



Research Article

Prospective cohort study and public health issues of exogenous Oxytocin in animal milk

M. Shahid Mahmood^{1*}, Iqra Yousaf¹, M. Luqman¹ and Faisal Masoud¹

¹Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan

*Correspondence: shahiduaf@gmail.com

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ABSTRACT

Oxytocin (OT) is a nona-peptide (having nine peptides) hormone commonly used in veterinary medicine as well as in human. It has many benefits regarding the biologic and behavioral effects such as inducing labor, increasing the lactation yield and supporting emotional and mental wellbeing. The main objectives of the study were to examine the concentration of OT in animal milk and evaluating its side effects in human beings through questionnaire. Milk samples (N=384) were collected from different 8 towns of district Faisalabad to determine OT concentration through UV spectrometry in milk. Samples were analyzed for mastitis through surf field mastitis test (SFMT), Somatic cell count (SCC) by Newsman staining and differential leukocytic count (DLC) by panoptic staining. For bacteriological analysis, standard plate count (SPC), total viable count (TVC), total coliform bacteria (TCB), and total staph count (TSC) were determined. Risk factors including age, sex, stage of labor and dose were analyzed through prospective cohort study by questionnaire. It was observed through UV spectrometry results that OT is not retaining in milk samples after heating up to boiling temperature for 3-5 minutes. The results of SCC and TVC were shown to be lowered in samples treated with OT as compared to negative control. Side effects of its direct use on human beings were seen to be vomiting, headache, lowering heartbeat. Based on these findings it can be concluded that OT is not imposing serious threats to human health directly.

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Introduction

Livestock is playing a crucial role in the revenue generations in the country. Its contribution in gross domestic production (GDP) is 11.2%. Dairy population of Pakistan comprises of 40, 47.8, 30.9 million of buffaloes, cattle and sheep, respectively which contribute almost 70% of milk production (GOP 2018-19).

Milk has a great nutritional value because it contains a large amount of water, fats, proteins, carbohydrates, calcium, phosphorus and trace elements. It is a lacteal secretion obtained from healthy animals in its natural form, containing highly rich contents of micro and macronutrients. It contains 87% water, 3.4% proteins, 4.8% lactose, 13% total solids, 4% fats and 0.8% minerals (Ijaz et al. 2019).

Milk collected from dairy animals is widely used for human consumption, but our daily production level cannot meet our needs due to large population size, improper storage, use of adulterants and compromised quality. High nutritious value of milk enables it to be a perfect medium for the growth of bacteria, mostly under unhygienic production and

improper storage. Good quality raw materials are also essential to make good dairy products. Presence of pathogenic bacteria in the milk can lead to the health threatening effects and deadly infectious diseases (Saxena and Poonam 2013). Milk yield is controlled by many factors, such as hormonal status, genetics, nutritional state, environment and milking frequency in dairy animals (Ijaz and Aleem 2006).

To maximize the good quality milk yield, some common practices are followed by farmers amongst which major one is use of OT injection in dairy animals (Abbas et al. 2014). Oxytocin is a hormone which stimulates the milk secretion by releasing through pituitary gland into the blood. It is bound with myoepithelial cells and induces the contraction of mammary gland for the expulsion of milk into the milk ducts (let down) (Abbas et al. 2014). The synthetic form of this hormone in the form of injections, is given to the animals before milking to improve deficient oxytocin release (Hameed et al. 2010). Hence enables the dairy sector to fulfill the daily needs of consumers.

OT was first time reported in 1909 by Sir Henry Dale (Perry 2014). It is a neuropeptide, synthesized in the

hypothalamus of the brain and transferred to the posterior pituitary gland in response to various physiological stimuli such as suckling and machine sound etc. It is also produced in the uterine epithelium, ovary, testis, vascular endothelium and heart other than the hypothalamus (Pullakhandam et al. 2014).

With the beginning of the endocrine system, OT starts releasing in the blood circulation. OT binds to specific receptors present in the mammary gland to affect the myoepithelial cell activity after systemic stimulation. Myoepithelial cells are present between epithelial cells and basement membrane of mammary alveoli as well as along the duct system of mammary gland. Myoepithelial cells contract due to OT binding, causing the expulsion of milk from mammary alveoli by increasing alveolar pressure (Ijaz and Aleem 2006). It is used for efficient fetal expulsion, milk-ejection reflex and for optimizing lactation in some species (Sabatier et al. 2013). It is considered to be a maternal hormone known for uterine contraction during childbirth. The synthetic form of OT is used to induce labor and to prevent bleeding from uterine tissue after delivery (Gordon et al. 2011). Level of OT is also directly proportional to the postpartum maternal behavior (Bakermans-Kranenburg and Van Ijzendoorn et al. 2013).

Our milk production is also deficient due to some diseases of udders that are caused by some microorganisms because of poor hygienic conditions. Mastitis is one of the most prevalent diseases worldwide. It results from the growth of germs in the udder. It occurs in two forms. First one is clinical mastitis in which visible swelling in the udder/teat and noticeable changes in milk such as flakes, clots can be observed. While the second one is sub-clinical, or hidden mastitis in which swelling is so mild and there is no visible change in the milk or in the udder/teat. The subclinical mastitis is 15-40 times more common than clinical mastitis and it is diagnosed through Surf Field Mastitis by adding milk sample in 3% surf solution (Muhammad and Rashid 2012).

One major role of oxytocin was explained to be helpful in curing subclinical mastitis caused by *Staphylococcus aureus* and other coliforms. These bacteria cause unending damages to the milk secretory cells in the udder tissue, which lead to altered milk composition and reduced milk yield. It has been shown that *Staph. aureus* can hide from immune system by changing its shape into micro abscesses and remains attached to the secretory cell wall. A high dose of this injection can rupture the walls of cells where the micro abscesses are hidden and allow them to be secreted in the milk. Hence helping in more and more bacterial outlet along with milk and ultimately lessen the chances of inflammation.

The genera *Escherichia*, *Serratia*, *Klebsiella*, *Citrobacter*, *Enterobacteria* and *Proteus*, although are the normal intestinal flora but many species of them are also opportunistic pathogens. Coliforms are also found in raw milk amongst which *E. coli* is most commonly isolated microorganism. The existence of these organisms in milk indicates unhygienic production or inappropriate handling of milk or milking apparatus (Zubeir and Ahmed 2007).

Contamination of bulk tank milk by coliforms is resulted by milking udder in wet environment or milking an animal suffering from sub-clinical mastitis. Hence laboratory tests show elevated coliform count (Salman and Hamad 2011).

It can be concluded by the above discussion that the milk quality should be tested by checking the number of *Staph.* and total coliforms in it in order to provide good quality milk to consumers, as these are the major microbiota which are compromising the milk quality.

This hormone is reported to be released in a lot of physiological and pathological functions for example male sex organ erection, ejaculatory process, pregnancy, contraction of uterus, milk ejection, maternal behavior, osteoporosis, cancer, diabetes and social bonding (Viero et al. 2010). The OT production is linked to axis involving the hypothalamic-pituitary-adrenals of the body. It has anti-stress property, needed for initiation of breast feeding and maternal-infant bonding (Feldman 2012). Schizophrenia, (abnormal social behavior) is also related to lower plasma OT levels. It serves as anti-psychotic medication in such patients and helps to overcome this disorder (Bakermans-Kranenburg and Van Ijzendoorn et al. 2013).

In human beings, it is involved in maternal bonding, sexual behavior and affiliation (Ishak et al. 2011). It is used for milk-let down during breastfeeding. It is also utilized for uterine contraction and to avoid bleeding during childbirth. In pregnant women, lower plasma OT levels during pregnancy results in complications in delivery (Gordon et al. 2011).

Owing to its adverse effects, extreme use of OT in dairy animals can result in reduction in milk ejection as well as addiction in the absence of OT therapy (Iqbal et al. 2013). Repeated or overdoses of oxytocin can lead to painful uterine contractions and possible uterine rupture (Perry 2014). The study was undertaken to evaluate the oxytocin concentration in milk, evaluate role of oxytocin in controlling mastitis and respective cohort study of public health concerns through questionnaire (Fig. 3).

Materials and Methods

Sampling

Total sample size (N=384) determined by using formula $N = 1.96^2 \times P_{exp}(1 - P_{exp}) / d^2$ where prevalence expected (P_{exp}) was 50%, degree of accuracy ($d=0.05$). All the samples collected from different 8 towns of Faisalabad, where animals were injected with oxytocin. Sample (10 ml) collected from dairy farms, local areas immediately after oxytocin injection (1 ml) in animals and transported in ice box to Hi-Tech Lab, UAF for further analysis. A questionnaire was also designed to evaluate the effects of oxytocin in human.

OT detection by UV spectrometry

Each sample was divided equally into two halves (3ml +3 ml) in test tubes for the OT detection via UV spectrometry, one set was heated till boiling of milk and second set tested without heating. PerkinElmer spectrophotometer at 220nm was used for evaluation of the presence of oxytocin.

Detection of Mastitis through Surf Field test:

For the detection of mastitis, 3% solution of household detergent was prepared and added same volume of milk sample and detergent solution and observed after 15-20 seconds of rotation (Ijaz et al. 2019). Thickening or any other change was examined from mixture.

Newman's staining for somatic cell count

For somatic cell count Newman's Lampert staining used and cell counted by using formula of somatic cell count=Working Factor (WF) × average number of cells per ml. Different values including area of microscopic field (0.00005 cm² or 1/ 20000 cm²), milk quantity (0.01 ml or 1/100 ml), microscopic factor (2x10⁶ cells per ml) and working factor (4x10⁴ cells per field) were calibrated for counting of cells.

Panoptic cell staining for differential leukocytic counting

For the hematological (Neutrophils, Lymphocytes, Macrophages) analysis of milk samples, panoptic staining method was used by preparing 25ul milk sample smear on slide and stained with undiluted for 1 min and with diluted (1:2 May-Gruenwald solution, Sigma-Aldrich, Steinheim, Germany) for 3 min respectively that followed by 3% Giemsa solution for 14 min. After staining, slides were washed with Sorensen buffer (0.01M) and cells were counted under microscope.

Bacteriological analysis

Standard plat count (SPC), total viable count (TVC), total coliform bacteria (TCB), total Staph count (TSC) were performed for the bacteriological analysis of milk samples.

Results

UV spectrometry

Total 384 milk samples were observed under 220nm in PerkinElmer spectrophotometer with the blank run 0.59 and positive control 1.945, a clear absorbance difference was observed between heated and non-heated milk for the OT detection (Fig. 1 and Fig.2).

Somatic cell count (SCC)

Somatic cell counted in all sample by after newsmen lampert staining with comparison to normal range of SCC (50,000-100,000) and <100,000 showed sub clinical mastitis. SCC done before and after heating of milk.

Differential Leukocytic count (DLC)

With high SCC milk samples showed high numbers of neutrophils (44.3 %), lymphocyte (21.5 %) and macrophages (34.2 %).

Total Viable Count (TVC)

Total viable count was measured by CFU/ml= average number of colonies × dilution factor/volume factor. Milk samples without heating has 5 × 10⁵ CFU/ml (50 × 1000/0.1) and after heating without OT has 9 × 10⁵ CFU/ml (90 × 1000/0.1).

Total Staph Count (TSC)

Average staph count in milk samples with the presence of OT was 0.53 × 10⁵ CFU/ml by using the formula CFU/ml= average number of colonies (53) × dilution factor (1000)/ volume factor (0.1).

Total Coliform Count (TCC)

Average coliform count in milk samples with the presence of OT was 0.79 × 10⁵ CFU/ml by using the formula CFU/ml= average number of colonies (79) × dilution factor (1000)/ volume factor (0.1).

Questionnaire analysis (factors) and statistical analysis

Questionnaire evaluated different factors including persons recommended use of oxytocin in humans (80%) and side effects caused by OT examined were nausea (40%), confusion (40%), uneven heartbeat rate (47%), memory loss (40%) and uterine rupture (9%). For the statistical analysis, t- test was applied for the spectroscopic values and SCC and the mean value, mean difference and t-value are given in the Fig. 2.

Discussion

The present study was related to a neuroendocrine hormone "Oxytocin" which was firstly reported by Sir Henry Dale in 1909. He described that extracts of posterior pituitary glands encouraged the uterine contractions. This hormone is produced in hypothalamus of brain and stored or released via posterior pituitary then comes in blood circulation. This hormone is reported to be involved in a wide variety of functions such as to increase the milk let-down, induce uterine contractions for timely fetal expulsion, to prevent postpartum hemorrhages and some physiological and pathological functions including sexual activity, penile erection, ejaculation, social bonding and anti-stress activity. Dairy sector being a backbone of Pakistan's economy is playing a crucial role in its GDP. But our milk production is not enough to meet our daily needs. To enhance milk production artificial oxytocin (in the form of injection) is given to dairy animals very frequently in Pakistan. This study was designed to check the hypothesis that whether oxytocin is involved in inducing negative effects in human beings or not. For this purpose, milk samples were taken from rural area of Faisalabad and OT detection was done by UV spectrometry at wavelength of 220nm. Somehow similar study was conducted by Adam (2009) reporting that ultraviolet (UV) spectrum of oxytocin revealed λ-max absorbance at approximately 275 nm with an additional peak at approximately 280nm with an additional region of increased absorbance occurring at between 200–240 nm. Therefore, the use of UV as a method of detection at a wavelength of approximately 220nm was satisfactory. The use of UV detection for the analysis of oxytocin is a relatively simple procedure without the need for sample derivatization. In present study, samples were divided into three groups: negative control, positive control and heat treated (Fig. 2). Comparing the values of absorbance of negative control (blank) with others, it may be concluded that OT does not retain in heat treated samples. Mostly blank showed the absorbance within range of 0.06-0.68 while that of positive controls (samples surely

having oxytocin) showed values to be within range of 1.85-2.01. While the heat-treated samples showed the range of 0.003-1.72 supporting the conclusion that boiling helped to deteriorate the OT residues in milk. Similar reporting was given by quality control experts of Parag Dairy; in 2012 that boiling helps to reduce oxytocin in milk. General Manager, Parag dairy, Lucknow, SK Prasad said: "though a lot of samples are weeded out at the level of society, but we do not have a specific hormone test to ascertain the presence of oxytocin in a sample".

Ijaz and Aleem in 2006 also supported the fact that the gut enzymes and very short retaining time of oxytocin do not allow it to be there in milk for longer time. Hence it may not impose any serious threat to human beings.

In contrast to all these, Abbas et al. (2014) reported that oxytocin may alter the milk composition due to which it is causing some unwanted effects in human beings but the effects were not reported in the study. Another animal study had shown oxytocin effects in stimulating the gonadotropin releasing hormone through prostaglandin E2, resulting in early puberty (Parent et al. 2008). But by the present study observations, we may conclude that oxytocin may not retain in milk due to short retaining time, gut enzyme activity and heat treatment of milk before consumption.

Concerning the sub-clinical mastitis control, the hypothesis that oxytocin helps to cure mastitis was proved to be true by the results of present study. Somatic cell count was done to check the udder health. Two groups of samples were made: (1) samples of animals that were not injected OT, (2) samples of animals injected with OT. Animals not given OT were more prone to the sub-clinical mastitis showing the SCC of range 8.2×10^4 to 1.9×10^5 . While the animals treated with OT showed SCC range of 2.9×10^4 to 9.2×10^4 . These results showed that oxytocin is playing the role in lowering mastitis in dairy cows as SCC up to 10^5 was normal. While more than this value, animals are considered to suffer from sub-clinical mastitis and SCC up to 5×10^5 shows that animal is diseased having clinical mastitis.

A similar study was conducted by Mishra et al. 2013 explaining the major role of oxytocin to be helpful in curing subclinical mastitis caused by *Staph. aureus* and other coliforms. These bacteria cause unending damages to the milk secretory cells in the udder tissue, which leads to altered milk composition and reduced milk yield. It has been shown that *Staph. aureus* can hide from immune system by changing its shape into micro abscesses and remains attached to the secretory cell wall. A high dose of this injection can rupture the walls of cells where the micro abscesses are hidden and allow them to be secreted in the milk. Hence helping out in more and more bacterial outlet along with milk and ultimately lessen the chances of inflammation. It also supports the results that regular use of oxytocin injection lowers the *Staph* and *Coliform* count in udder hence the chance of infection gets lowered. At the start of sub-clinical mastitis the total *Staph* count and *Coliform* count were observed to be higher due to the rupturing of micro abscesses walls and with the passage of time this count was observed to be lowered hence, showing

the values of TSC (total *Staph.* count), TCC (total *Coliform* count) and SCC (somatic cell count) within the range of normal values.

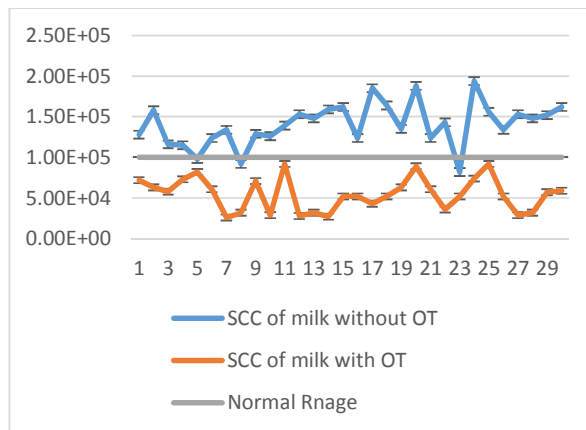


Fig. 1: Somatic cell count of the milk samples with and without Oxytocin treatment

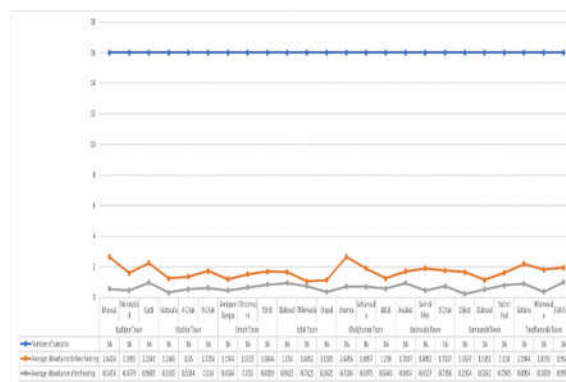


Fig. 2: Absorbance of milk samples before and after heat treatment

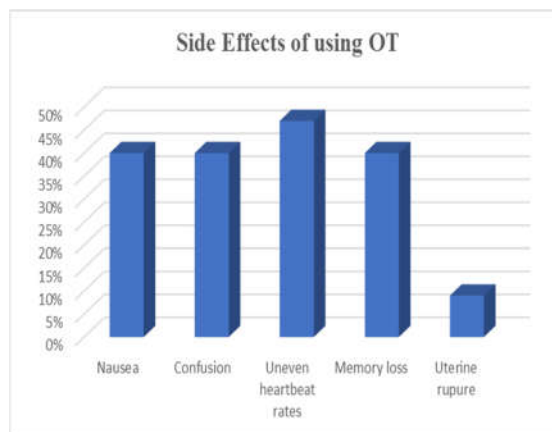


Fig. 3: Questionnaire analysis of the data collected for the side/adverse effects of Oxytocin

Concerning the questionnaire evaluation showing the effects of direct use of oxytocin, 80 % doctors are recommending the use of oxytocin. Mostly observed side effects were nausea, confusions, uneven heartbeat rate, memory loss and uterine rupture with the percentages of 40, 40, 47, 40 and 13%, respectively for a very short period of time. These percentages showed that the observed side effects were not life threatening. Hence, oxytocin is not showing any serious hazard towards the health of human beings.

Conclusions

In the present study, it has been observed that recommended doses of oxytocin for limited time have no injurious effect to the quality of milk in term of the composition of the milk that has been synthesized by the udder tissue, as the half-life of oxytocin is 5-7 minutes and it may be degraded by gut enzymes.

Authors contribution

All authors contributed equally

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