1998) and cardiac muscle (Schulz et al. 2004) and also in I/R mediated endothelial dysfunction (Pernow et al. 2003). However, the study of effectiveness of arginine on ovarian I/R injury is still pending. Moreover, we hypothesize that the L-arginine/ NO pathway might seem significant during the I/R practice in ovary.

Therefore, in the present study, we investigated whether L-arginine, a nitric oxide donor, can reduce ischaemia-reperfusion injury of the ovary. For this purpose, the protective effects of L-arginine in rats subjected to 3-h-long total ovarian vascular exclusion followed by 3-h-long reperfusion were evaluated.

Materials and methods

Chemicals and reagents

L-arginine was purchased from Sigma-Aldrich. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), xanthine, bovine serum albumin, nitrobluetetrazolium (NBT), xanthine oxidase, sulfanilamide, phosphoric acid, naphthyl ethylene diaminedihydrochloride were bought from Merck (Darmstadt, Germany). All of the reagents were of analytical grade.

Experimental animals

Eight-week-old female Adult albino rats (Wistar strain) with body weight 130 to 150 g were used for the study. The rats were acclimatized in an experimental animal house for seven days before the commencement of the experiment. The animals housed in cages under standard laboratory conditions of temperature (25 ± 2 °C) and humidity (55 ± 5%) and in a 12 h light/dark cycle schedule with free access to water supply. Animals were fed with a standard diet containing 71% carbohydrate, 18% protein, 7% fat and 4% salt mixture. All of the rats were allowed free access to food and water *ad libitum*, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from the cages daily.

All the experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee of Serampore College.

Experimental design

Rats were divided into three groups (*n* = 5): group 1 – control group (sham operated group); group 2 – ischaemia/ reperfusion group (I/R); and group 3 – L-arginine + I/R group.

The surgical procedures on rats were performed under anaesthesia with 50 mg kg^{-1} ketamine hydrochloride solution injected intraperitonially. Sterile appropriate laboratory conditions were maintained. We waited for the convenient time to execute the surgical intervention, which was considered to be when the animals were motionless in a supine position. At that point, the ovaries of 15 rats were obtained by performing a 2.0 to 2.5 cm

lower abdominal vertical incision. Ischaemia was created by applying vascular clips to the lower part of both of the ovaries in the region where the ovaries are connected to the uterus) (Yapca et al. 2014). No ischaemia was applied to sham operated group ovaries. The abdomen was only sham operated and kept for 3 h. After 3 h, the rats were killed with an overdose of anaesthesia and ovaries were removed and collected. The second group of animals (I/R group) received reperfusion. In this group no drug was administered and 3 h of ischaemia was followed by 3 h of reperfusion. In the third group, which was the L-arginine + I/R group, 3 h of ischaemia and 3 h of reperfusion were executed. L-Arginine, in a quantity of 200 mg kg^{-1} (Li, Liang 2003), was administered intraperitoneally 5 min before reperfusion. After reperfusion, all rats of group 2 and 3 were sacrificed with an overdose of anaesthesia and both of their ovaries were surgically removed. The ovaries were then used for histological and various biochemical assays. One ovary from each animal was used for histological examination and one from each animal was taken for various biochemical assays.

Preparation of ovarian tissue extract

The ovaries were quickly removed. For the estimation of different oxidative stress markers, ovarian tissue extract was prepared in ice-cold Tris-HCl buffer (pH 7.4) (Mukherjee et al. 2006). For the estimation of SOD and CAT activity, the tissues were homogenized in ice-cold isotonic phosphate buffer (pH 7.0 and pH 8.0, respectively).

Preparation of permanent slides for histological examination

Permanent slides of the ovaries were prepared and stained with eosin–hematoxylin for histopathological evaluation. Ovarian tissues from all groups of animals were taken and were Bouin's fixed. Paraffin blocks were made, and 4 to 5 mm thin sections were cut with a rotary microtome, and routine microscopic slides were prepared. Hematoxylin and eosin-stained slides were microscopically examined.

Estimation of nitric oxide production

The role of nitric oxide synthase was indirectly evaluated by estimating the amount of NO production. NO decomposes rapidly in aerated solutions to form stable nitrite/nitrate products. Nitrite accumulation was estimated by Griess reaction (Raso et al. 1999) and was used as a marker of NO production. Ovarian tissue homogenates (100 μL) were loaded into a microtitre plate followed by adding 100 μL Griess reagent [1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthyl ethylene diaminedihydrochloride] and incubated at room temperature for 10 min. Later, the absorbance was measured at 550 nm using an ELISA Reader (Thermo Scientific, USA). The amount of nitrite in the sample (micromolar unit) was calculated from a sodium nitrite standard curve.

Estimation of lipid peroxidation

Malondialdehyde (MDA) concentration was estimated by the thiobarbituric acid reactive substance (TBARS) test, which was used as an indicator of lipid peroxidation. Two milliliters of tissue homogenate was mixed with 1 mL 20% (v/v) TCA and 1 mL 0.67% (v/v) thiobarbituric acid and then boiled for 10 min. After cooling, the mixture was filtered through Whatman filter paper and the reading was taken at 530 nm. The concentration of MDA mmol mg^{-1} protein was quantified as an index of lipid peroxidation (Wills 1987).

Estimation of superoxide dismutase activity

SOD activity was measured by the nitrobluetetrazolium (NBT) method, which is based on inhibition of NBT reduction by SOD. In this reaction, 2.5 mL 0.05 mol L^{-1} sodium carbonate buffer (pH 10) was mixed with 0.1 mL 3 mmol L^{-1} EDTA, 3 mmol L^{-1} xanthine, 1.5 mg mL^{-1} bovine serum albumin (BSA), 0.75 mmol L^{-1} NBT, and the homogenate. Reaction was initiated after adding 0.1 mL 56 mU mL^{-1} xanthine oxidase. After 30-min incubation, the reaction was stopped by mixing with 6 mmol L^{-1} CuCl$_2$ and the reaction mixture was centrifuged at 350 g for 10 min. Absorbance of blue formazan was recorded at 560 nm (Sun et al. 1988). The relative absorbance was then converted into units of SOD activity per mg protein. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate.

Estimation of catalase activity

CAT activity was determined by the decomposition of H$_2$O$_2$ at 25 °C (Aebi 1984). The difference in absorbance at 240 nm per unit time was used as a measure of CAT activity. The values were expressed as U mg^{-1} protein. One unit of catalase is the amount of enzyme that will decompose 1.0 mmol of H$_2$O$_2$ per min at pH 7.0 at 25 °C.

Estimation of reduced glutathione concentration

For the estimation of ovarian reduced glutathione (GSH) concentration, in 1000 mL PBS, 100 mL of homogenate was mixed with 50 mL 5,5-dithiobis-2-nitrobenzoic acid (4 mg mL^{-1} in methanol) and then incubated at room temperature for 15 min. Then, readings were taken at 412 nm (Sedlak, Lindsay 1968). Results were expressed as mmol GSH mg^{-1} protein.

Estimation of protein

Protein in the ovarian tissue extract was estimated by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Statistical analysis

Data were expressed as mean ± SE. The Kruskal-Wallis nonparametric ANOVA test was performed to find significant differences between groups significantly. To

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test inter-group significant differences, the Mann-Whitney U multiple comparison test was performed. Statdirect 2.7.2. was used for statistical analysis. Differences were considered significant if $p < 0.05$.

Results

To scrutinize the involvement of oxidative damage in the mechanism underlying the detrimental effects induced by I/R injury of ovary, nitric oxide production, lipid peroxidation, glutathione concentration, CAT) and SOD activity in ovarian tissue homogenates were evaluated.

The results affirmed that MDA concentration, an indication of lipid peroxidation, was significantly ($p < 0.01$) higher in the I/R group compared to the sham operated group (Fig. 1B). Pretreatment with L-arginine caused a significant ($p < 0.01$) reduction in MDA concentration in the ovarian tissue extract of animals of the I/R group. The present study results demonstrated that ischaemia-reperfusion caused a significant ($p < 0.01$) decrease in the tissue concentration of NO (Fig. 1A) compared to the sham

operated group. Ovarian tissue concentration of NO was found to be elevated ($p < 0.01$) in animals of the I/R group pretreated with L-arginine.

Activity of both antioxidative enzymes, SOD and CAT, were found to be altered in animals of the I/R group. Ischaemia-reperfusion caused a significant ($p < 0.01$) reduction in the activity of SOD in ovarian tissue (Fig. 2A), which was recovered significantly ($p < 0.01$) by pretreatment
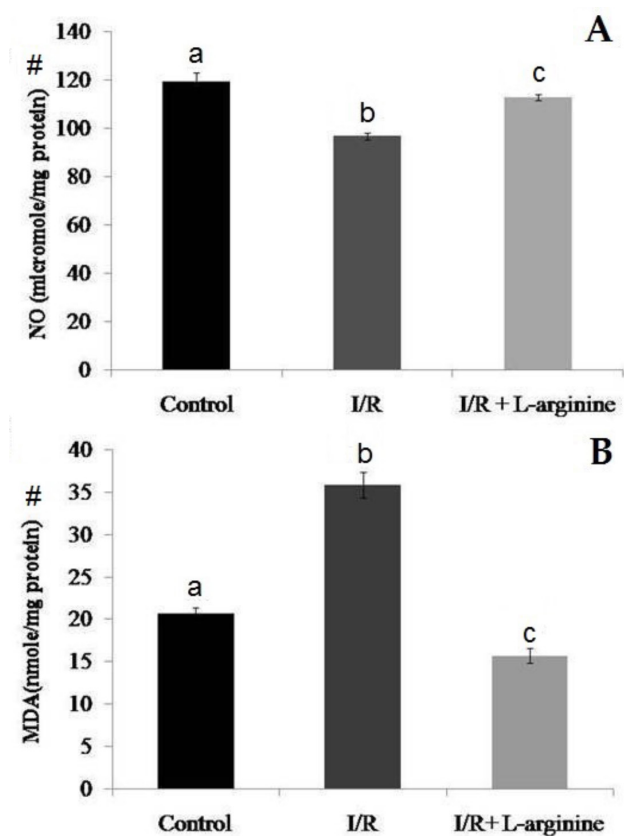

Fig. 1. Effect of L-arginine (single dose of 200 mg kg^{-1} body weight) on (A) NO level and (B) MDA level during ischaemia-reperfusion (I/R) injury in rat ovary. # Significance level based on the Kruskal–Wallis test ($p < 0.05$). Different letters indicate statistically significant differences between groups (mean ± SE, n = 5, Mann-Whitney U multiple comparison test, $p < 0.05$).

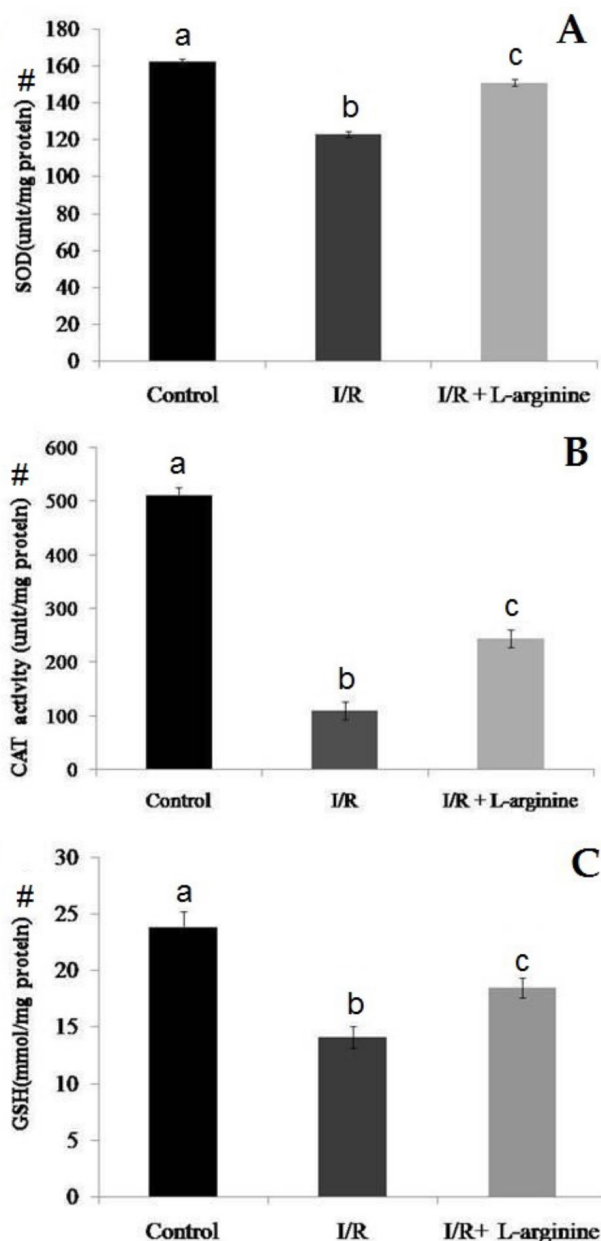**Fig. 2.** Effect of L-arginine (single dose of 200 mg kg^{-1} body weight) on SOD activity (A) CAT activity (B) and GSH level (C) during ischaemia-reperfusion (I/R) injury in rat ovary. # Significance level based on the Kruskal–Wallis test ($p < 0.05$). Different letters indicate statistically significant differences between groups (mean ± SE, n = 5, Mann-Whitney U multiple comparison test, $p < 0.05$).
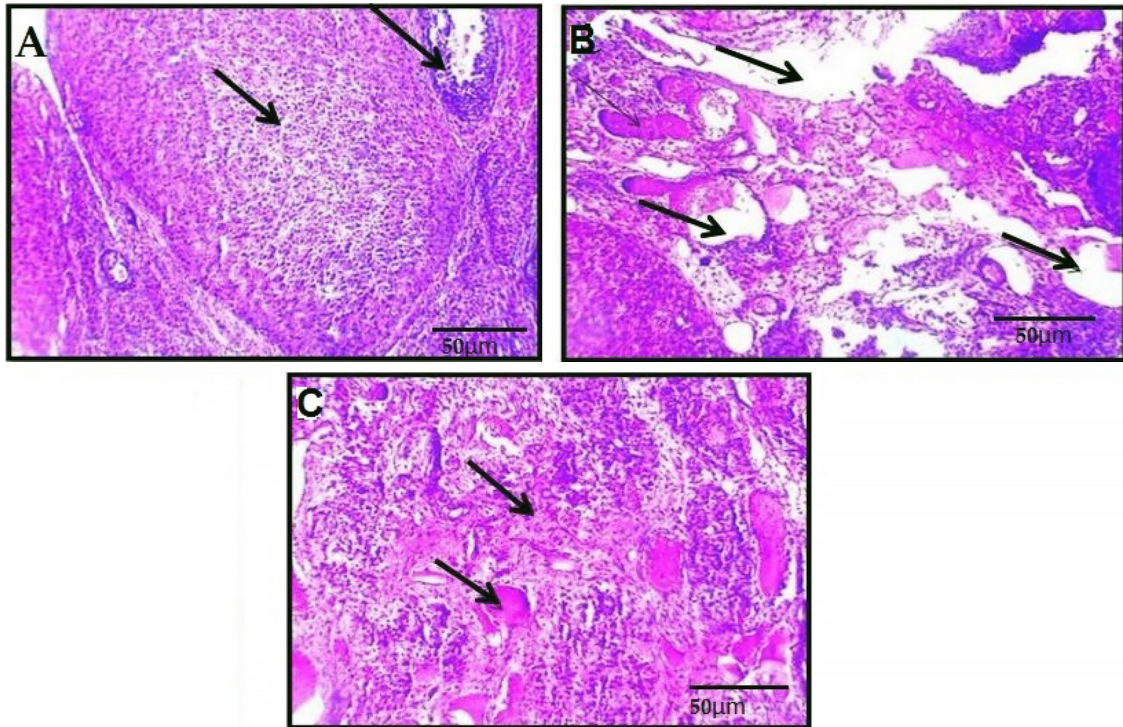
Fig. 3. Representative photomicrograph of haematoxylene and eosine stained section ($\times$ 10) showing morphology of ovary in control (A), I/R (B) and I/R + L-arginine (C). In (A) no pathological changes were seen. Normal architecture of ovary tissue was maintained. In (B) I/R group: vascular congestion, oedema and haemorrhagic foci were present (marked by black arrow). Ovarian follicles were degenerated. (C) L-arginine + I/R group shows decreased/moderate damage.

with L-arginine. Similarly, a significant decline in the activity of CAT (p <0 .001) was observed (Fig. 2B) in the I/R group, compared with the sham operated group. I/R induced a decline in the CAT activity, which was rescued with the supplementation of L-arginine, resulting in similar activity as observed in sham operated control animals (p < 0.001). Concentration of GSH, a non enzymatic antioxidant, was significantly (p < 0.001) decreased in ovarian tissue extract of animals in the I/R group when compared with the sham operated control group (Fig. 2C). Reduction in ovarian tissue GSH concentration in animals of the I/R group was anticipated by the pretreatment of L-arginine (p < 0.01).

Typical morphologic characteristics of ovarian tissues were found in the sham operated group of animals. The follicles with their cellular components appeared normal. The histopathologic alterations in the I/R group were comprehensive. Severe vascular congestion, interstitial edema, haemorrhage, and follicular degeneration were encountered in the ovary tissues. Haematoxylene and eosin stained ovarian tissue sections in the I/R group contained fields of edema and intensive haemorrhage (Fig. 3B). The follicles were also degenerated. The histological features of the follicles were partly recovered in the L-arginine + I/R group, even though some degrees of edema and haemorrhage were still instant, but still the follicles were conserved well. Small haemorrhagic foci were observed in the ovary of the L-arginine pre-treated I/R group.

Discussion

Ovarian torsion, a gynaecologic emergency situation that might affect any woman, is generally detected in women of reproductive age (Becker et al. 2009). Ovarian ischaemia is considered as an outcome of torsion and progresses to cell death because of inadequate blood flow to the tissue (Halici et al. 2008; Kara et al. 2012). There is a high chance of complete ovarian loss and infertility if a delay is made in early diagnosis and treatment. As the ischaemic tissue needs to recover blood supply in order to regenerate the cells and get rid of the toxic metabolites, the pathophysiologic condition termed ischaemia/reperfusion injury is confronted. However, the impairment caused by reperfusion of the ischaemic tissue is much harsher than the damage caused by ischaemia alone (Abramov et al. 2007). This ischaemic mechanism is alarming for tissues because of increased production of various toxic metabolites such as MDA and ROS (Demircioglu et al. 2011). ROS, such as superoxide anions, hydrogen peroxide and hydroxyl radicals, generated after reperfusion cause significant damage to DNA, cell membrane and mitochondria through the increased level of lipid peroxidation and cytokine production from activated neutrophils (Sun et al. 2018). This ultimately results in tissue damage. The cytoprotective effect of NO on I/R damage in different organs has recently been described (Zhang, Galiñanes 2000). Earlier, many studies reported the protective effects of numerous agents

against ovarian I/R injuries (Hortu et al. 2020; Sağsöz et al. 2002; Colak et al. 2020). In this study, we assessed whether NO has a protective role against I/R injury with the use of L-arginine, which is considered to be a noble amino acid precursor of nitric oxide. The function of NO against I/R injury on other organs such as liver (Lucas et al. 2015) and heart (Schulz et al. 2004) has been described in many of studies. Here we clearly show the protective role of NO against I/R injury of ovary by using L-arginine.

Lipid peroxidation, as a free radical-generating system has been implied to be firmly associated to I/R-induced tissue damage, and MDA concentration in tissue can be considered as a good marker of the rate of lipid peroxidation (Grundt et al. 2003). MDA, a highly toxic metabolite of the ROS-mediated lipid peroxidation cascade, imparts oxidative stress. It is known that MDA concentration significantly increases in I/R injury, which causes disruption in wall fluidity and permeability of the damaged cells (Ozer et al. 2005). Consistent with this previous observation, the results obtained in our present study confirm that tissue MDA concentration was significantly increased in post ischemic reperfusion. Our results also demonstrated that administration of L-arginine caused a significant decrease in MDA concentration, which implies that the protective effect of the drug might be conferred by its scavenging property. Further, this result is in good agreement with the earlier studies reporting beneficial effect of L-arginine on other tissue systems (Huk et al. 1998; Hubert et al. 2002; Schulz et al. 2004; Lucas et al. 2015). Intracellular enzymes such as SOD and CAT serve as weapons of cells to provide protection against the detrimental effects of ROS injury. There have been earlier reports showing that ischemic tissue reciprocates by boosting the activities of SOD, CAT, and concentration of GSH to counter the adverse outcome of toxic substances (Nita et al. 2001; Kara et al. 2012). Glutathione administers extensive protection to cells by aiding in a cellular cascade of defence against oxidative damage. Tissue injury brought about by distinct catalysts are conjoined with glutathione reduction (Kaçmaz et al. 2005). Therefore, to curtail the level of oxidative injury during organ reoxygenation, the preservation of high levels of glutathione is crucial (Biasi et al. 1995; Bilzer et al. 1999). It was previously shown that GSH, a critical antioxidant, is suppressed in ischaemic tissue following reperfusion (Celik et al. 2004). Hence, the decrease in glutathione concentration during I/R injury was perhaps due to its utilization amid oxidative stress. In this study, involvement of GSH in oxidative stress was confirmed by a decrease in GSH concentration in the I/R group with respect to the control group and GSH cocentration was found to be significantly increased in the I/R group pretreated with L-arginine.

Similarly, catalase, an antioxidant enzyme that scavenges the pro-radical hydrogen peroxide, was used for the determination of the antioxidant level. The results revealed that catalase activity was significantly lower in the I/R group compared to the control. SOD is an antioxidant enzyme that provides protection to tissues from the adverse effects of free radicals and active oxygen species by triggering the conversion of superoxide anion radicals into hydrogen peroxide and molecular oxygen (Arosio 2000). In this prospective study, we observed a decline in SOD activity in the I/R group compared to the sham operated group, confirming previous findings (Yapca et al. 2014). Further, pretreatment with L-arginine recovered the depleted SOD and CAT levels of the ovary in the respective group.

The current study showed that L-arginine exhibits significant protection against I/R injury of the rat's ovarian tissue, and that NO plays a significant role in this mechanism. These findings are well in line with the reported protective effects of NO in other organs, including heart and liver, undergoing I/R injury (Lucas et al. 2015; Schulz et al. 2004). Our results highlighted the role of NO during ovarian I/R treatment under the experimental conditions used.

NO, a colourless gas is a product of L-arginine amino acid, which serves as a substrate of nitric oxide synthase enzyme (Diesen, Kuo 2010). As discussed earlier, NO has a puzzling role during I/R injury, depending upon its concentration. Earlier experiments have proposed the protective efficacy of NO in I/R injury (Shimamura et al. 1999). Previous study revealed that endogenously produced NO through the action of nitric oxide synthase as well as exogenously administered NO during an ischaemic abuse can curb the intensity of the reoxygenetaion damage in hepatic I/R injury, thereby clearly suggesting a constructive role of NO against I/R injury (Phillips et al. 2009). Further, NO is a useful antioxidant that has some beneficial activity against ROS like H_2O_2 and O_2^- (Wink et al. 2001). It has been observed that the antioxidant effects of NO can be markedly elevated if specific pathways are activated, resulting in increased endogenous antioxidant production (Patel et al. 2000). NO has various biologic actions, such as vasodilation (vascular smooth muscle relaxation) and inhibition of platelet aggregation; a mechanism for both involves the elevation of cyclic guanosine monophosphate following soluble guanylate cyclase activation by NO.

Our hypothesis in this study was based on evidence that a decrease in NO production by injured endothelial cells through the attack of reactive oxygen species produced during reperfusion period is one of the major paths for organ injury caused by ischaemia and reperfusion (Tsao et al. 1990). In consistency with this hypothesis, we observed an elevation of the NO level by using the NO precursor L-arginine in the I/R group compared to the I/R group without pretreatment with L-arginine. Therefore, we can speculate that the NO concentration increase through treatment with L-arginine reduced ischaemia and reperfusion injury of the rat ovary. The precise role of NO

in ovarian I/R injury is yet to be elucidated.

It was previously reported that histopathological changes such as vascular congestion, oedema, haemorrhage, and follicular degeneration were found to be present in a ischemic group following reperfusion (Bas et al. 2013). In the present study, similar results were found in the case of the I/R group, which became reversed with pretreatment of L-arginine, suggesting protecting efficacy of L-arginine on structural damage of ovary.

In conclusion, our results demonstrated that L-arginine decreases I/R injury in rat ovary and functions as a protective agent for ovarian tissue in I/R injury. However, further studies are needed to explore the benefits of arginine in both ischemic ovarian tissues and thus reproduction. Moreover, further studies may also provide greater amount of knowledge about the beneficial clinical effects of L-arginine on ovarian tissues exposed to ischaemia-reperfusion injury. Our findings indicate that L-arginine might be useful in clinical practice, particularly in treatment of damage resulting from ovarian torsion.

Acknowledgements

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