

Research Article

Oxytocin Induced Protection Against Non-Alcoholic Fatty Liver Disease in Rats

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Abstract:

Background and aim: Non-alcoholic fatty liver disease (NAFLD) is a worldwide medical problem affecting about 30% percent of obese and 10% of normal persons. Based on the suggested metabolic effects of oxytocin, the present study was designed to determine the effect of treatment with oxytocin on NAFLD induced by high fat diet. **Methods:** Male Wistar rats were divided into three groups: 1- Control group: fed standard pellet chow diet and injected with the vehicle. 2- High fat diet group (HFD): fed a high fat diet containing cholesterol 5% for 8 weeks. 3- High fat diet plus oxytocin treated group: fed a high fat diet and were subcutaneously injected with oxytocin (1.6 µg/kg bodyweight/day) for 8 weeks. Liver tissues and serum were collected and evaluated by histopathological and biochemical assay for fatty changes and biochemical parameters (liver transaminases, alkaline phosphatase, triglycerides, lipid peroxides, total nitrite and total anti-oxidant capacity). **Results:** Oxytocin treatment decreased the fatty changes in the liver, decreased the elevated liver transaminases, alkaline phosphatase, triglyceride level, lipid peroxides, and total nitrite and increased the total antioxidant capacity **Conclusions:** The results of the present study demonstrated that treatment with oxytocin induced protection against NAFLD most probably via its anti-inflammatory, antioxidant effect and its effect on fat metabolism. These findings are likely to motivate further research and indicate new approaches for treating NAFLD via modulating adipose tissue morphology and metabolism.

Key words: Oxytocin, NAFLD, Obesity, Nitric oxide and Antioxidant

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent causes of abnormal liver dysfunction in clinic. Along with the change of life style and dietary pattern, its prevalence has markedly increased. It represents a spectrum of conditions that are histologically characterized by macrovesicular hepatic steatosis^(1,2). NAFLD affects individuals who have not consumed alcohol in amounts considered harmful to the liver. The spectrum of NAFLD is broad, extending from simple steatosis through nonalcoholic steatohepatitis (NASH) to cirrhosis and liver failure^(3,4). Among them, the term non-alcoholic steatohepatitis has recently been proposed to identify a fatty liver disease that is accompanied with diffuse fatty infiltration and inflammation⁽⁵⁾. Although the pathologic

findings in NASH and alcoholic liver disease are similar, the exact mechanisms that

cause NASH are still unclear. Recently, a 2-hit hypothesis was proposed to explain the development of NASH. Steatosis is the first hit followed by a second hit that includes inflammation, oxidative damage, and fibrosis⁽⁶⁾. Many factors, such as cytokines, endotoxin, oxidative stress, and insulin resistance, have been proposed as the causes for the second hits of NASH⁽⁷⁾. Several therapies, including diet⁽⁸⁾ and anti-oxidants, have been tried to treat patients with NASH⁽⁹⁾, however, no therapeutic strategy has been established for NASH as yet.

Oxytocin (oxy) is a non-peptide commonly known for its role in childbirth, breast feeding, pair-bonding, social behaviors, feeding, drinking, and the stress response^(10,11). This peptide is produced both peripherally at the

uterus, ovaries, corpus luteum, prostate gland, testis, kidneys, adrenal gland and heart, and in the central nervous system in neurons of the paraventricular and supraoptic nuclei of the hypo-thalamus⁽⁶⁾. The current use of oxytocin as a therapeutic agent is limited to obstetric practice corresponding to its main physiological effects, namely the induction of labor and milk ejection. However, there is accumulating evidence indicating a significant role for oxytocin outside of pregnancy, and these actions of oxytocin may, if adequately understood, become targets for potential therapeutic strategies⁽¹¹⁾.

Oxytocin reported to exerts insulin-like effects in isolated adipocytes, for example, stimulation of glucose oxidation, lipogenesis and pyruvate dehydrogenase activity^(12,13), although oxytocin has also been found to have an antiinsulin action⁽¹⁴⁾. In addition, oxytocin has been shown to stimulate glucose uptake in rat skeletal muscles and neonatal cardiomyocytes^(15,16). Interestingly, concentrations of oxytocin in plasma were found to be significantly higher in obese men and women compared with control subjects⁽¹⁷⁾. Further, mice deficient in oxytocin receptors have been found to develop obesity⁽¹⁸⁾.

The very recent publications have also reported that intraperitoneal (ip) injection of Oxy suppresses food intake^(19,20). These reports taken together suggest that Oxy is a catabolic, as well as anorectic peptide giving the base for our study which was designed to investigate the effect of oxytocin on fatty liver disease which is a worldwide medical problem nowadays.

Materials and Methods

Chemicals:

Oxytocin and cholesterol were obtained from sigma chemical (St Louis, MO, USA); Triglyceride, Malondialdehyde, ALT, AST and ALP kits were obtained from Biodiagnostic (Cairo, Egypt). Other chemicals were obtained from EL-Gomhoria Company (Cairo, Egypt).

Animals:

Adult male Wister rats weighing 140 – 160 g were purchased from the National Center of Research, El-Giza, Egypt. Rats were housed in a light and temperature controlled room on a 12-hr light/dark 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. Experimental procedures and care of animals were carried out according to the guidelines of the Animal Care and Use Committee of Faculty of Medicine, Minia University.

Experimental protocol

After acclimatization for one week, rats were divided randomly into three groups (4 rats each) and treated for 4 weeks as follow:

1. Control group: rats were fed standard pellet chow and received physiological saline injection.
2. High fat diet group (HFD): rats were fed on a 2% cholesterol diet to induce fatty liver⁽²¹⁾ and received physiological saline injection
3. Oxytocin treated group (Oxy-treated): rats were fed on a 2% cholesterol diet and injected daily with oxytocin (1.6 µg/kg subcutaneously)⁽²²⁾.

At the end of the experimental protocol, rats were anesthetized by inhalation of ether and blood samples were collected from abdominal aorta and processed for biochemical measurements. Then, rats were sacrificed and their livers were rapidly collected, blotted dry, weighed and divided into 2 parts. One part was put in 10% formalin for histopathological examination and the second part was kept at -80°C and used for biochemical measurements.

Measurement of liver index:

Liver index was calculated from the equation: (liver weight/body weight) in g × 100 (Xu *et al.*, 2007).

Biochemical measurements:

Determination of serum liver transaminases

Serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by using commercially available kit (Biodiagnostic, Egypt)

Determination of serum alkaline phosphatase

Serum level of alkaline phosphatase was measured colorimetrically using commercially available kit (Biodiagnostic, Egypt) following the manufacturer protocol procedures.

Determination of serum total antioxidant capacity

Serum total antioxidant capacity was measured using colorimetric method using commercially available kit (Biodiagnostic, Egypt) according to the manufacturer protocol procedures.

Determination of liver tissue lipid peroxides.

Hepatic tissues were homogenized in ice-cold phosphate buffered saline (pH 7.4), centrifuged at 4000 r.p.m for 10 min, the supernatant was removed and stored on ice. Malonaldehyde level (MDA) was measured colorimetrically by using commercially available kit (Biodiagnostic, Egypt) according to the manufacturer protocol.

Determination of liver triglycerides content

Quantitative determination of liver tissue homogenate content of triglycerides was done by enzymatic colorimetric method using available kit (Diamond DP international, Hannover, Germany) according to the manufacturer protocol.

Determination of liver tissue total nitrite level

Colorimetric determination of nitrite in hepatic tissue samples was done from liver tissue homogenate using commercially available kit (Biodiagnostic, Egypt) according to the manufacturer protocol.

Histopathological examination

For the histopathological study, rat liver specimens were taken 2 mm away from the edge of the largest hepatic lobe, fixed with 10% formaldehyde; embedded in paraffin wax, stained with hematoxylin and eosin (H&E) and then observed under the light microscope for histo-pathological changes.

Statistical analysis of the data

Data are presented as means \pm SEM. The results were analyzed by one way analysis of variant (ANOVA) followed by student t-test with $P \leq 0.05$ selected as the criterion for statistical significance using Software GraphPad Prism Version 5 (GraphPad Software Inc, La Jolla, CA, USA).

Results

Effect of oxytocin treatment on liver index:

Liver index was significantly increased in cholesterol-fed group compared with normal control group. In oxytocin treated group, liver

index was significantly decreased compared with cholesterol-fed group (figure 1).

Effect of oxytocin on serum liver transeaminases

Cholesterol fed diet increased level of serum ALT and AST significantly compared to control group. Treatment with oxytocin significantly reduced the elevated liver transeaminases nearly to control level (table 1).

Effect of oxytocin on serum alkaline phosphatase level

Table (1) shows effect of oxytocin treatment on serum alkaline phosphatase, Cholesterol fed diet increased significantly serum alkaline phosphatase level comparable to control group. Oxytocin treatment significantly reduced the elevated alkaline phosphatase level compared to cholesterol fed group.

Effect of oxytocin on total anti-oxidant capacity

Cholesterol feeding decreased significantly total antioxidant capacity compared to control group while oxytocin treatment significantly elevated it to the near control value (table 1).

Effect of oxytocin on hepatic triglycerides content

Table (2) shows the effect of oxytocin treatment on hepatic triglyceride level, Cholesterol-fed diet increased significantly triglycerides level comparable to control group, oxytocin treatment significantly reduced hepatic triglycerides levels compared to cholesterol fed diet group.

Effect of oxytocin on hepatic lipid peroxides and nitrite content

Table (2) illustrates the effect of cholesterol feeding on lipid peroxides and nitrite content and the effect of oxytocin treatment on these parameters. Cholesterol feeding increased lipid peroxidation products (MDA) and total nitrite level significantly compared to control group. Treatment with oxytocin significantly reduced the elevated lipid peroxides and total nitrite compared to cholesterol fed group.

Effect of oxytocin on histopathological changes

Figure 2 shows microscopic photography of hepatic sections A; Control group that receive standard diet where hepatocytes are arranged in

trabecules running radiantly from central vein and are separated by sinusoids (S). B; Hepatic sections showing hepatocyte hypertrophy, ballooning (black arrows) and sever cytoplasmic vacuolations (red arrows), degeneration of some hepatocytes (yellow arrows) with darkening of

their nuclei (pyknosis). C; Oxytocin regained normal hepatocyte structure with only mild hepatocyte vacuolation (black arrow head). All figures are stained by haematoxylin and eosin (x400).

Table (1): Effect of high fat diet on total antioxidant capacity, liver transaminases, alkaline phosphatase and its modulation by oxytocin treatment

	Control	HFD	Oxy-treated
TAO	1.17 ± 0.11	0.78 ± 0.08*	1.47 ± 0.07#
ALT (U/L)	23.43 ± 2.9	51.07 ± 7.77*	20.76 ± 4.40#
AST (U/L)	140.43 ± 0.97	172.40 ± 0.47*	120.77 ± 7.34#
ALP (U/L)	77.96 ± 8.08	100.30 ± 10.32*	77.78 ± 7.94#

Values presented as mean ± SEM

* P < 0.05 vs. control group

P < 0.05 vs. HFD group

TAO: Total anti-oxidant capacity, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase

Table (2): Effect of high fat diet on hepatic content of triglycerides, malonedialdehyde, total nitrite and its modulation by oxytocin treatment

	Control	HFD	Oxy-treated
TGs (mg/g tissue)	13.8 ± 2.11	20.86 ± 3.2*	10.07 ± 1.07#
MDA (µg/g tissue)	2.12 ± 0.1	3.27 ± 0.34*	2.3 ± 0.27#
NO (µM/g tissue)	74.43 ± 8.7	101.06 ± 3.80*	50.06 ± 7.09#

Values presented as mean ± SEM,

* P < 0.05 vs. control group

P < 0.05 vs. HFD group

TGs: Triglyceride, MDA: Malonedialdehyde, NO: total nitrite

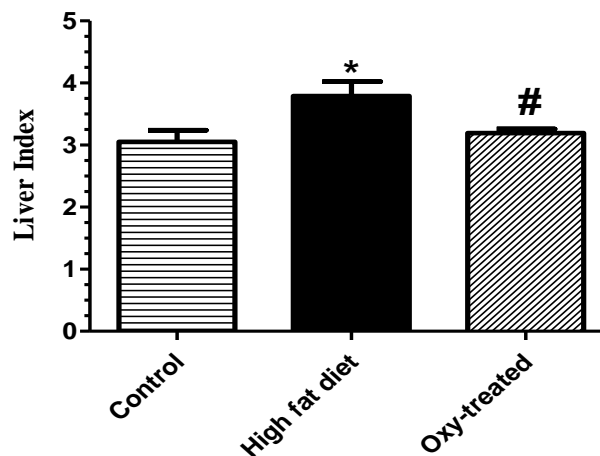


Fig. (1): Effect of high fat diet on liver index and its modulation by oxytocin treatment

Values presented as mean \pm SEM,* P < 0.05 vs. control group; # P < 0.05 vs. HFD group

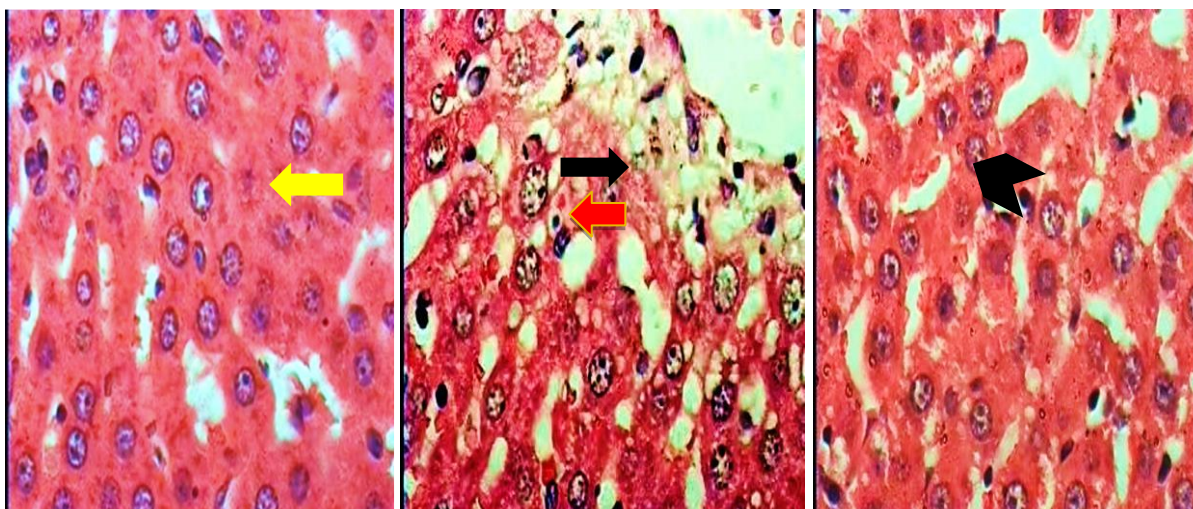


Figure (5): Photomicrographs of hepatic sections showing effect of HFD on hepatic histology and its modulation by Oxy treatment. A: Control group; B: HFD group and C: Oxy-treated group.

Discussion

NAFLD is a disease characterized by excessive accumulation of triglycerides in hepatocytes, followed by lipid peroxidation and release of inflammatory mediators which are potential players in the pathogenesis of liver injury^(24,25).

In the present study we investigated the potential effect of oxytocin treatment on the pathogenesis of NAFLD. The results of the study demonstrated that, 2% cholesterol diet successfully induced steatohepatitis after 8 weeks as evidenced physically by significant increase in liver weigh/body weight ratio (liver index) and confirmed histopathologically by increased deposition of fat droplets in hepatocytes with inflammatory cell infiltration. Administration of oxytocin significantly attenuated both physical and histopathological evidences of steatohepatitis. To explore the possible mechanism involved in the protection by oxytocin in NAFLD we measured some biochemical parameters such as serum liver transaminases (ALT-AST), alkaline phosphatase (ALP) and total antioxidant capacity (TAO), liver tissue content of triglycerides (TGs), lipid peroxidation products (MDA), and total nitrite (NO).

AST, ALT and ALP enzymes are frequently used to evaluate the status of liver damage and considered more sensitive parameters to

measure liver injury in rodent species^(24,25,26). When the hepatocyte is injured, plasma membrane is disrupted and the leakage through extracellular fluid of the enzyme occurs where they can be detected at abnormal levels in the serum. Our results showed that high cholesterol diet elevated serum ALT, AST and ALP levels. Similar finding has been reported by other⁽²⁷⁾. Rats receiving oxytocin showed almost normalization of serum ALT, AST and ALP levels suggesting that oxytocin may have anti-inflammatory effect.

A similar anti-inflammatory effect for oxytocin was reported in various inflammatory conditions^(28,29,30).

Fatty liver impairs glucose and lipid metabolism, thereby promoting type 2 diabetes mellitus, metabolic syndrome and cardiovascular disease, and also increases the risk of cirrhosis and hepatic cancer⁽³¹⁾. Inhibition of accumulation of fat in liver contributes to prevention of these diseases. In our study high cholesterol diet led to a significant increase in triglycerides in liver tissues compared with normal control group. It has been reported that the increase in hepatic triglycerides was due to failure of the liver to synthesize apolipoprotein required for packaging and exporting fat from the liver thus accumulates triglycerides in the liver⁽³²⁾. In oxytocin-treated rats, there was a

significant decrease in liver triglycerides. Regarding the mechanisms through which peripherally administered Oxy acts on the liver, it has been reported that reduction of hepatic triglyceride biosynthesis and redistribution of lipids from ectopic sites to adipose tissue are suggested mechanism for oxytocin lowering effect of TG level. This leads to positive metabolic changes, such as increased peripheral insulin sensitivity⁽¹⁷⁾. Also Oxy directly affects the glycogen synthesis in hepatocytes⁽¹⁸⁾ and that Oxy evokes central regulation of hepatic cholesterol metabolism⁽¹⁹⁾, suggesting possible involvement of direct effect of Oxy and/or indirect action of Oxy mediated by the central nervous system. The fatty liver-correcting effect provides peripherally administrated Oxy with an advantage as a potential agent to treat obesity and metabolic syndrome, although the underlying mechanisms remain to be clarified.

In our study, oxytocin decreased the body weight by 0.9% and 19.4% compared with normal control and cholesterol-lfed groups, respectively (data not shown). The multiple effects of oxytocin that include insulin sensitization, antidyslipidemia and decreased body weight explains, at least partially, why oxytocin decreased liver tissue content of TG near the values of normal control group.

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⁽²⁰⁾. Our results showed that oxytocin significantly reduced MDA in liver tissues. Previous studies suggested that oxytocin has a lipid peroxidation chain breaking anti-oxidant effect^(21,22,23).

NO is a multifunctional molecule and its role in liver injury is an issue of debate⁽²⁴⁾. In the present study, induction of NAFLD in rats was accompanied with significant elevation of NO content in liver tissues. Emerging evidence shows that NO regulates fatty acids metabolism in mammals. At physiological levels, NO enhances lipolysis in and inhibits synthesis of fat in target tissues (e.g., liver and adipose tissue). However, at pathological levels, NO inhibits nearly all enzyme-catalyzed reactions through protein oxidation⁽²⁵⁾. Oxytocin treatment significantly increased the total antioxidant capacity together with reduction of the

pathologically elevated NO content in liver tissue suggesting that oxytocin might have antioxidant effect^(26,27).

Conclusion

The results of the study demonstrate that peripheral Oxy treatment reduces body weight, ameliorates fatty liver and improves antioxidant status. Peripheral Oxy treatment provides a new therapeutic avenue for treating fatty liver and obesity.

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