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Mukkuddu Maathrai (Triple Tablet), An Indigenous Medicine Elevates Serum Prolactin Level Of Female Rats (*Rattus norvegicus*)

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Abstract: *The Mukkuddu Maathrai is a mixture of Kasturi, Saffron and Korosanai. Ayurvedic doctors in Jaffna peninsula have been prescribing this mixture to patients for their good health. The prolactin (PRL) can be used as a marker in hypothalamo-pituitary axis. The objective of the present study was to determine the effect of Mukkuddu Maathrai treatment on serum prolactin level of female rats at estrus stage. Female rats were maintained under uniform conditions. Stages of oestrus cycle were determined by using vaginal smear cytology. Initial blood sample was collected by tail bleeding at the estrus stage and were treated with oral feeding. Tests and controls were fed with 5% w/v of the Mukkuddu Maathrai mixture solution and distilled water alone respectively. Treatments were done continuously as once per day until one oestrus cycle complete. At the next estrus stage, second blood sampling was performed. Serum PRL levels were measured by enzyme immunoassay and the results were compared by using paired't' test at 95% confident limits. All experiments were conducted with their duplicates. During the treatment, prolactin secretion increased significantly in female rats at estrus stage. Mukkuddu Maathrai treatment triggers hypothalamo-pituitary axis of female rats and thus influences on serum prolactin level.*

Key Words: *Estrus, Kasturi, Korosanai, Prolactin, Saffron.*

I. Introduction

The Mukkudu Maathrai (triple tablet) is an indigenous medicine (practices in South India and Northern Sri Lanka) which is a mixture of Kasturi (Musk), Saffron and Korosanai (Bezoar) in 1:2:3 ratios respectively. Kasturi is an animal product which gets from a musk gland of the adult male Musk deer, Moschus moschiferus which belongs to family Moschidae [1]. The musk gland is situated beneath the skin of the abdomen between the reproductive organ and umbilicus in males. It opens to the exterior through an orifice that lies anterior to the urethra [2]. Saffron is an herbal product, the stigma of flower of Crocus sativus, which is a perennial and an autumnal herbs of the family Iridaceae [3, 4]. Korosanai is an animal product, light and easily broken concretion in the intestine and bile of a cow or ox and also obtained from the ruminants animals, fish and snakes [5]. Since ancient period, Ayurvedic doctors in Jaffna peninsula of Northern Sri Lanka have been prescribing this mixture for six month old babies to adults for their good health such as fever and cold etc. It has been believed by the Ayurvedic practitioners that the above mixture causes a strong effect on hypothalamo-pituitary axis. Prolactin (PRL) is a peptide hormone, secreted from the pituitary gland and is reported to have over 300 separated biological activities [6]. Moreover PRL has been also used as a marker to monitor the functional status of the hypothalamo-pituitary-gonadal axis [7]. By considering the drastic health benefits and the safety of natural remedies, scientists and medical professionals are showing great interest in studying the medicines values in indigenous drugs. But so far no studies have investigated the possible effect of Mukkuddu Maathrai on endocrine physiology. The objective of the present study was to determine the effect of Mukkuddu Maathrai treatment on serum prolactin secretion in female rats at estrus stage.

II. Materials and Methods

2.1. Selection and rearing of animals

Domestic laboratory rat, *Rattus norvegicus*, which belongs to family Muridae were selected for this investigation. Healthy, sexually mature, virgin, estrus staged female rats (3months old and 120 ± 10 g of body weight) were used in the whole experiments. Reproductive cycle stages of virgin female rats were determined

by using of vaginal smear cytology. Selected animals were maintained under uniform conditions in 30±1˚C with approximately 12 hours light and 12 hours dark cycles and with ad-libitum feeding of pelleted food and water at the Animal House, Department of Zoology, Faculty of Science, University of Jaffna. The study was approved by Institutional Review Board. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

2.2. Vaginal smear cytology

On the days of experiment, vaginal smear cytology was performed between 8.30 am to 10.00 am. A cotton bud was dipped into normal saline (0.9 % w/v) and then vaginal cells were collected by means of rotating (2 times) the cotton bud gently into the vagina (1cm of the bud) with the angles of 45^0. Then the bud was rotated the end 2 or 3 times. After the bud was withdrawn from the vagina, collected cells were spreaded carefully on to a clean glass slide. It was allowed to air dried for 10 minutes. Slides were immersed in 0.05 % (w/v) of Methylene blue solution for 15 minutes. The slides were then washed gently using distilled water and observed under binocular light microscope to determine the stage of oestrus cycle.

2.3. Preparation Of Mukkuddu Maathrai

All three natural products, such as *Kasturi* (Lata Kasturi, S.V.Traders, India), Saffron (Moon brand saffron, Selected Export Quality, Kashmir Saffron Co. Srinagar, India) and *Korosanai* (India) were weighed separately by using electronic balance (PG203-S, Mettler Toledo, Switzerland). *Kasturi*, Saffron and *Korosanai* were mixed and crushed in 1:2:3 ratios by using Motor and Pestle, according to standard protocol practiced by Ayurvedic doctors in Northern Province of Sri Lanka (Personnel communication). 50 mg of Cocktail powder was liquated with 1000 µl of deionized distilled water in eppendorf tube (5 % w/v).

2.4. Treatment for rats

Treatments were performed from one estrus stage to next estrus stage (continuously for 6 days until one oestrus cycle completes) by oral feeding. The freshly prepared *Mukkuddu Maathrai* mixture solution (5 % w/v, 21mg/kg, body weight/ day) was used for treatments of rats. Six rats were taken and divided into two groups such as group- I (3 control rats) and group-II (3 tests). Test rats were fed with 50 µl of the freshly prepared *Mukkuddu Maathrai* mixture solution. Control rats were fed with 50 µl of distilled water. All rats were treated once in every 24 hours for 6 days. The whole treatment experiments were repeated six times with their duplicates.

2.5. Blood collection and serum separation

Blood samples were collected at estrus stage by tail bleeding [8]. Animal was restrained by using the restrainer cage and partially anesthetized by ether. Blood was collected from the tail vein by slicing the tip of the tail (0.2 mm thickness) using a sharp sterile blades with the help of regiform in the presence of warm condition. Initial blood sample was collected by tail bleeding at the estrus stage (0 day) and second blood sample was collected at the next estrus stage (consecutive, 6^{th} day). Serum was separated by centrifugation (TC 4815 D, Eltek, Microcentrifuger, India) at 3000 rpm for 10 minutes and transferred into a sterile eppendorf tube and stored at -20 ˚C until further use.

2.6. Enzyme immune assay for rat prolactin

Serum PRL levels were measured by using commercially available rat prolactin enzyme immunoassay kit (Cat. No. 589701-96 wells, SPI bio, de la bonde, Massy Cedex, France) and absorbance was read by using ELISA microplate reader (Type: 355, REF: 51118170, Thermo electro corporation, Shanghai, China) at 405 nm when the Maximum Binding (B_o) wells was reached an absorbance of 0.5 unit.

2.7. Analysis of data

Statistical analyses were done by using Microsoft Excel. The results were compared by using paired‘t’ test at 95% confident limits. Mean ± SD was used to describe the data.

III. Results

Various stages of oestrus cycle were determined by vaginal smear cytology. Four significant reproductive stages were observed. The three types of cells, Polynuclear leukocytes, epithelial cells and keratinized cells were observed under the microscope. The interpretations of the stages of oestrus cycle were based on the relative quantities of different types of above cells. The stages are proestrus, estrus, metestrus and diestrus. Proestrus is characterized by nucleated epithelial and few keratinized and leukocytes cells. Estrus stage is contained only predominantly keratinized cells (Fig 1). Metestrus is contained leucocytes and keratinized cells. Diestrus is contained leucocytes only [9]. Serum prolactin levels show at the successive estrus stage of

female rats (Table 1). Serum prolactin level significantly increased after treatment (P<0.05) compared with before treatment (0 day) and control (6 day) in female rats (Fig 2).

Table 1:- The serum PRL concentrations at consecutive estrus stage in female rats (P<0.05)**.**

Time	Concentration of serum PRL (in ng /ml)	
	Test	control
Pretreatment (0 day) at estrus	5.5763 ± 0.1155	5.5684 ± 0.1142
After treatment (6 day) at estrus	11.8763 ± 0.1155	5.8087 ± 0.2732

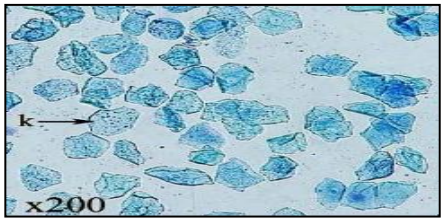

Figure 1:- Vaginal smear cytology: estrus stage in female rats (methylene blue staining) under the light microscope. Anuclear cornified or keratinized epithelial cells (k) present predominantly.

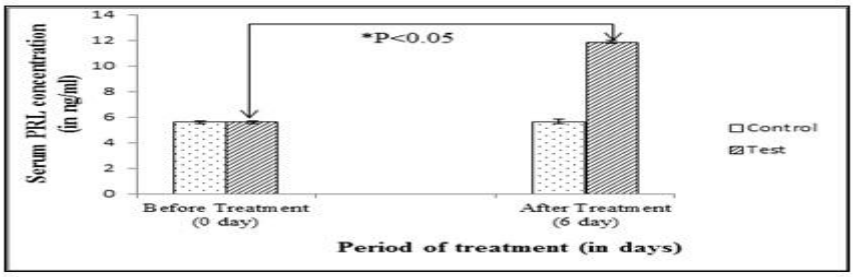

Figure 2:- Serum PRL level at consecutive estrus stage in female rats (*P<0.05).

IV. Discussion

The oestrus cycle is characterized by cyclical changes in uterus, ovaries, vaginal mucosa, behaviour and hormone levels. The oestrus cycle of rat can be divided into four stages: proestrus, estrus, metestrus and diestrus. Wide variation of female hormone levels observed during the oestrus cycle, stage of oestrus cycle is emerging as an important consideration when working with female animals in clinical scenarios and in several research areas [10]. In species with short oestrus cycles, the cycle involves only a follicular phase that culminates in a spontaneous ovulation. The timing of behavioural receptivity in the female is tightly linked to that ovulation and is under hormonal control [11]. Maturation of pre-ovulatory follicles and ovulation are under the combined and balanced influences of ovarian and extra ovarian hormones. Imbalances or alterations in these hormones lead to irregularity in the ovarian functions and duration of oestrus cycle [12]. Prolactin (PRL) is a peptide hormone that is synthesized and secreted from specialized cells (lactotrophs) of the anterior pituitary gland [6, 13]. Plasma PRL levels were low during diestrus and during the morning (09.00) and noon (12.00) of proestrus. There was a significant (P<0.001) surge of PRL during the afternoon (17.00) of proestrus. No significant alterations in PRL levels were observed at various times (09.00, 12.00 and 17.00 h) of estrus, though the values were slightly higher than diestrus and proestrus morning and noon concentrations [14]. During the oestrus cycle, serum prolactin level of rat was found to be highest in estrus and lowest in diestrus. Serum prolactin levels during proestrus, estrus and metestrus were not significantly different [15]. Length of oestrus cycle, changes of ovary and uterus are varying with mating, ageing and status of reproduction [16]. Therefore in the present study, healthy, sexually mature, virgin, estrus staged female rats were used for the experiments.

Ayurvedic doctors in Jaffna peninsula (Northern Province in Sri Lanka) have been prescribing this mixture from one day old babies to adults. Six month baby has been prescribing with 15 mg of *Mukkudu Maathrai*. This

amount of *Mukkudu Maathrai* was converted to the rat based on body weight. Rat is smallest animal and the basal metabolic rate is higher than human [17, 18]. Therefore 4 times (by weight) of *Mukkuddu Maathrai* were used for treatment in rats. Oral feeding was performed for 6 days because average length of oestrus cycle in female rats was 6 days in our Animal House conditions. In Ayurvedic medicines, natural *kasturi* (musk) is used within indigenous systems of medicine as a stimulant to cure bronchitis, pneumonia, impotence, typhoid, typhus and as a sedative to treat asthma, epilepsy, hysteria and other nervous disorders. As an anti-inflammatory agent, musk is a more effective antidote for snake venom than hydrocortisone [19, 2]. Musk is also used in the treatment of malaria, high fever and heart ailments. The lactating mother is given a pinch of *kasturi* with the belief that the breast feeding babies develop immunity to diseases. Therefore the musk is believed to bolster the immune system in children [1]. The stigmas of the saffron flower contain many chemical substances. Carbohydrates, minerals, mucilage, vitamins (especially riboflavin and thiamine) and pigments including crocin, anthocyanin, carotene, lycopene, zigzantin, flavonoids, amino acids, proteins, starch, gums, and other chemical compounds have also been described in saffron [3, 20, 21, 22, 23].

As a medicinal plant, saffron has traditionally been considered an anodyne, antidepressant, eupeptic, anti-catarrhal, carminative, stimulant, stomachic, a respiratory decongestant, antispasmodic, aphrodisiac, diaphoretic, emmenagogue, expectorant and sedative such as gingival sedative and nerve sedative [22]. It was used in folk remedy against scarlet fever, smallpox, colds, asthma, eye and heart diseases and cancer. Saffron can also be used topically to help clear up cancer sores and to reduce the discomfort of teething infants [3, 20]. Crude saffron extracts present anti-tumor and anti-carcinogenic activities as well as cytotoxic and anti-mutagenic effects [24, 25, 26]. Modern pharmacological studies have demonstrated that saffron extract or its active constituents have anticonvulsant [27], antidepressant [28], anti-inflammatory [29] and antitumour effects, radical scavenger as well as learning and memory improving properties [22, 20, 30, 31, 32]and promote the diffusivity of oxygen in different tissues [22]. It is also very important to note that suitable chemo-preventive natural agents should have little or no toxicity, a high efficacy, to be orally administrable, to have a known mechanism of action and of low cost [33]. Korosanai (Bezoar) is laxative, anti-spasmodic, cholagogue, cooling and aromatic. It is specially indicated in measles and small-pox, to reduce excessive heat in the body, whooping cough and watery stools and choleraic symptoms. It is used in convulsions, hysteria, spasmodic diseases, melancholia and intestinal disorders with deficient secretion of bile, in jaundice, and in abortion, etc. It is given to infants for stopping green stools and as a laxative in small doses. The usual adult dose is from 5 to 10 grains. It enters into the composition of some medicines used for skin diseases [4]. Medicinal properties of Kasturi, Saffron and *Korosanai* were reported exclusively but there are no reports on their collective impact in their combination. Prolactin is an anterior pituitary hormone which secretion controls by the hypothalamo-pituitary axis. The present study reports for the first time that the *Mukkuddu Maathrai* on the serum PRL secretion significantly increased after treatment (6 day) compared with before treatment (0 day) and the control in the consecutive estrus stages. Prolactin helps to initiate breast development by inducing lobuloalveolar growth of the mammary gland and also stimulates lactogenesis. In some mammals, particularly rodents, PRL is also important for the maintenance and secretory activity of the corpus luteum and affects other actions related to reproduction such as mating and maternal behaviors. Dopamine serves as the major-inhibiting factor or break on prolactin secretion. High prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating and gonadotropic-releasing hormones (GnRH) [34] which are necessary for ovulation. Such increase in prolactin level may inhibit ovulation and promote the loss of menstrual periods which will hinder conception. Further actions of PRL, plays a role in maintaining the constancy of the internal environment by regulation of the immune system, osmotic balance and angiogenesis. PRL is a common mediator of the immunoneuroendocrine network, where nervous, endocrine and immune systems communicate with each other. PRL plays a significant role in regulation of the humoral and cellular immune responses in physiological as well as pathological states, such as autoimmune diseases [13]. Therefore when took the *Mukkuddu Maathrai*, raised the serum PRL level in rat. This lead to increase the immune responses during disease conditions (cold, fever) in human. *Mukkuddu Maathrai* can give to baby directly or when mother take this medicine, raised milk production and immune responses in mother and transfer to the baby through milk during lactation.

V. Conclusion

The elevated level of prolactin in this study justifies the folkloric use of the natural plant and animal products (Mukkuddu Maathrai) in stimulating lactation, immune responses, infertility and contraception in hormone dependent organs like the ovary and mammary glands. Our findings in this study have important implications for lactation, development of contraceptive in mother and produce immune responses in mother and child. Natural products used as contraceptive will be more acceptable for economic reasons and side effects that are less than chemical agents.

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