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Mechanism of action of oxytocin as cardioprotection in rat model of myocardial infarction

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Abstract*: Oxytocin (OT) is well known for its role in reproduction. However, evidence has emerged suggesting a role in cardiovascular system but less is known about the role of this hormone in the injured heart. We elucidate oxytocin cardioprotective effects against myocardial infarction (MI). Male rats were divided into six groups: control without surgery, sham without occlusion, MI, OT pretreated then MI, combined OT and L-NAME (NO synthase inhibitor) then MI and combined OT and indomethacin (cyclooxygenase blocker) then MI. Twenty-four hours post-MI induction, hemodynamics parameters, inflammation markers, oxidative stress markers, apoptotic gene expression, brain naturitic peptide (BNP), and histopathological assessment were carried out. When compared to MI model group, OT significantly reduced LVEDP and increased LVSP and ± dP/dt.Also, it significantly decreased serum levels of BNP, TNF-α, IL-6, and TBARS with an increase in the activities of SOD and GPx. Furthermore, BAX and p53 mRNA were decreased. Interestingly, no significant improvement in any of the markers was detectable when we administrated OT with L-NAME. While the same results observed when we treated the rat with OT and indomethacin. We conclude that OT protected against the sequelae of myocardial infarction. These findings provide new insight into therapeutic strategies for myocardial infarction.*

Keywords*: Apoptosis, L-NAME , Myocardial infarction, Oxidative stress, Oxytocin.*

I. Introduction

Acute myocardial infarction (MI) due to coronary artery occlusion represents a major cause of morbidity and mortality in humans. MI related complications such as heart failure are of great socio-economic burdens to society and healthcare systems [1].

Till now, no single mechanism that could fully explain the development of post MI depressed cardiac function and development of HF. It is likely that the cause is multi-factorial. In this regard, there has been surge of studies that have shown involvement of increased oxidative stress [2], inflammation [3], apoptosis and left ventricular (LV) myocardial remodeling, which are collectively characterized by chamber dilatation and impaired ventricular function [4] in both humans and animals.

During the first few days after myocardial infarction (MI), the dominant pathological processes are inflammation and cell death. The inflammatory response after MI is integral to the healing process and contributes to left ventricular (LV) remodeling [5]. However, no effective therapeutic strategy against cardiac inflammation has been established [6].

Oxytocin (OT) is known as a neurohypophyseal hormone and is not only important to the central nervous system (CNS), but also has physiological actions in peripheral organs in addition to its role in female reproduction [7].

Cardiovascular system belongs to important targets of OT action that is supported by the presence of oxytocin receptors in the heart and vascular endothelial cells [8]. Moreover, the rat heart has been shown to be a site of oxytocin synthesis and release [9].

Cardiovascular effects of OT include its influence on blood pressure [10,11], heart rate and contractility [12].

Cardiac OT is structurally identical to that found in the hypothalamus, indicating that this active form of OT is derived from the same gene. The abundance of OT and its receptors in atrial myocytes suggests that, directly and/or via the release of the cardiac hormone atrial natriuretic peptide, this hormone regulates the force of cardiac contractions [13].

The mechanisms responsible for cardioprotective action of oxytocin are not understood. So far, the involvement of negative chronotropic action of oxytocin [12] and activation of mitochondrial ATP-dependent potassium channels [14] has been suggested. Oxytocin treatment prior to myocardial infarction can modify

several other pathways playing a role in ischemia/reperfusion injury, such as enzyme systems of protein kinases [15,16].

Peripheral administration of oxytocin resulted in a transient increase followed by a prolonged decrease in blood pressure [11]. Oxytocin treatment also produced a reduction in heart rate and contractility. Perfusion with oxytocin induced a concentration-dependent negative inotropic effect in isolated rat hearts [10].

NO was shown to be a mediator/protector of ischemia and reperfusion injury in many organs, such as the heart, liver, lungs, and kidneys. Some of the protective actions of NO in ischemia and reperfusion are due to its potential as an antioxidant and anti-inflammatory agent, along with its beneficial effects on cell signaling and inhibition of nuclear proteins, such as NF-κ B and AP-1 [17].

The present study was designed to evaluate the cardioprotective effects of oxytocin administration on myocardial infarction in rat model and the possible role of nitric oxide (NO), reactive oxygen species (ROS) and cyclooxygenase in this regard.

II. Materials And Methods

2.1 Drugs and chemicals

Oxytocin as lyophilized powder (C43H66N12O12S2, 50 IU/mg solid-Cat No. O3251), L-N^G-nitroarginine methyl ester (L-NAME) (an NO synthase inhibitor) (C7H15N5O4 · HCl, Cat No. N5751) and indomethacin (a non specific cyclooxygenase inhibitor) (C19H16ClNO4, Cat No. I7378) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were prepared as described in normal saline. Assay kits for determination of malondialdehyde (MDA, Cat No. NWK-MDA01) were purchased from NWLSS (Vancouver, WA, USA). An assay kit for the determination of superoxide dismutase activity (SOD, Cat No. 706002) was purchased from Cayman Chemical (Ann Arbor, MI, USA). An assay kit for the determination of glutathione peroxidase activity (GPx, Cat No. 703102) was purchased from Cayman Chemical (Ann Arbor, MI, USA). ELISA kits for the determination of the levels of IL-6 (Cat No. ELR-IL6-001) and TNF-α (Cat No. ab46070) were purchased from RayBio MO, USA and Abcam, Cambridge, MA, USA, respectively. Assay kit for the determination of Brain Natriuretic Peptide (BNP Elisa Kit, Cat. No. KT-8580) was purchased from Kamiya Biomedical Company (Seattle, WA.).

2.2 Animals

Male young Wistar rats (n= 36, age 3 weeks, weighing 60-75 g) were supplied by the animal house facility at King Khalid University, Abha, Kingdom of Saudi Arabia. They were housed four per cage in a controlled environment (23 ± 1◦C; 45%–50% relative humidity; fixed 12/12 h light/dark cycle, lights on at 08:00 h) with food and water ad libitum. The experimental protocols were approved by the Animal Care and Use Committee of the King Khalid University. All surgical and treatment procedures were consistent with the guidelines of the National Institute of Health (NIH publication No. 85-32, revised 1996). (Institute of Laboratory Animal Resources, 1996).

2.3 Experimental Design

After seven-day acclimatization, rats were randomized into 6 groups (n=6 each) as follows: 1) Control un-infarcted group: Control rats which were not exposed to any surgical procedure; 2) Sham operated group: Rats underwent same surgical procedure used to ligate left anterior descending coronary artery (LAD) except that the silk suture was placed around the left coronary artery without being tied; 3) MI model group: Rats underwent LAD ligation; 4) Oxytocin pre-treated group (OT then MI): Rats pre-treated with OT (3.6 μg/100 g body weight/day, subcutaneous) for seven consecutive days on daily basis and then underwent LAD ligation; 5) Oxytocin and L-NAME pre-treated group: (OT+L-NAME then MI): received concomitant dose of OT and L-NAME (20 mg/kg, I.V) for seven consecutive days on daily basis and then underwent LAD ligation; 6) Oxytocin and indomethacin pre-treated group: (OT+Indomethacin then MI): received concomitant dose of OT and indomethacin (1 mg/kg, I.V) for seven consecutive days on daily basis and then underwent LAD ligation. Each treatment was administered to animals in a final volume of 0.2 ml according to the rout of administration and the intravenous administrations were administered to animal through the rat's tail. Dose selection and routs of administration were based on previous studies which showed a cardioprotective role of these drugs at theses doses using these routs of administration [18,19,20].

2.4 Rat MI Model (LAD ligation)

Myocardial infarction was produced using a previously described method by Xing et al. [21]. Thirty rats were anaesthetized by intraperitoneal (i.p) injection of a 1% solution of chlorohydrate (375 mg/kg). After the anaesthesia ensued, rats were placed on a heating pad to maintain normothermia (36°C) and then fixed in the supine position by tying the legs and the upper jaw. The trachea was incubated by a non-invasive method via the mouth and mechanically ventilated with room air with a small-rodent ventilator (Harvard Rodent Ventilator,

Model 863, Harvard Apparatus, Holliston, MA) to perform a left thoracotomy to expose the heart. The electrocardiogram (ECG, Lead II), heart rate and respiratory rate were continuously monitored. MI was induced by permanent ligation of the LAD coronary artery at the location between the pulmonary cone and the left atrial appendage under its origin 2-3 mm, using an 8-0 polypropylene suture passed about 2–3 mm from the inferior margin of left auricle. MI was confirmed by myocardial blanching and ECG ST-segment elevation. In the process of operation, physiological saline and gel were dripped on conjunctiva and cornea to prevent blindness caused by corneal drying in rats. After successful ligation of the coronary artery, the thorax was closed in layers starting from ribs, muscles and then skin with standard produces using 5-0 sutures. Then, the rats were given intramuscular injection of penicillin and subcutaneous injection of an analgesic (Buprenorphine 0.1 mg/kg). After restoring spontaneous breathing, rats were pulled out the endotracheal intubation, and then rats were placed on electric blanket waiting for their revival and then returned to their cages.

2.5 Cardiac Hemodynamic Measurements 24 hours post MI

Cardiac hemodynamic measurements were assessed at 24 h post coronary ligation in the MI or treated groups as well as in the Control and Sham operation. In brief, rats were anaesthetized by intraperitoneal (i.p) injection of a 1% solution of sodium pentobarbital (50 mg/kg). Then, they were placed on a heating pad to maintain body temperature (about 36oC) and then affixed in the supine position by tying the legs and the upper jaw. After performing tracheal intubation and successful ventilation of the rats as described above, an open chest surgery was performed and SPR-320 pressure catheter was inserted directly into the left ventricle (LV) to measure LV systolic pressure, LV end diastolic pressure (LVEDP), maximal rate of rise in LV pressure (+dP/dt) and maximal rate of decline in LV pressure (-dP/dt). Calibration of the Millar catheter was verified before each measurement. All data were recorded for 10 minutes with the help of on PowerLab Data Acquisition System (ML780 PowerLab/8channels, AD Instruments Ltd, Australia).

2.6 Determination of Brain Natriuretic Peptide

Following the measurements of the left ventricle hemodynamic parameters, a 1 ml blood was withdrawn from the aorta into ethylenediaminetetraacetic acid (EDTA)-treated tubes and then the aorta was closed temporarily by a clamp. Then, all blood samples were centrifuged at 4000 rpm for 10 min to obtain the plasma, which was directly used to determine the levels of Brain Natriuretic Peptide (BNP) using Kamiya ELISA kits according to manufacturer's instructions.

2.7 Preparation of Left Ventricle (LV) Homogenates and Biochemical Measurements

Parts of the LV obtained from the rats in all groups were freshly washed with cold phosphate buffered saline (PBS), pH 7.4 and weighted. 100 mg LVs Specimens were homogenized with an ultrasonic homogenizer in one ml cold phosphate buffer, pH 7.4, containing EDTA. For determination of tissue TNF-α, the myocardial homogenate was suspended in PBS solution containing protease inhibitors.The homogenate was then centrifuged at 5000 xg for 20 min at 4^{o}C.An aliquotwas used for total protein measurement (Bio-Rad, Hercules, California, USA). Results were expressed for TNF-α as picogram/mg total protein. The supernatant obtained was distributed in separate tubes and stored at –70 °C for later determination of the levels of MDA (TBARS), IL-6, TNF-α and activities of SOD and GPs as per manufacturer's instructions. Other parts of these LVs were frozen in liquid nitrogen and stored at -80°C and used later for mRNA extraction.

2.8 RNA Extraction and RT-PCR

The procedure was optimized for semiquantitative detection using the primer pairs and conditions described in Table II. Published sequences of PCR primers used for the detection of p53 and Bcl-2 associated X protein (Bax) were used according to the procedure established already in our labs, where β-actin was used to control for loading [22]. Total RNA was extracted from the frozen parts of LV (30 mg) using an RNeasy Mini Kit (Qiagen Pty. Ltd., Victoria, Australia) according to manufacturer's directions. The concentration of total RNA was measured by absorbance at 260 nm using a UV1240 spectrophotometer (Shimadzu, Kyoto, Japan). The purity was estimated by the 260/280nm absorbance ratio. Single-strand cDNA synthesis was performed as follows: 30 µl of reverse transcription mixture contained 1 µg of DNase I pretreated total RNA, 0.75 µg of oligo d(T) primer, 6 µl of 5x RT buffer, 10 mMdithiothreitol, 0.5 mMdeoxynucleotides, 50 U of RNase inhibitor, and 240 U of Reverse Transcriptase (Invitrogen). The Reverse Transcription (RT) reaction was carried out at 40°C for 70 min followed by heat inactivation at 95°C for 3 min. The tested genes and the internal control (β-actin) were amplified by PCR using 2 µl RT reaction products from each sample in a 20 µl reaction containing Taq polymerase (0.01 U/ml), dNTPs (100 mM), $MgCl_2$ (1.5 mM) and buffer (50 mMTris-HCl). PCR reactions consisted of a first denaturing cycle at 97°C for 5 min, followed by a variable number of cycles of amplification, consisting of denaturation at 96°C for 30 sec, annealing for 30 sec, and extension at 72°C for 1 min. A final extension cycle of 72°C for 15 min was included. Annealing temperature was adjusted for each target: 60°C for

p53 and 55°C for Bax and β-actin. A control reaction without reverse transcriptase was included for every sample of RNA isolated to verify the absence of contamination. PCR products (10 µl) were electrophoresed on 2% agarose gels containing 100 ng/ml ethidium bromide, and photographed with a Polaroid camera under ultraviolet illumination.

2.9 Histopathological evaluation

Some longitudinal section from each heart from each group was rapidly fixed in 10% formaldehyde, dehydrated and embedded in paraffin, then cut into 4µm slices that were stained with hematoxylin and eosin for histological assessment.

2.10 Statistical Analysis

Statistical analysis was performed using Graphpad Prism Statistical Software package (version 6). Data was presented as means with their standard deviations (mean±SD). Normality and homogeneity of the data were confirmed before ANOVA. Differences among the experimental groups were assessed by one-way ANOVA followed by Tukey's test.

III. Results

3.1 ST Elevation

As shown in Fig. 1, normal ECG waves with normal ST segment height were seen in the control and sham groups of rats (A and B, respectively). The success of left anterior descending coronary artery (LAD) ligation in MI model group was confirmed by ST segment elevation (Fig. C). However, high ST segment similar to the one seen in the MI group was clearly evident in oxytocin + L-NAME pretreated then MI group (Fig. E). Normal ECG waves without any significant ST elevation were seen in control, sham, oxyotcin pre-treated then MI and oxytocin+indomethacin pre-treated then MI groups (Figs. D & F).

3.2 Left ventricular trace recordings

As shown in Fig. 2, normal left ventricular trace recordings (normal LV pressures) were seen in the control, sham, oxytocin pre-treated then MI and oxytocin+indomethacin pre-treated then MI groups of rats (A, B, D & F respectively). In MI and oxytocin + L-NAME pretreated then MI groups, there are a decrease in LV end systolic pressure (LVESP) and an increase in LV end diastolic pressure (LVEDP).

3.3 Cardiac Hemodynamic Measurements 24 hr Post MI

As shown in table I, LVESP, LVEDP, maximal rate of rise in LV pressure (+dP/dt) and maximal rate of decline in LV pressure (-dP/dt) were not significantly different between the control and sham operated rats. Also, the heart rate is not significantly different between all groups. However, compared with sham group, the rats of MI group showed a significant increase of LVEDP 15.4 ±1.9 mmHg versus 3.4 ± 1.0 mmHg in the sham group as well as a significant decreases in LVESP (61.4±2.9 versus 109.4±5.7), LV +dP/dt (901±88 versus 1567±254) and LV -dp/dt (793±95 versus 1120 ± 113). Oxytocin pre-administration to MI induced rats resulted in a significant improvement in the levels of these hemodynamic parameters. Oxytocin significantly increased LVESP, LV +dP/dt and LV -dp/dt and significantly decreased LVEDP. In OT + L-NAME pretreated then MI group, L-NAME abolished the effect of oxytocin. There were a significant decrease of LVESP, LV +dP/dt and LV -dp/dt and a significant increase of LVEDP when compared to sham and OT then MI group but still significantly higher LVESP than in MI group but no significant changes of LVEDP, LV +dP/dt and LV -dp/dt when compared to MI group. In oxytocin+indomethacin pre-treated then MI group, indomethacin failed to abolish the effect of oxytocin on the hemodynamic parameters. So, no significant changes of these parameters when compared to sham and OT pretreated then MI group.

3.4 plasma Brain Natriuretic Peptide (BNP)

As shown in Fig. 3, no significant change in the levels of plasma BNP was seen in sham operated group when compared to control group. BNP levels were significantly elevated in the MI and OT + L-NAME then MI groups. Interestingly, the administration of oxytocin to MI group significantly decreased BNP levels. Also, in OT + IND then MI group, there were significantly lower levels of BNP. But still the levels of BNP in OT then MI and OT + IND then MI groups higher than in sham group.

3.5 LV Oxidative Stress Markers

Malondialdehyde (MDA) levels as measured by measuring levels of thiobarbituric acid reactive substances (TBARS) and activities of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) were measured in the LV homogenates obtained from all groups of rats as shown in Fig. 4. No significant difference was detected between control and sham groups in regards to the levels of TBARS, SOD or GPx activities.

However, in the MI and OT+L-NAME groups a significant increase in the levels of TBARS and a significant decrease in SOD and GPx activities were detected. On the other hand, TBARS, SOD and GPx activities remained at sham group levels when MI group was pre-treated with OT or OT+IND.

3.6 LV Inflammatory Markers Levels

As shown in Fig. 5, LV levels of Tumor necrosis factor-α (TNF-α) and Interleukin 6 (IL-6) were measured. Low levels of TNF-α and IL-6 were detected in the LV homogenate from control group. Sham operated group showed no significant difference in the levels of the 2 markers. TNF-α and IL-6 levels were significantly elevated in the MI and OT+L-NAME then MI groups**.** No significant changes in the levels of TNF-α and IL-6 between MI and OT+L-NAME then MI groups. The administration of oxytocin to MI group significantly decreased LV levels of TNF-α and IL-6. Also, significant decrease in LV levels of TNF-α and IL-6 were seen in the MI induced rats pre-treated with OT+IND but still significantly higher in the two groups when compared to sham group.

3.7 Levels of p53 mRNA and BAX mRNA

Fig. 6 shows the transcriptional changes of p53 and BAX mRNAs in the LVs obtained from all groups of rats. All tested transcripts were detected, and RT-PCR resulted in fragments similar in size to those expected (Table II). The levels of the β-actin transcript remained relatively constant in all groups.

In the control or sham operated groups, p53 and BAX mRNA were barely detectable. However, it is noteworthy that the levels of BAX mRNA, and p53 mRNA increased in the MI group rats. Their levels remained consistently elevated in the OT+L-NAME then MI group but slightly lower than in MT group. Both p53 mRNA and BAX mRNA were significantly reduced upon OT administration (i.e., alone or in combination with IND). Notably, while OT nearly returned p53 levels to sham group levels, BAX mRNA remained slightly elevated.

3.8 Left Ventricle Histology and Histopathology

Histological changes in the left ventricle in rats from all groups were assessed (Fig. 7). Both control (Fig. 7A) and sham (Fig. 7B) groups showed normal myocardial tissue with orderly striated heart muscle fibers, intercalated discs, and a clear nuclear and muscle bands staining. However, sections obtained from MI (Fig. 7C & D) were lacking transverse band structure, shrinkage, fragmentation, or disappearance of nucleus. Many nuclei have become pyknotic (shrunken and dark) and have then undergone karyorrhexis (fragmentation) and karyolysis (dissolution). Myocardial necrosis and apoptosis were evident as shown in the extradark pink staining of the cardiomyocytes. Also, inflammatory cell infiltration was seen in this group. On the other hand, sections obtained from OT (alone; Fig. 7E) or in combination with indomethacin (Fig. H) showed almost normal architecture of cardiac cells, normal fiber striation, and clear nuclear and fiber staining. Necrosis, apoptosis, and inflammatory cell invasion were rarely seen at 1000X magnification.Sections from oxytocin and L-name pre-treated groups (Fig. 7F & G) showed small area of infarcted myocardium replaced by macrophages and surrounded by large wavy myocardial fibers.

IV. Discussion

In the present study we have investigated the protective effects of oxytocin (3.6 μg/100 g body weight/day) pretreatment in improving the early cardiac dysfunction in a rat model of myocardial infarction (MI) induced by left anterior descending coronary (LDA) ligation in rats. We also study the possible mechanism of action of OT either through NO or cyclooxygenase pathway.

The current study demonstrated that OT treatment 7 days to rats with MI ameliorated echocardiographic parameters. The conferred cardioprotection was characterized by improved cardiac muscles structure, and normalized hemodynamics as well as contractility of LV parameters. Oxytocin decreased LVEDP and increased LVESP and ±dP/dtmax. This is in agreement with the study of Ondrejcakova et al. [18].

In our study, administration of L-NAME with oxytocin for 7 days and then underwent LAD ligation, incompletely abolished the effects of OT on LVESP as there were a significant decrease in LVESP and ±dP/dtmax and increase LVEDP when compared to OT then MI. But there were still significantly higher LVESP when compared to MI group and no significant changes of LVEDP and ±dP/dtmax when compared to MI group.

On the other hand, in OT and indomethacin pretreated group, There were no significant changes of LVESP, LVEDP and ±dP/dtmax when compared to OT then MI group. This indicates that IND did not abolish the effects of OT.

The results of the present study show that one-week treatment of rats with oxytocin leads to decreased plasma level of BNP. This is consistent with the findings of Jankowski et al. [6].

Brain natriuretic peptide (BNP) is secreted predominantly from the ventricles in response to increased wall stress, which is known to be one of the major forces driving left ventricular (LV) remodeling. Because a high level of NP is a powerful marker of LV systolic dysfunction and poor prognosis after MI [23], these results support the conclusion that OT infusion has a beneficial effect on heart function after injury.

In our current study, there was a significant increase in the levels of lipid peroxides (TBARS) and inflammatory markers TNF-α and IL-6 with a concomitant decrease of the endogenous antioxidant enzymes (SOD and GPx) activities, in the cardiac homogenates of the infracted areas taken from the MI group. The MI is recognized as a definitive factor for amplification of an excessive and unnecessary inflammatory response [24]. Considerable evidence indicates that the reactive oxygen species (ROS), such as superoxide anion (O_2-), hydrogen radicals, and hydrogen peroxide, play an important role in mediating myocardial ischemic injury and cardiac cell apoptosis.

Ischemic stress represents a potent trigger for cytokine production. It was found that direct myocardial mechanical stretch which is maximal in the infarct and peri-infarct zone is also a potent regulator that leads to the prompt production of TNF-α and IL-6 in the myocardium through three major intracellular cross-talking signal transduction pathways, mitogen-activated protein kinase (MAPK), JAK-signal transducer and activator of transcription (STAT), and calcineurin-dependent pathways [25].

Lipid peroxidation, as a free radical-producing system, has been proposed to be tightly linked to IR-triggered tissue damage, and MDA is an important parameter of oxidative stress and a good pointer of lipid peroxidation [26]. In Hekimoglu et al. [27] research, in renal IR group, the level of MDA notably elevated in the liver tissue, while subcutaneously administered OT repressed MDA rising notably and improved lipid peroxidation.

Interestingly, cytokines are capable of decreasing left ventricle performance and myocyte contractility directly and indirectly. In the setting of injury, the reduction in contractility mediated by TNF-α and IL-6 may be an adaptive response to decrease myocardial energy demand. TNF-α and IL-6 can attenuate myocyte contractility directly through the immediate reduction of systolic cytosolic [Ca^{2+}] via alterations in sarcoplasmic reticulum function. However, TNF-α is also capable of decreasing myocyte contractility indirectly through nitric oxide-dependent attenuation of myofilament Ca^{2+} sensitivity. Early, within minutes after cardiac injury, TNF decreases systolic function by altering calcium-induced calcium release by the sarcoplasmic reticulum and by disrupting the L-type calcium channel [28].

In agreement with our results, Gutkowska et al. [29] have shown that treatment with OT reduced the expression of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6).

Data of Jankowski et al. [6] study suggest that improvement of cardiac contractile function in response to OT treatment is associated with reduced TNF-α expression in the injured myocardium.

Our observation is also consistent with experiments demonstrating inhibition of IL-6 release by OT in pituitary cells [30], macrophages and endothelial cells [31] and the infarcted site of the rat heart [6].

Our results are consistent with Anvari et al. [32] who found that the administration of OT significantly decreased MDA level as compared to IR group. OT ameliorated immediate myocardial injury in heart grafts, through downregulation of the inflammatory response, of reactive oxygen species, and of neutrophil dependent apoptosis [13].

In a study by Das and Sarkar [33], the infarct size-reducing and anti-arrhythmic effects of OT were achieved via selective activation of OTR, CM mitoK_{ATP} channels, and NO. The NO contribution suggests the role of cGMP and protein kinase G (PKG) in OT effects on mitochondria.

NO has protective effects on cells during IR. NO has been demonstrated to inhibit oxidative stress, cytokine release, apoptosis, adhesion and aggregation of neutrophil leukocytes [34]. A reduction of NO during IR is generally caused by endothelial dysfunction and reduction of endothelial nitric oxide synthase activity. OT, which is revealed to be protective in IR induced remote liver injury, may exhibit its effect by increasing NO synthase activity and releasing NO from vascular endothelium [27].

In the present study, we found that administration of L-NAME with OT returned the high levels of serum BNP, cardiac MDA, TNF-α and IL-6 as in MI group. While IND did not abolish the effect of OT on these parameters.

Apoptosis is a physiological phenomenon. However, the increase in myocardial apoptosis following MI is one of the mechanisms involved in aggravating cardiac tissue injury [35].

During the first few days after MI, the dominant pathological processes are inflammation and apoptosis. Indeed, increased cardiomyocyte apoptosis has been observed in HF after MI both in animal models and in humans [36]. Excessive apoptosis can accelerate the loss of myocardial cells, deteriorate heart function, and promote the development of ventricular remodeling. In particular, ventricular remodeling and myocardial apoptosis are the primary reasons leading to decreased LV function and heart failure following MI, which ultimately causes death [37].

The intrinsic apoptotic pathway was dominated by the Bcl-2 family of antiapoptotic proteins, namely, Bcl-XL and Bcl-2, as well as proapoptotic proteins, namely, BAX, p53, noxa [38]. p53 can translocate to mitochondria, where it binds to Bcl-2, thereby counteracting the antiapoptotic function of Bcl-2. In addition, p53 can stimulate reactive oxygen species production and Fas/CD95 to redistribute to the cell surface [39]. Thus, it appears that p53 may use multiple pathways to convey its death signals.

NO has also been shown to inhibit apoptosis by downregulating the expression of gene p53, which normally promotes apoptosis in the kidneys. Animals with worse histopathological features, after I/R injury, were found to have elevated levels of p53 gene expression. From this study we confirmed the beneficial effects of an exogenous NO donor during an ischemic insult and demonstrated that the downregulation of gene p53 correlated with a decrease in apoptosis [40].

Interestingly, in the current study, the levels of proapoptotic genes were elevated. In effect, OT neutralized the pathological apoptosis observed in the infarcted left ventricle (Fig. 7C & D) by downregulating the proapoptotic driving force in the cell, BAX and p53. This intervention reinstated the homeostatic apoptosis under permanent occlusion-induced MI of the LV.

We also observed that L-NAME administration with OT partially abolished the effects of OT. This indicates that the effects of OT are via NO and other mechanisms. On the other hand, administration of indomethacin with OT did not abolish its effects. Khansari et al. [41] found that infusion of L-NAME and indomethacin inhibited oxytocin from exerting its effects. Our explanation may be due to the difference in the dose of indomethacin in this study.

Our results are in agreement with Jankowski et al. [6] who found that OT treatment led to the absence of haemorrhage, inflammatory cell infiltration and apoptosis in infarcted cardiac areas. Because myocardium starts to undergo irreversible injury within 20 min of ischemia, we initiated the OT infusion before production of MI. It is therefore possible that OT exposure before coronary ligation contributes to myocardial protection in rats.

V. Figures And Tables

Table I: Cardiac hemodynamic parameters in the control, sham and all other experimental groups of rats, 24 hours post induction of MI.

Group	HR (Beats/min)	LVESP (mmHg)	LVEDP (mmHg)	+dp/dt (mmHg/s)	-dp/dt (mmHg/s)
Control	351.6±22.8	114.8±9.4	3.3±1.1	1687±158	1257±103
Sham	359.8±24.3	109.4±5.7	3.4±1.0	1567±254	1120±113
MI	350.2±22.1	61.4±2.9[ab]	15.4±1.9[ab]	901±88[ab]	793±95[ab]
OT then MI	341.1±25.4	107.1±6.9[c]	3.4±0.9[c]	1577±244[c]	1243±226[c]
OT+L-NAME then MI	345.0±24.9	70.6±3.8[abcd]	17.4±3.35[abd]	932±93[abd]	793±71[abd]
OT+IND then MI	347.0±25.4	108.5±4.5[ce]	3.2±1.2[ce]	1513±288[ce]	1184±147[ce]

Values are expressed as Mean ± SD for 6 rats in each group and considered significantly different at $P < 0.05$. [a]Significantly different when compared to control group [b]Significantly different when compared to sham group. [c]Significantly different when compared to MI group. [d]Significantly different when compared to OT then MI group. [e]Significantly different when compared to OT+L-NAME then MI. MI: Myocardial Infarction, OT: Oxytocin, IND: indomethacin. HR: Heart Rate, LVESP: Left Ventricle End Systolic Pressure, LEEDP: Left Ventricle End Diastolic Pressure, +dp/dt: Maximal Rate of Rise in LV Pressure -dp/dt: Maximal Rate of Decline in LV Pressure.

Table II: Primers and conditions used in PCR reactions.

Target	Primer sequence (5' to 3')	AT (C)	Size (bp)
p53	5 - CTACTAAGGTCGTGAGACGCTGCC-3[c] 5 - TCAGCATACAGGTTTCCTTCCACC-3[d]	60	106
Bax	5- 5_-GGTTGCCCTCTTCTACTTT-3 [c] 5 - AGGCAGGCTGCTCTTTC-3[d]	55	143
β-actin	5-CGTTGACATCCGTAAAGAC-3[c] 5-TAGGAGCCAGGGCAGTA-3[d]	55	110

AT; Annealing temperature.
c; Sense.
d; Antisense.

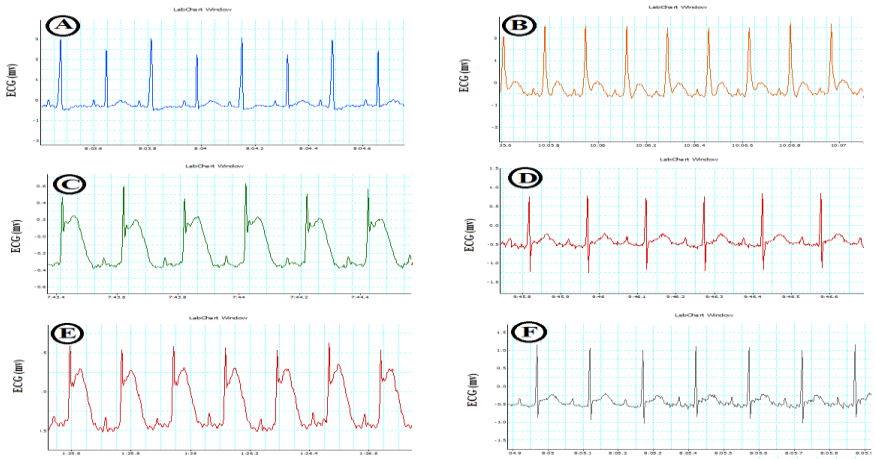

Figure 1: Examples of ECG trace recordings obtained from all experimental groups using PowerLab Instrument (Australia) using three limbs electrodes. Data were recording by lead II. A: control group, B: Sham operated group, C: MI group, D: Oxytocin pre-treated then MI group, E: Oxytocin + L-NAME pretreated then MI group. F: Oxytocin+Indomethacin pre-treated then MI group. Clear ST segment elevations were seen in groups C and E.

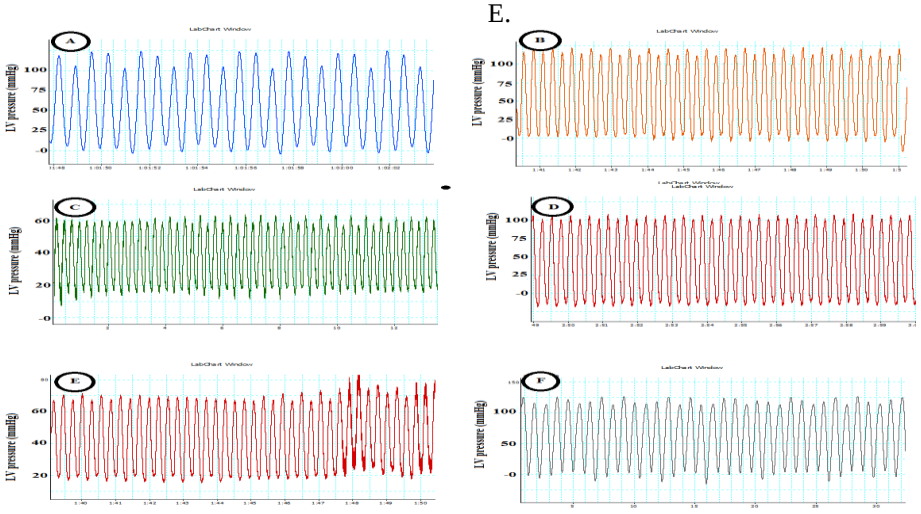

Figure 2: Examples of left ventricular trace recordings obtained from all experimental groups using PowerLab Instrument (Australia). A: control group, B: Sham operated group, C: MI group, D: Oxytocin pre-treated then MI group, E: Oxytocin + L-NAME pretreated then MI group. F: Oxytocin+Indomethacin pre-treated then MI group.

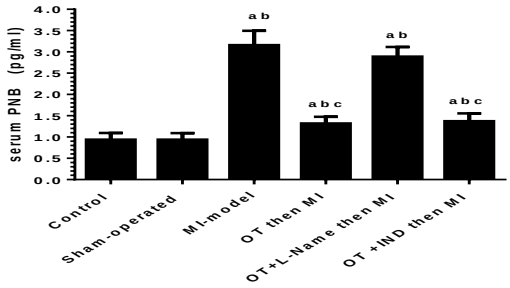

Figure 3: Levels of Brain Natriuretic Peptide (BNP) in the serum of the control and all experimental groups of rats. Values are expressed as Mean ± SD for 6 rats in each group. Values are expressed as Mean ± SD for 12 rats in each group and considered significantly different at $P < 0.05$. [a]Significantly different when compared to control group [b]Significantly different when compared to sham group. [c]Significantly different when compared to

MI group. [d]Significantly different when compared to OT then MI group. [e]significantly different when compared to OT+L-NAME then MI. MI: Myocardial Infarction, OT: Oxytocin, IND: indomethacin.

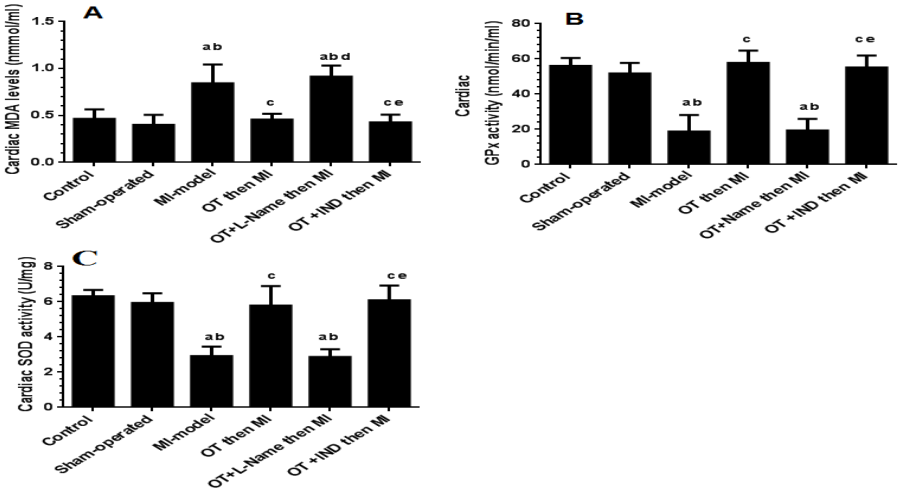

Figure 4: Levels of Thiobarbituric Acid reactive substances (TBARS)(A) and activities of superoxide dismutase (SOD)(C) and glutathione peroxidise (GPx)(B) in the left ventricle homogenates of the control and all experimental groups of rats. Values are expressed as Means ± SD for 6 rats in each group and considered significantly different at $P < 0.05$. [a] Significantly different when compared to control group b Significantly different when compared to sham group. [c]Significantly different when compared to MI group. d Significantly different when compared to OT then MI group. [e] significantly different when compared to OT+L-NAME then MI. MI: Myocardial Infarction, OT: Oxytocin, IND: indomethacin.

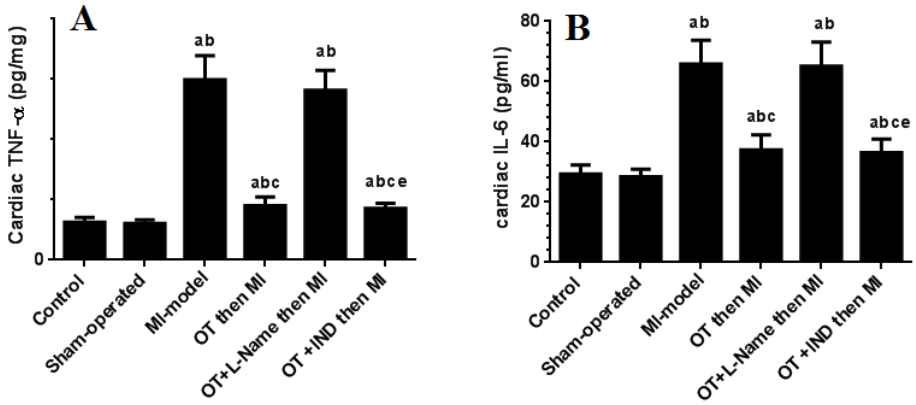

Figure 5: Levels of Tumor Necrosis Factor-α (TNF-α)(A) and Interleukin 6 (IL-6)(B) in the homogenate of the left ventricle of the control and all experimental groups of rats. Values are expressed as Means ± SD for 6 rats in each group and considered significantly different at $P < 0.05$. [a]Significantly different when compared to control group [b] Significantly different when compared to sham group. [c] Significantly different when compared to MI group. [d] Significantly different when compared to OT then MI group. [e] significantly different when compared to OT+L-NAME then MI. MI: Myocardial Infarction, OT: Oxytocin, IND: indomethacin.

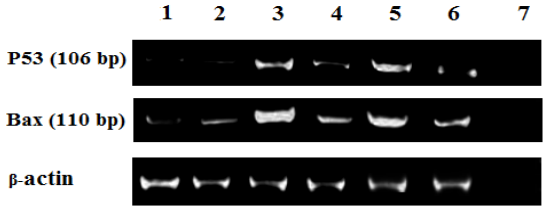

Figure 6: Semiquantitative reverse transcription PCR products and relative expression of LV mRNA of Bax and p53 in reference to β-actin mRNA (housekeeping gene). The RT-PCR products obtained from all groups were separated by 2% agarose gel electrophoresis with 100 ng/ml ethidium bromide. 1: Control group. 2: Sham operated group. 3: MI model group. 4: OT then MI. 5: OT+L-name then MI . 6: OT+IND then MI. 7: Negative

control in which reverse transcriptase was omitted. MI: Myocardial Infarction, OT: Oxytocin, IND: indomethacin.

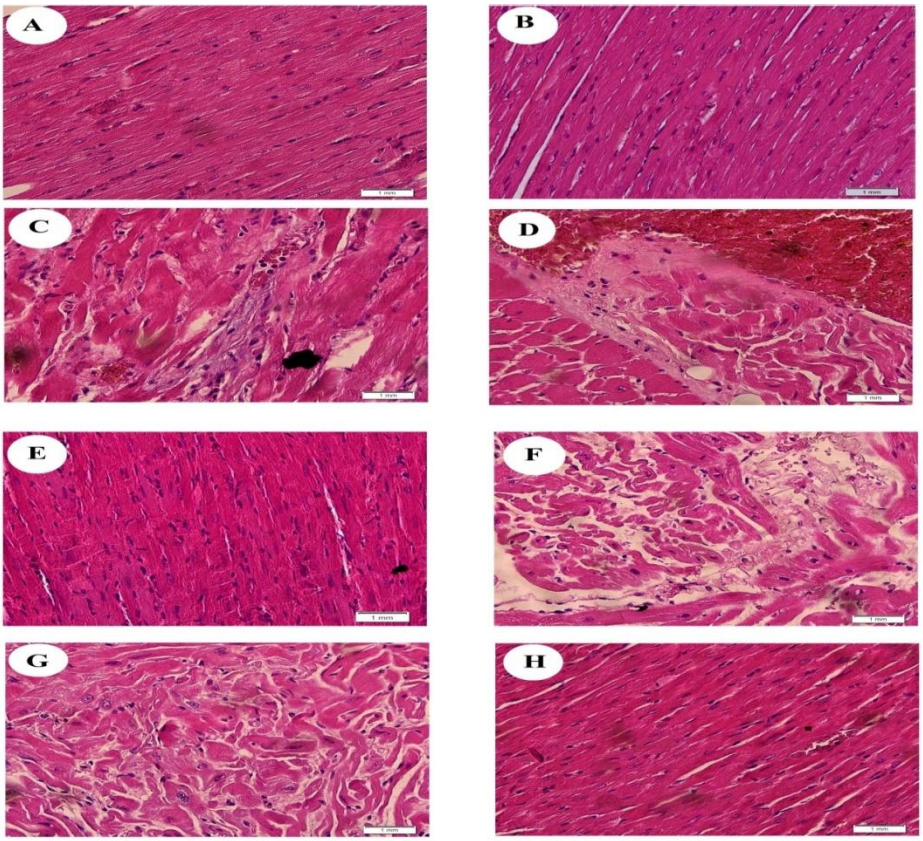

Figure 7: Histology and pathohistology of left ventricle in all experimental groups at 400X final magnification light microscopy. Sections **A and B** were taken from the control and sham operated rats, respectively,. The myocardial tissue appears normal with orderly striated heart muscle fibers and a clear nuclear and muscle bands staining. **C and D** sections from the MI group show areas of coagulative necrosis. The cytoplasm of myocardium is shrinked and deeply eosinophilic with lack of transver band structure. The nucleus shows pyknosis (shrunken and dark satin), karyorrhexis (fragmentation) and karyolysis (dissolution). Areas of hemorrhage, acute inflammatory infiltrate are seen. Few myocardial cells are apoptotic. **E** Section from ischemic area from Oxytocin pre-tread group show degenerative changes with partial preservation of striation of muscle fibers and appearance of intercalated discs. Hemorrhage and inflammatory cell infiltrates were completely absent. Also, no apoptosis was seen and the nuclei retained their normal appearance but remained in some sections disorganized. **F and G** Sections from oxytocin and L-name pre-treated groups showed small area of infarcted myocardium replaced by macrophages and surrounded by large wavy myocardial fibers. **H**Section taken from oxytocin and indomethacin pre-treated ligation induced cardiac ischemia group exhibit normal architectures of cardiac cells, normal fiber striation and clear nuclear and fiber staining. Necrosis, apoptosis and inflammatory cells infiltration are not detected.

VI. Conclusion

The present study demonstrated that pre-administration of OT seven days prior to LAD permanent occlusion-induced MI is profoundly protective to LV myocardium. The evidence supporting this conclusion includes: 1) normalized hemodynamic parameters and ST elevation; 2) reduced BNP, TNF-α, and IL-6 levels; 3) normalized levels of oxidative stress markers; 4) re-instated homeostatic apoptosis via reducing Bax and p53 gene expression, and 5) preserving LV myocardial architecture. Infusion of L-NAME partially inhibited oxytocin from exerting its effects but infusion of indomethacin did not inhibit it. This indicates that OT exerts its action partially by increasing NO synthase activity.

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