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Oxytocin Hormone Administration Attenuates Cardiac Oxidative Injury and Inflammation in Hypercholesterolemic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author HMI designed the study and wrote the protocol. Authors WHN and AHS managed the animals, collected all data, performed the statistical analysis and wrote the first draft of the manuscript. Author AEDRAR did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Oxytocin (OT) hormone has been recently recognized as a cardiovascular hormone with diverse regulatory roles. The present study examined the potential protective effect of exogenous OT administration on hypercholesterolemia-induced injury in rat heart and possible mechanisms involved.
Methodology: Hypercholesterolemia was induced in adult male albino rats fed high cholesterol diet 2% either with or without daily subcutaneous injection of OT (1.6 µg/kg body weight/day) for 8 weeks. Serum parameters included; Serum lipid profile, C-reactive protein, cardiac injury markers including lactate dehydrogenase (LDH) and creatine kinase (CK) levels were measured. Cardiac

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tissue parameters included malondialdehyde (MDA), total anti-oxidant capacity (TAC), nitric oxide (NO) and tumor necrosis factor-α (TNF-α) contents.
Results: Hypercholesterolemic rats showed marked dyslipidemia, significantly high serum levels of C-reactive protein, LDH and CK. Cardiac tissue samples showed enhanced oxidative stress and inflammation in terms of increased MDA and TNF-α alongside decreased TAC and NO contents. OT–treated animals exhibited significantly lower serum C-reactive protein, LDH and CK levels but without significant change in serum lipids. Cardiac tissue samples showed significantly lower MDA and TNF-α alongside significantly higher TAC and NO contents.
Conclusion: These results demonstrate a potent cardioprotective effect of OT hormone against hypercholesterolemia-induced cardiac injury probably via anti-inflammatory and antioxidant mechanisms.

Keywords: Oxytocin; cholesterol; heart; antioxidant; anti-inflammatory.

1. INTRODUCTION

Hypercholesterolemia has been widely accepted as an important risk factor in the development of cardiovascular diseases (CVDs) since it leads to development of hyperlipidemia, atherosclerosis, and ischemic heart disease [1]. Although the focus of research so far has been mainly on the vascular effects of hyperlipidemia, i.e. arteriosclerosis, evidence shows that hyperlipidemia exerts direct negative effects on the myocardium itself in addition to the development of atherosclerosis [2]. Intracellular lipid accumulation in cardiomyocytes and several alterations in the structural and functional properties of the myocardium have been observed in response to cholesterol diet [3]. Studies have shown that hyperlipidemia attenuates the cardioprotective effect of ischemic preconditioning via a mechanism independent from atherosclerosis and other vascular effects of hyperlipidemia [4,5]. Furthermore, it has been shown that a moderate hypercholesterolemia combined with a marked hypertriglyceridemia leads to a moderate contractile dysfunction in isolated rat hearts [1], and marked alterations in the expression of several genes of various functional clusters in the myocardium [3]. These data suggest that hyperlipidemia exerts complex effects on the myocardium.

Hyperlipidemia is often linked to oxidative/nitrosative stress in the vasculature as well as in the myocardium [6]. It has been previously shown an increased formation of peroxynitrite, a toxic reaction product of superoxide and nitric oxide, in the rat myocardium in cholesterol-enriched diet-induced hyperlipidemia [1]. Peroxynitrite has been reported to induce DNA damage, to increase lipid peroxidation, and to cause post-translational modification on proteins (e.g. nitration, oxidation of thiol groups), thereby activating (e.g. poly-ADP-ribose polimerase, matrix metalloproteinases) or inhibiting (e.g. aconitase, superoxide dismutase) certain enzymes [6]. These cellular effects of peroxynitrite may contribute to the development of contractile dysfunction seen in hyperlipidemic rats.

Oxytocin (OT) is a neurohypophyseal peptide traditionally associated with female reproductive functioning, and more recently with prosocial behavior. Recent evidence has also proposed that OT is a cardiovascular hormone that plays an important role in normal homeostatic mechanisms [7,8]. OT hormone is synthesized and released in the heart and vasculature of rats and human, and these tissues also express OT receptors (OTR) [9]. Animal studies investigating the effects of peripheral OT administration in models of inflammatory diseases have provided evidence for the existence of potentially anti-inflammatory and cytoprotective properties involving OT. For example, it was demonstrated that OT protects against sepsis-induced multiple organ damage and inhibits chronic colitis in rats [10]. OT has also been shown to alleviate oxidative renal injury in pyelonephritic rats via a neutrophil-dependent mechanism [11]. Furthermore, *in vitro* studies have shown that OT decreases NADPH-dependent superoxide production and proinflammatory cytokine release from vascular endothelial cells and macrophages [12,13] suggesting that OT may attenuate pathophysiological processes involved with atherosclerotic lesion formation and raising the possibility of a direct cardioprotective effect of oxytocin against hyperlipidemia- induced adverse effects.

Therefore, the present study aimed to investigate the potential protective effect and possible

underlying mechanisms of chronic exogenous OT administration on the cardiac oxidative injury and inflammation in hypercholesterolemic male albino rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Oxytocin and cholesterol were obtained from Sigma Aldrich chemical Co. (Germany). Other chemicals were obtained from EL-Gomhoria Company (Cairo, Egypt).

2.2 Animals

18 adult male albino (Sprague dawley strain) rats weighing (150-200) g and 4 months old were used in the present study. Rats were purchased from the National Research Center, Cairo, Egypt. All animals were housed in stainless steel cages with normal hour's dark: light cycle and allowed ad libitum access to water and fed with a standard pellet chow. Rats were left to acclimatize for one week before inclusion into the experiment. The use of animals Ethical issues were addressed according to the guidelines of the Animal Care and Use Committee of Faculty of Medicine, Minia University.

2.3 Experimental Protocol

Rats were divided randomly into three groups (6 rats each) and were treated for 8 weeks as follows:

i. **Control group:** Rats were fed standard pellet chow and received daily subcutaneous injection of physiological saline (Nacl 0.9%) with the same volume of prepared oxytocin [14].
ii. **High cholesterol Diet (HCD) group:** Rats were fed on a 2% cholesterol diet [1] and received physiological saline.
iii. **HCD and OT-treated group:** Rats were fed on a 2% cholesterol diet and concurrently received daily subcutaneous injection of OT (1.6 µg/kg) [14].

At the end of the experiment, blood samples from retro-orbital vein were collected in glass tubes, left to clot and centrifuged. Sera were obtained and stored at -20℃ until analysis. Then, the rats were sacrificed and the thorax was opened. The heart was rapidly removed, blotted dry, weighed, and kept at -80℃ until analysis.

2.4 Serum Analysis

Serum samples were used for determination of the following parameters;

- Total cholesterol (TC), Triglycerides (TGs), Low density lipoprotein cholesterol (LDL-c) and High density lipoprotein cholesterol (HDL-c) by enzymatic colorimetric methods using commercial kits (Biodiagnostic, Egypt).
- C-reactive protein (CRP) was performed with an ELISA Kit (Helica Biosystems, Inc. Fullerton, CA.) according to manufacturer's instructions.
- Lactate dehydrogenase (LDH) and creatine kinase (CK) were determined by kinetic method as previously described [15,16].

2.5 Cardiac Tissue Analysis

Specimens from the heart were weighed (in gm) and homogenized in potassium phosphate buffer 10 mM pH (7.4). The ratio of tissue weight to homogenization buffer was 1:10. The homogenates were centrifuged at 5000 rpm for 10 min at 4℃. The resultant supernatant was used for determination of the following;

- The total amount of peroxides were assayed by the thiobarbituric acid method described by Ohkawa et al. [17]. This measures the malondialdehyde equivalent substances which are breakdown products of lipid peroxides.
- Determnation of NO: The biodiagnostic nitrite assay kit (Egypt) was used to measure endogenous nitrite; the metabolite of NO as indicator of NO level. It depends on the addition of Griess reagent, which forms with nitrite a deep purple azo compound. The intensity of this colour depends on nitrite concentration and is deteremined by spectrophotometric reading at 540 nm against a sample blank and standard.
- Total Antioxidant Capacity (TAC) level was determined by using Biodiagnostic kit (Egypt) method. The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with

a defined amount of exogenously provide hydrogen peroxide (H2O2). The antioxidants in the sample eliminate a certain amount of provided H2O2. The residual H2O2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5-dichloro-2-hydroxybenzensulphate to a colored product can be measured at 505 nm.

- Measurement of tumor necrosis factor α (TNF-α) was done by using an ELISA Kit (Glory Science Co., Ltd, USA.). The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Rat Tumor necrosis factor α (TNF-α) in samples. Add Tumor necrosis factor α (TNF-α) to pre-coated Tumor necrosis factor α (TNF-α) monoclonal antibody microelisa well, incubation; washing. Add HRP tagged TNF-α antibodies. After another incubation and washing, remove the unbound enzyme, add Chromogen Solution A and B, the color of the liquid change into blue, and the color finally become yellow at the effect of acid. The depth of the color is positively correlated with concentration of the Rat.

2.6 Statistical Analysis

Data were represented as means ± standard errors of the mean (SEM). Statistical analysis was performed using Prism computer program (Graph pad Prism 6, software Inc., San Diego, CA, USA). Significant difference between groups was done by one-way ANOVA followed by Tukey-Kramar post hoc test for multiple comparisons with a value of $P \leq 0.05$ considered statistically significant.

3. RESULTS

3.1 Changes in Serum Lipid Profile in Experimental Groups

As shown in Table 1, HCD caused marked alterations in serum lipid profile in terms of significant increases in serum TC (138.17%), TGs (136.08%) and LDL-c (210.75%) along with significant reduction in serum HDL-c (-33.01%) levels in comparison to the control normal fed group. OT treated group did not show any significant difference in serum lipid profile from that of the corresponding non-treated group although it was still significantly different from that of control normal fed group (Table 1).

3.2 Changes in Serum Cardiac Injury Markers (LDH & CK) and CRP Levels

Cholesterol enriched diet induced cardiac injury, as judged by the significantly elevated cardiac injury markers LDH (128.64%) and CK (98.96%), ($p \leq 0.05$). A significant rise in serum level of CRP (208.02%), as a marker of systemic inflammation, was also observed ($p \leq 0.05$). Treatment with OT reversed this increase in serum LDH (54.93%) and CK (-45.27%) levels, ($p \leq 0.05$) and it also successfully attenuated the HCD-induced increase in serum CRP level (-61.83%), ($p \leq 0.05$) and brought it back nearly to the control level (Table 2).

3.3 Changes in Cardiac TNF-α Content

In Fig. 1, the hearts of hypercholesterolemic rats showed a significant increase in their content of TNF-α (118.28%) in comparison to the control normal fed group. On the other hand, concurrent OT administration prevented this increase (-43.05%), ($p \leq 0.05$) and kept its level near to control level (Fig. 1).

Table 1. Effect of high cholesterol diet (HCD) on serum lipid profile and its modulation by oxytocin treatment

	Control	HCD	Oxy-treated
TC (mmol/L)	2.41 ± 0.07	5.74 ± 0.14*	5.42 ± 0.11
TGs (mmol/L)	1.94 ± 0.12	4.58 ± 0.14*	4.26 ± 0.15
HDL-c (mmol/L)	1.03 ± 0.07	0.69 ± 0.05*	0.63 ± 0.03
LDL-c (mmol/L)	0.93 ± 0.04	2.89 ± 0.14*	2.49 ± 0.15

*Data are expressed as M±SEM of 6 rats in each group; * significant from control group, P≤0.05, HCD: high cholesterol diet; Oxy: oxytocin; TC: total cholesterol; TGs: triglycerides; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol*

Table 2. Effect of high cholesterol diet on serum cardiac injury markers (LDH & CK) and CRP levels and their modulation by oxytocin treatment

	Control	HCD	Oxy-treated
LDH (U/L)	413.8 ± 5.75	946.1 ± 14.89*	426.4 ± 6.16●
CK (U/L)	12.48 ± 0.97	24.83 ± 1.79*	13.59 ± 0.73●
CRP (mg/L)	2.62 ± 0.22	8.07 ± 0.43*	3.08 ± 0.23●

*Data are expressed as M±SEM of 6 rats in each group; *: significant from control group; ●: significant from HCD group, P≤0.05; HCD: high cholesterol diet; Oxy: oxytocin; LDH: lactate dehydrogenase; CK: creatine kinase; CRP: C-reactive protein*

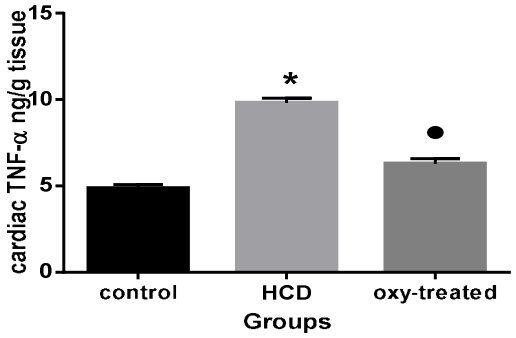

Fig. 1. Effect of HCD on cardiac TNF-α content and its modulation by oxytocin treatment

*Data are expressed as M±SEM of 6 rats in each group; *: significant from control group; ●: significant from HCD group, P≤0.05; HCD: high cholesterol diet; Oxy: oxytocin; TNF-α: tumor necrosis factor alpha*

3.4 Changes in Cardiac Oxidative Status and No Content

Figs. 2-4 illustrate the effects of HCD on lipid peroxides, TAC, and NO contents and their modulation by OT treatment. Feeding rats cholesterol 2% for 8 weeks resulted in significant increase in lipid peroxidation products (MDA) (95.86%) along with significant reductions in both TAC (-54.79%) and NO (-58.08%) contents as compared to the control normal fed group, (p≤0.05). Treatment with OT reversed the condition and successfully reduced the HCD-induced elevation in MDA (-43.18%) and restored TAC (94.92%) as well as NO (118.48%) contents almost nearly to the control level, (p≤0.05) (Figs. 2-4).

4. DISCUSSION

Apart from its well known vascular effects, hypercholesterolemia proved to have direct negative effects on the cardiac muscle. In the present study, signs of inflammation and oxidative stress were evident in the hearts of hypercholesterolemic rats. These adverse effects were significantly attenuated by concurrent OT administration. Thus, OT hormone seems to have a potent cardioprotective effect against hypercholesterolemia-induced adverse effects. Suggested mechanisms may involve increased NO production, suppression of inflammation, inhibition of lipid peroxidation along with enhanced antioxidant capacity thereby increasing tissue resistance to injury.

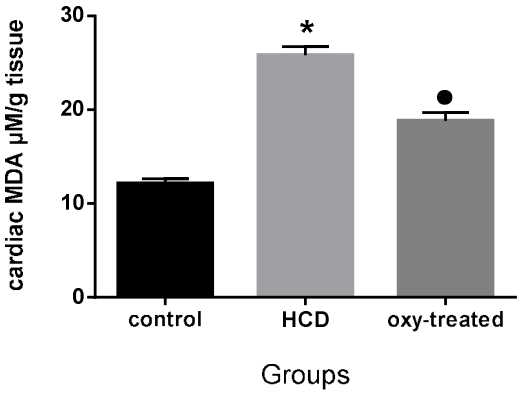

Fig. 2. Effect of HCD on cardiac MDA content and its modulation by oxytocin treatment

*Data are expressed as M±SEM of 6 rats in each group; *: significant from control group; ●: significant from HCD group, P≤0.05; HCD: high cholesterol diet; Oxy: oxytocin; MDA: malondialdehyde*

In the present study, High cholesterol diet (HCD) for 8 weeks produced a state of systemic inflammation as evidenced by significant elevation in serum CRP level, accompanied with disturbed lipid profile. Serum LDH and CK levels were significantly elevated indicating cardiac injury, along with signs of inflammation and oxidative stress as evidenced by significant rise in the pro-inflammatory cytokine; TNF-α and lipid peroxidation products; MDA, and significant reduction in both TAC and NO contents in cardiac tissue of HCD group. These results are consistent with previous reports showing the detrimental effects of hypercholesterolemia on

cardiac tissue causing cardiac dysfunction [18,19].

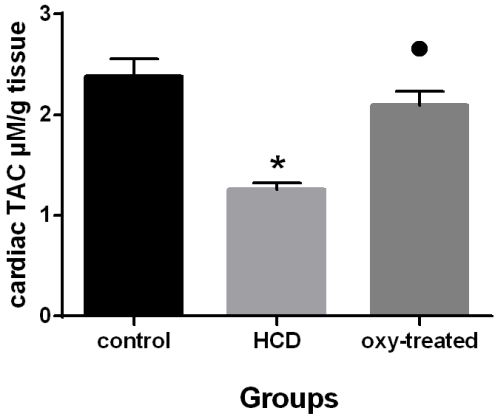

Fig. 3. Effect of HCD on cardiac TAC and its modulation by oxytocin treatment

*Data are expressed as M±SEM of 6 rats in each group; *: significant from control group; ●: significant from HCD group, P≤0.05; HCD: high cholesterol diet; Oxy: oxytocin; TAC: total antioxidant capacity*

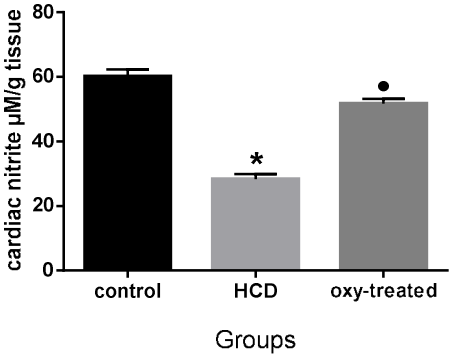

Fig. 4. Effect of HCD on cardiac NO content and its modulation by oxytocin treatment

*Data are expressed as M±SEM of 6 rats in each group; *: significant from control group; ●: significant from HCD group, P≤0.05; HCD: high cholesterol diet; Oxy: oxytocin; NO: nitric oxide*

Inflammation has a pivotal role in the development of CVD [20]. Measurement of the inflammatory marker CRP may provide a useful method of assessing risk of CVD in apparently healthy persons particularly when lipid levels are normal [21]. This was confirmed in the present study by the significantly elevated serum CRP level as well as cardiac TNF-α content in HCD rats as compared with normal fed rats indicating both systemic and cardiac inflammation.

Gurgan [22] said that LDH and CK served as markers of myocardial tissue damage as they leak out from the damaged tissue to the blood stream when the cell membrane become permeable or rupture. Significant increase in LDH and CK in HCD thought to be due to the atherogenic lipoprotein subclasses commonly associated with hyperlipidemia [23]. In the present study, feeding of rats HCD for 8 weeks produced significant rise in serum LDH and CK indicating cardiac injury. Concurrent OT treatment significantly attenuated HFD-induced cardiac injury and almost normalized serum LDH and CK levels suggesting a potent cardioprotective effect of OT in hyperlipidemic conditions; findings which are in accordance with other similar researches [13,24].

One possible mechanism of OT-induced cardioprotection against hypercholesterolemia adverse effects could be attributed to its antiinflammatory effect; the results of our study revealed that OT treatment to HCD rats decreased significantly serum levels of CRP as well as cardiac TNF-α content indicating suppression of inflammatory response. Similar results were reported by Szeto et al. [12], Jankowski et al. [24]; and Nation et al. [25]. They found that chronic OT infusion in Apo-E knockout mice attenuated aortic atherosclerosis and plasma CRP and inhibited the secretion of the proinflammatory cytokine IL-6 in visceral adipose tissue. Furthermore, Al-Amran and Shahkolahi [26] have demonstrated that OT ameliorates myocardial injury in heart transplant through down-regulation the myocardial inflammatory response, reactive oxygen species, and neutrophil-dependent myocardial apoptosis. These former studies agree with our results and suggest a potent anti-inflammatory effect of OT hormone.

Lipid peroxidation products; (MDA) serves as a marker of cellular oxidative stress and had long been recognized as a major causative factor of oxidative damage in chronic diseases[27]. In the present study, cardiac tissue of HCD rats showed signs of oxidative stress as evidenced by the significant elevation in oxidative markers, namely MDA along with depletion of cardiac TAC and NO contents. Hyperlipidemia has been shown to increase production of reactive oxygen species (ROS) including peroxynitrite ($ONOO^-$) [1]. It seems that hypercholesterolemia induces NADPH oxidase and increases cardiac superoxide. In turn, superoxide radical reacts with nitric oxide leading

to formation of the more toxic radical, peroxynitrite, resulting in oxidative stress and cardiac dysfunction [28].

The direct link between serum cholesterol and the activation of NADPH oxidase is not entirely clear in the myocardium. One possible mechanism may involve the activation of the phagocyte NADPH oxidase [29]. It has been suggested that cholesterol-nriched microdomains in the membrane act to recruit and/or organize the cytosolic NADPH oxidase factors in the assembly of the active NADPH oxidase [18].

Our results revealed that OT administration to HCD group prevented lipid peroxidation, as evidenced by the significant reduction in MDA levels, and augmented TAC in cardiac tissue suggesting a potent antioxidant effect of OT on the heart during hyperlipidemic conditions. Similar results were reported by Anvari et al. [30] who found that administration of OT during early reperfusion, dose-dependently protects the isolated male rat heart against ischemia/reperfusion injury. Also Akdemir et al. [31] found that OT administration to rats after ischemia and ischemia-reperfusion of the ovaries by using torsion /de-torsion technique significantly decreased the tissue malondialdehyde (MDA) levels in both the torsion and OT group, and torsion/de-torsion OT group in comparison with the torsion-only group and torsion/de-torsion group.

Previous studies have suggested that OT has a lipid peroxidation chain breaking antioxidant effect [10,32]. Furthermore, it has been shown that OT exerts antioxidant effects on vascular smooth muscle cells, aortic endothelial cells and macrophages through attenuation of NADPH-oxidase-dependent superoxide production and preserved antioxidant capacity thereby supports the maintenance of cellular integrity [25].

Hypercholesterolemia is always linked to impaired NO-cGMP signaling in both endothelial and non-endothelial cells [1]. In the normal heart, nitric oxide (NO) is synthesized by Ca2+-dependent NO synthases in cardiac myocytes, vascular and endocardial endothelium (NO synthase III) as well as in specific cardiac neurons (NO synthase I) and plays an important role in the regulation of coronary circulation and cardiac contractile function [33].

In the present study, cardiac NO level was significantly decreased in the hearts of hypercholesterolemiac rats. Similar results were reported in previous researches [34,35]. The exact mechanism of reduced NO level in the heart is not clear. However, Deliconstantinos et al. [36] reported that incorporation of high concentrations of cholesterol into endothelial cell membranes caused down regulation of NO synthase (NOS). In addition, reduced vascular NO release in hyperlipidemia could be a consequence of increased formation of superoxide, which then reacts with NO to form peroxynitrite (ONOO−) [1,35]. Thus, it could be stated that the HCD-induced decrease in cardiac NO, observed in the present study, may be either due to an enhanced elimination of NO and/or diminished enzymatic synthesis by NOS.

Another possible mechanism of OT-induced cardioprotection during hypercholesterolemic conditions may be attributed to an increased NO production, as evidenced by the significant elevation in cardiac NO content in OT-treated rats along with attenuated oxidative stress and inflammatory status in the heart. NO inhibits the adhesion and aggravation of neutrophil leukocytes. In turn, this inhibitory action on tissue neutrophil infiltration thereby inhibits the release of ROS and inactivating inflammatory cytokines and thus alleviating tissue injury [37]. This OT-induced increase in NO level may be either secondary to reduced lipid peroxidation (as evidenced by the significant reduction in cardiac MDA levels), and subsequent preservation of NO and/or increased expression of NOS [38].

Unfortunately, OT treatment to HCD group failed to produce any significant changes in serum lipid profile. These results are in accordance with Szeto et al. [13] who also found no changes in serum lipids in OT-treated hyperlipidemic rabbits. On this basis, we could say that the cardioprotective effect of external OT administration in hypercholesterolemic rats is unlikely related to changes in serum lipids (at least in the present study).

5. CONCLUSION

In conclusion, Hypercholesterolemia has detrimental effects on the heart including oxidative injury and inflammation. Chronic OT administration can protect the heart against these adverse effects probably via anti-inflammatory and antioxidant mechanisms as well as increased NO production. Thus, OT

hormone may have potential for therapeutic use in hyperlipidemia-induced cardiac dysfunction. Further studies must be done to determine the therapeutic dose of oxytocin.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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