LONG TERM EXPOSURE OF SYNTHETIC OXYTOCIN ON HAEMATOLOGICAL PARAMETERS OF FEMALE ALBINO MICE

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**ABSTRACT**

Oxytocin (OT) possesses vasodilator and anti-inflammatory properties but these are controversial till now. To the best of the knowledge, the relevant data regarding to the haematology were scared. The research study was tried to investigate the chronic effects of low dose OT on haematological parameters by using a rodent model i.e. albino mice. Thirty six immature female albino mice, Mus musculus (weight 8±2g and age 10days) were divided into 2 groups as control (n=18) and treated (n=18). The treated and control groups were received intraperitonial (i.p.) dose of synthetic oxytocin (50mIU/5µl/g body weight) and physiological saline (5µl/g body weight) respectively for different intervals i.e. 30, 60 and 90 days. And hematological parameters including total count white blood cells (TC-WBC), total count red blood cells (TC-RBC) and haemoglobin% (Hb%) in blood as well as total bilirubin in serum were measured. The major observations of the research study were that, OT did not show much significant variations (P>0.05) in TC-WBC, TC-RBC, Hb% and total bilirubin as compared to control group. Finally the research study was concluded that, synthetic OT did not alter haematological parameters as well as total bilirubin in female albino mice at selected dose and duration apart from this a care must be taken on usage of higher dose.

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INTRODUCTION

Almost all synthetic oxytocin (OT) are same as nonapeptide endogenous OT, only pharmacology is different in which synthetic oxytocin Pitocin is one of them. OT is effective after administration by any parenteral route such as i.p., s.c., i.m., i.v., intranasal and orally. The cells of gastrointestinal tract are equipped with OT receptors which may facilitate the absorption of orally administered OT [1]. Receptors for OT belong to a G-protein coupled receptor of class I group and present in the cells of different organs such as blood capillary, kidney, brain, gastro-intestinal tract, lung etc. Moreover, cross reactivity of OT also noted with vasopressin receptors viz., V1 subtype (V1a and V1b) and V2 type [2]. OT and vasopressin both peptides are structurally very similar, each having nine amino acids and a disulphide bond, and differing by only two amino acids (in positions 3 and 8) [3].

Exogenous OT decreases renal, coronary and cerebral blood pressure due to these endothelial OT receptors produce a calcium-dependent vasodilatory response via stimulation of the nitric oxide pathway [4]. This can be achieved binding of OT with vasopressin V1a receptor or OT receptor which is present on vascular endothelial cells of blood vessel [5,6]. OT receptors have already been detected in human vascular endothelial cells [5] and in the rat great vessels [7]. Exogenous OT increases different serum or plasma biochemicals such as total protein, glucose, cholesterol and c-reactive proteins; liver enzymes viz., aspartate transaminase (AST) and alanine transaminase (ALT); as well as metabolic hormones including triiodothyronine (T3) and thyroxin (T4) in lactating buffaloes [8]; creatinine and urea in rat [6]. Several studies also proven that, synthetic OT cause water intoxication, increase kaliuresis, natriuresis, urine osmality and decrease urine output [6,9]. Different reasons behind to the water intoxication; firstly, OT is potent vasodilator and decrease angiotensin II stimulated vasoconstriction via increasing the prostaglandin level [10].

Exogenous OT was showed anti-inflammatory responses and protective effect against ischemia-reperfusion injury in different organs such as liver, kidney etc., [11-15]. Decreased inflammations by OT was mediated through an increase in corticosterone levels; decrease the release of interleukin-6; inhibits platelet aggregation via increasing prostacyclin release; inhibits adhesion and aggregation of neutrophils; and decrease myeloperoxidase activity via increasing nitric oxide [16]. OT may also decrease the release of interleukin-6, and influence the coagulation and the fibrinolytic system. OT may be involved immune and inflammatory processes because OT and OT receptors are located in the thymus [16]. The anti-inflammatory effect of OT become controversial due to several previous research studies show that OT mediated inflammatory responses via attenuate monocytes or macrophages inflammatory cytokine production following lipopolysaccharide stimulation [11-15]. On the other hand, the recent research study of Ross et. al., were not agreed with this [17]. Moreover, exogenous OT induces ADP-induced platelet aggregation [18].

Its safe at low dose and short term administration, but it has cause adverse effects on high dose and long term administration by any mode of administration such as ovarian anomalies [19], mammary gland degeneration [20], renal toxicity and cardiac cell hypertrophy [6] etc. Exogenous OT may also hinder oxygen supply to the body parts or organ due to it show contractile response on different air ways of lungs via formation of complex with OTR present in smooth muscle cells of airways [2]. A report was also showed that, OT infusion induces pulmonary edema in postpartum period [21]. The authors present a case, in which a large and maternal overdose of OT (17,300 mU over 20 min) during end-stage labor was associated with neonatal death and extensive hepatic infarction [22]. OT may cross the blood brain barrier and the fetal placental barriers may be due to its small size (1KDa) and reduced effectiveness in block the drugs in infants especially during stressful experiences including birth [19,23]. Oxytocin (OT) possesses vasodilator and anti-inflammatory properties but these are controversial till now. On the basis of above background detail related to effects of OT on serum biochemistry, different organs and vasomodulator properties; the present study tried to explore the chronic effect of OT on haematological and a serobiochemical parameter by using the immature female albino mice model.

MATERIAL AND METHODS

Experimental Chemical

The experimental chemical was synthetic oxytocin (OT) which commercially available and purchased from the registered medical shop of Bhopal (India), trade name Pitocin IP injection (10USP equivalent to 10IU/ml) manufactured by Pfizer Ltd., Nani Daman- 396210.

Animals and Treatment Protocol

Clinically healthy mice, Mus musculus of Parke (P) strain were obtained from the animal breeding colony of the animal house of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, Madhya Pradesh, India. The animal were housed into polypropylene cages and acclimatized to standard laboratory condition i.e. temperature 23-25˚C with 10h: 14h light: dark cycle and had free access to mice food and water ad libitum. Maintenance and experimentations for care and use of laboratory animals were carried out as per the guidelines of Institutional Animal Ethical Committee (IAEC), Bhopal, MP, India. The experiment was carried out on female pups of age 10±5 day and body weight 8±2g. The thirty six female albino mice pups (along with their mother till weaning) were randomly divided into 2 groups as control and treated of 18 each. The treated and control groups were administered with an intraperitoniol (i.p.) dose of synthetic OT (50IU/5µl/g body weight) and physiological saline (5µl/g body weight) respectively via insulin syringe (100U of 1 ml) upto 30, 60 and 90 days to avoid any skin damage. After the treatments, the mice were sacrificed on 31st, 61st, 91st day in the morning time by cervical dislocation and as per guidelines of IAEC.
Haematological and Serobiochemical Analysis

End of the treatment, blood was isolated form sacrificed mice by cardiac puncture method and kept into two type of storing vials as with and without blood anticoagulating agent for haematological and serological study respectively. For haematological analysis such as total count of white blood cell (TC- WBC) and total count of red blood cell (TC- RBC) were done by new improved Neubauer counting chamber method [24] and haemoglobin percentage (Hb %) was done by Sahli’s acid haematin method [25]. Serum preparation, blood (without anticoagulating agent) was incubated at 37 °C for 1 hr. It was further incubated at 4°C for 2 hr; then centrifuged at 10,000 rpm by cooling centrifuge (Remi C-24) for 10 minutes; finally serum was aspirated from the cells/blood clot. For quantifying serum bilirubin (total) diazotized sulfanilic acid colorimetric assay [26] was performed and reading taken by the help of semi automatic biochemistry analyzer (ERBA-CHEM-7).

Statistical Study

One way analysis of variance (ANOVA) was done by Sigma Stat statistical software version 3.5 and standard error of mean (SEM) were done to compare and assess any/at all differences at P-values *P<0.05, **P<0.01 and ***P<0.001 as less significant, more significant and highly significant respectively between the numerical data which were collected from the control and experimental groups by the method of Tukey’s test. [27]

OBSERVATION & RESULTS

Effect of oxytocin on Total count WBC (TC-WBC) of female albino mice, Mus musculus

IP administration of OT (0.05 IU/g) to female mice was showed non significant (P>0.05) variation in WBCs count after different intervals i.e. 30 days (4.13 ± 0.18 NS), 60 days (4.25 ± 0.22 NS) and 90 days (4.38 ± 0.21 NS) in comparison to the control group as 30 days (4.11 ± 0.21), 60 days (4.23 ± 0.16) and 90 days (4.48 ± 0.15) (Figure 1).

Effect of oxytocin on total count RBC (TC-RBC) of female albino mice, Mus musculus

IP administration of OT (0.05 IU/g) to female mice was showed non significant (P>0.05) variation in RBCs count after different intervals i.e. 30 days (9.15 ± 0.20 NS), 60 days (9.01 ± 0.22 NS) and 90 days (9.4 ± 0.19 NS) in comparison to the control group as 30 days (9.12 ± 0.21), 60 days (9.10 ± 0.20) and 90 days (9.65 ± 0.16) (Figure 2).

Effect of oxytocin on haemoglobin percentage (Hb %) of female albino mice, Mus musculus

IP administration of OT (0.05 IU/g) to female mice was showed non significant (P>0.05) changes in Hb content after different intervals i.e. 30 days (10.81 ± 0.20 NS), 60 days (11.30 ± 0.09 NS) and 90 days (11.80 ± 0.11 NS) in comparison to the control group as 30 days (10.98 ± 0.22), 60 days (11.40 ± 0.10) and (11.90 ± 0.21) (Figure 3).

Effect of oxytocin on total bilirubin of female albino mice, Mus musculus

IP administration of OT (0.05 IU/g) to female mice was showed non significant (P>0.05) alteration in total bilirubin after different intervals i.e. 30 days (0.42 ± 0.02 NS), 60 days (0.49 ± 0.03 NS) and 90 days (0.54 ± 0.02 NS) in comparison to the control group as 30 days (0.42 ± 0.03), 60 days (0.45 ± 0.02) and 90 days (0.54 ± 0.01) (Figure 4).

DISCUSSION

During the research study, OT treated group was showed non significant changes in TC-WBCs as compared to control group. The study on OT related to leucocytes count is rare; the available research study related to leucocytes was only based on inflammatory responses. Petersson et. al., research study was showed that, decreased inflammation in rat model by OT was mediated through inhibition of adhesion and aggregation of neutrophils via decreasing the secretion of myeloperoxidase enzyme [16]. As well as in the research study, synthetic OT treated mice were showed non significant differences in TC-RBCs and haemoglobin content as compared to control mice. The research study of Ozor and Omorogiwa were showed that, exogenous OT did not cause any significant difference (p>0.05) in the red cell count, related RBC indices, relative plasma viscosity and fibrinogen concentration in OT augmented labor woman [28]. On the other hand, OT induced labor woman showed significantly higher values of Hb content, red blood cell count, haematocrit, whole blood relative viscosity and relative plasma viscosity but significantly lower values of erythrocyte sedimentation rates [29]. One more report was available that, in which OT infusion to labor mother significantly increased Hb content, RBCs count and haematocrit in cord blood and neonatal blood [30]. The direct role of OT on haematopoiesis not yet discovered but the present study may suggest a possible mechanism toward relation of OT on haematopoiesis which is that, OT enhances prostaglandin as PGE-2 synthesis via increasing COX-2 enzymes [19,31] and this PGE-2 increases intracellular cyclic AMP levels in target cells and alters hematopoietic cells proliferation and maturation [32]. In addition to, cyclic AMP and PGEO block neutrophils recruitment also [33].
The serum biochemical such as total bilirubin was also carried out in this study. Bilirubin is formed by the breakdown of Hb in hemolyzed or senescent RBCs. Bilirubin then enters the liver and is modified to an extractable conjugated form that enters the intestinal lumen but can be deconjugated by bacteria so that the bilirubin is reabsorbed into the blood circulation. Its presence in blood is necessary due to some antioxidant benefits. Moreover, medical reports are suggesting that it should not be completely eliminated otherwise a sometimes deadly kernicterus can be developed [34]. During research study the administration of OT did not significantly alter total bilirubin amount. Although a report was available that, OT infusion to labor mother significantly increased bilirubin level in cord blood and neonatal blood [30]. This is due to the vasopressin-like action of OT which further causes activation of electrolyte and water transport across the erythrocyte membrane with consequent osmotic swelling leading to decreased deformability and hence more rapid destruction of RBCs with resultant hyperbilirubinaemia [35]. Neonatal hyperbilirubinaemia can cause due to other several reasons including they (especially preterm infants) have higher rates of bilirubin production than adults; they have red blood cells with a higher turnover and a shorter life span; impaired conjugation of bilirubin; unconjugated bilirubin is not readily excreted; deficient hepatic uptake of bilirubin; increased enterohepatic circulation of bilirubin; structural defects of the erythrocytes; deficiency of uridine diphosphate glucuronosyl transferase enzyme which is required for the conjugation of bilirubin; and blood-group incompatibilities of mother to child [34]. If exogenous oxytocin causes hyperbilirubinaemia so it may be associated with increased levels of cellular lactate, decreased levels of cellular glucose, and impaired cerebral glucose metabolism, encephalopathy or neurotoxic effects (neurons undergoing differentiation are particularly susceptible to injury from bilirubin) [34]. Therefore, more study relevant to these must be required.

Figure 1: White Blood Cells (WBCs) count after different intervals i.e. 30, 60 and 90 days of control and OT treated groups in female mice, Mus musculus (P). Mean ± Standard Error of Mean (SEM) of n = 6 animals of control and treated groups. NS=Non significant differences at P>0.05 from the control with treated group by one way ANOVA.

Figure 2: Red Blood Cells (RBCs) count after different intervals i.e. 30, 60 and 90 days of control and OT treated groups in female mice, Mus musculus (P). Mean ± Standard Error of Mean (SEM) of n = 6 animals of control and treated groups. NS=Non significant differences at P>0.05 from the control with treated group by one way ANOVA.
Figure 3: Haemoglobin percentage (Hb %) after different intervals i.e. 30, 60 and 90 days of control and OT treated groups in female mice, *Mus musculus* (P). Mean ± Standard Error of Mean (SEM) of n = 6 animals of control and treated groups. NS= Non significant differences at P>0.05 from the control with treated group by one way ANOVA.

Figure 4: Total bilirubin level in serum after different intervals i.e. 30, 60 and 90 days of control and OT treated groups in female mice, *Mus musculus* (P). Mean ± Standard Error of Mean (SEM) of n = 6 animals of control and treated groups. NS= Non significant differences at P>0.05 from the control with treated group by one way ANOVA.

CONCLUSION
Finally the research study was concluded that, exogenous OT was not showed any significant changes in total count of white blood cells and red blood cells as well as haemoglobin % and total bilirubin in female albino mice at a test dose 50mIU/g body weight and selected experimental duration. Therefore all these results suggested that, synthetic oxytocin is a safe drug at low dose but a care must be taken on usage of higher dose.

AUTHOR'S STATEMENT
The authors declare no conflict of interest.

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