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Role of the silymarin on the prolactin gene expression in the pituitary, estrogen and progesterone hormones of western rats during lactation

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Abstract---Objective: To see how dose of Silymarin aqueous extracts affected prolactin hormone gene expression in pituitary glands and Androgen hormone in serum samples of female experimental rats throughout nursing. Methods: 24 adult healthy, Two sets of pregnant rats were created (12 each group) and treated as follows for ten days postnatal. G 1 were intubated orally distal water serving as control while, G 2 were intubated orally Silymarin (200 mg/kg/day). Results: The findings show that Silymarin had a strong influence on mRNA-PRL expression in pituitary glands, with a rise in Estrogen and a decline in Progesterone levels. Conclusion: The focus of this study mention a new evidence of the role of Silymarin on the prolactin gene expression for increasing the milk production and level of estrogen & progesterone hormones.

Keywords---Effects, Silymarin, Prolactin gene Expression.

Introduction

For nearly 2,000 years, milk thistle (*Silybum marianum* L. Gaertn, Asteraceae) has been utilized in medicine. It's been widely utilized to treat a variety of liver

problems. anti-inflammatory and antioxidant properties (Wu *et al.* ,2011; Gharban, 2021). Silymarin is made up of 4 flavonolignans in a complex mixture (isosilybin- silybin- silychristin and silydianin). The most important and active component of silymarin is silybin, which is essential for the pharmacological properties of silymarin (Farmer *et al.*,2014). Silymarin has a variety of benefits for treating liver disorders, including the protect the liver from injury and ability to inhibit hepatotoxin binding to receptor sites, the ability to decrease glutathione oxidation and increase its own level in the liver, increase hepatocyte protein synthesis. additionally and the ability to stabilize the liver cell membrane , By lowering blood lipids and decreasing platelet aggregation, silymarin can help to avoid diabetes and atheroma (Farmer *et al.*,2014). its content varies significantly amongst silymarin extracts, potentially altering its biological function. However, silymarin extract has been shown to boost milk yield in nursing mothers, as well as to improve bovine, β -casein gene expression and murine mammary cell proliferation. (starvaggi *et al.* ,2010). Because silymarin dramatically elevated prolactin in female rats, this effect was done in part through dopamine receptors, the observed favorable effect of silymarin on breastfeeding performance could be attributed to higher prolactin concentration (Wu *et al.* ,2011; feng *et al.* ,2016). There are numerous mechanisms by which silymarin can oxidative state and increase antioxidant defense mechanisms (freeman *et al.*,2000), and its protective impact against systemic oxidative stress has recently been established in late-pregnancy animals (Farmer *et al.*,2014). The dose of silymarin required to elicit an antioxidant effect is expected to be less than that required to increase milk supply (feng *et al.* ,2016) .

The pituitary gland is a small structure below the temporal lobe of the brain that produces hormones (Hong *et al.* ,2016). The anterior, middle, and posterior lobes of the pituitary gland are separated into three divisions (Amar and Weiss,2003). Many hormones, such as (Prolactin, oxytocin, ADH), GH, LH and FSH), are emitted by these glands and move all across the body, directing biological activities (Blackwell *et al.* , 1986; Hong *et al.* ,2016). The anterior lobe is primarily responsible for bodily growth, associated with puberty, and fertility (Ben and Hnasko, 2001). The adrenal and thyroid glands, and even the ovaries and testes, are all stimulated by the anterior lobe.

Prolactin (PRL), a 23-kDa polypeptide hormone released by mammatrophs cells of the anterior lobe (Ben and Hnasko, 2001). Endocrine neurons in the hypothalamus modulate prolactin excretion by secreting dopamine, which acts on dopamine receptors on mammatrophs to limit PRL synthesis (Rillema, 1980). PRL is a hormone that affects both men's and women maternal health. It encourages mammary glands to produce milk; higher PRL serum levels through gestation promote expansion of the mammary glands of the breasts and prepare them for dairy production. Prolactin regulates androgens hormone secretion (Freeman *et al.*, 2000). PRL levels that are far too excessive lower estrogen concentrations in female and testosterone amounts in males. The consequences of modestly raised PRL levels are far more varied, as estrogen levels in women can increase or drop significantly (Mcmurray ,2001).

Lactotroph cells in the anterior pituitary are the principal producers of PRL. Dopamine produced from tuberoinfundibular dopaminergic neurons has an

inhibitory effect on PRL (Bole-feysot,1998). Despite numerous attempts to identify PRL in neurological areas using various methodologies, the brain's ability to synthesize PRL has really been called into question. Immunocytochemistry was used to locate PRL responsive neurons in men and females of several mammals (Ben and Hnasko, 2001).

PCR was used to identify prolactin mRNA in the PVN and supraoptic nucleus (Bole-feysot ,1998) of mother rats, as well as qPCR in the hypothalamus, genes implicated sheep, as well as the periventricular preoptic nucleus, bed nucleus of the stria posterior part, PVN, as well as the lateral septum (Clap *etal.*,1994;Freeman *et al.*,2000; Torner,2004). Alternative therapies appear to be one of the techniques to promote milk production. The significance of lactation during first two years after birth as well as the difficulties that females face in this area highlight necessity future investigations into the accessibility and development of medications that increase milk production (Roselli,2008). Nevertheless, the Organization (Who) estimates approximately 1.5 million infants die each and every year due to a lack of lactation or insufficient dairy (Chaiseha *etal.*,2012). Phenotypically, prolactin release rises throughout pregnancy and after nursing to the point where prolactin quantity is 20 times greater than usual throughout gestation (Jakob,2014).

The focus of this study was to learn more about the reproductive hormones level, in addition including PRL expression levels during administration of Silymarin during pregnancy and lactation.

Material & Methods

Experimental protocol

Twenty-four female rats weight of rats (190-250 g) and their ages between (10 -12 weeks) , The animals were kept in an unique plastic cage in the (University of Karbala - College of Veterinary Medicine) animal house, provided the animals with the appropriate conditions. Females were mated with adult males in order to induce pregnancy. The females were randomly divided into two groups (12 each group). After the end of pregnancy and the occurrence of childbirth, the lactating females were started to be dosed from the first day of birth until the tenth day after birth., G 1 were intubated orally tap water serving as control while, G 2 were intubated orally silymarin (200 mg\kg\day) (Farmer *et al.* ,2017).

Preparation of primers

The primers (originally lyophilized) were dissolved in free ddH₂O to reach a final rate of 100 pM/l, which acted as a standard solutions that was kept at -20 ° C, according with primer synthesizer company's instructions. The stock primers were concentrated to a content of 10 pM/l to be utilized as a work primer (Table 1).

Protocol of GoTaq 1-Step RT-qPCR System for Real-Time qPCR (Gene expression assay):

Program a real-time apparatus for one-step RTqPCR in normal or fast mode. Thaw the GoTaq 1-Step RT-qPCR System components, as well as the RNA templates and primer pair, on ice, at room temperature or at (37) °C. Mix each thawing element well right away. Mix at a moderate speed with a vortex mixer to avoid aeration. We extract the RNA sample data (mRNA [500fg–100ng]) in water or any qPCR suitable diluent after thawing the reagents on ice. In a non-stick, eppendorf tubes on ice, mix the reactive ingredients. Out after every additional, stir carefully. Pipet reaction quantities to an ice-cold plate with care. Transmit the plate from either the ice to the pre-programmed apparatus, meanwhile. Begin the run as soon as possible, then gather the data and analyze the outcomes (Table 2).

Estimation of Hormone Estrogen & Progesterone in the serum

The hormone levels of Estrogen and Progesterone were determined using a Kit Specific to Measure Hormone Levels based on the mode of use. In accordance with the producer's guidelines of ELIZA Kit, (Kinn *et al.*,2017).

Statistical analysis

The data were normally distributed, as influenced by the D'Agostino and Pearson normality tests, and were reported as means with \pm standard error of mean SEM. Student's t-test was used to establish statistical significance between two conditions. The data was analyzed with Graph Pad Prism 9.0 for Windows, and the statistical significance level was ($P < 0.05$).

Table 1: Primers of gene expression experiment

Target gene	Primer name	5'-3'	PCR Product	Accession number
Prolactin	F	CCTGAAGACAAGGA ACAAGCC	344 bp	XM_032885212.1
	R	TGGGAATCCCTGCG CAGGCA		
CYPA	F	TATCTGCACTGCCAA GACTGAGTG	127 bp	M19533
	R	CTTCTTGCTGGTCTT GCCATTCC		

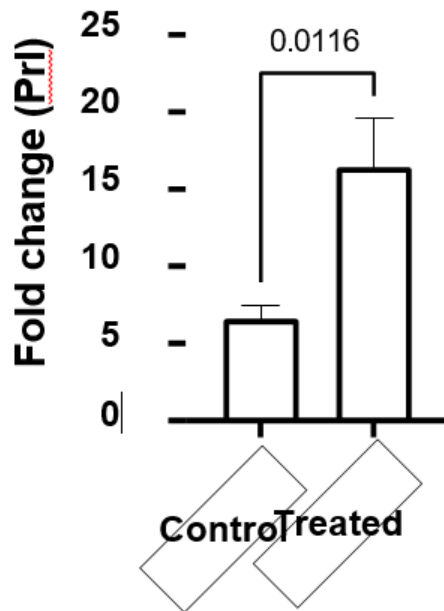
Table 2: Preparation of Real-Time PCR solutions

Components	Concentration	Volume (20µl)
GoTaq™ qPCR master mix, 2X	1X	10 µl
Forward primer	10 µM/µl	2µl
Reverse primer	10 µM/µl	2 µl
GoScript™ RT mix for 1-step RT-qPCR	1X	0.4 µl
ddH ₂ O	-	3.6 µl
RNA template	250 ng	2µl

Results & Discussions

Prolactin gene Expression

The effects of Silymarin on the prolactin gene transcription in the pituitary gland was investigated via real-time PCR in this study. In compared to the control group, Silymarin significantly accelerated Prl gene expression in dosage (p 0.05). (Figure 1). The focus of this study was to see how Silymarin extract affected milk supply in breast tissue. Silymarin raised the level of prolactin in all the other mother rats in this investigation, according to the findings. The amount of prolactin hormone rises throughout after birth, and it has a variety of functions, including secretory activities of the lactotropic cell in the pituitary glands and stimulation of the pituitary glands (Figure 1). (Table, 3). This corresponds to the findings of (Akinloye and Oke ,2012) who found that herbal extracts raised the quantity of prolactin in female mice via acting on endocrinological glands such like different regions of the breast tissues. In addition, this investigation (Dill *etal.*,2017) confirmed that herbal medication had an influence on prolactin concentration.



Figure, 1: Statistics using GRAPH PAD PRISM 9 software

Table 3: Descriptive analysis of prolactin gene expression in pituitary gland

Table Analyzed	Prolactin gene expression
Group 1	Treated
vs.	vs.
Group 2	Control
Unpaired t test	
P value	0.0116
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=5.535, df=3
How big is the difference?	
Mean of Group 1	6.586
Mean of Group 2	16.43
Difference between means (G1 – G2) ± SEM	9.843 ± 1.778
95% confidence interval	4.184 to 15.50
R squared (eta squared)	0.9108

Reproductive hormone

The influence of Silymarin on progesterone and estrogen hormones was investigated in the present study. The findings demonstrate that progesterone levels are declining whereas estrogen levels are significantly gained in postnatal treated groups with Silymarin in all treated groups comparison to the control group (p 0.05) (Figure 2). This work supports the findings of (Peinnequin *etal*.,2004 ; Sriranga *etal*.,2021) showing prolactin has a variety of functions, including maintaining mammary gland production, acting in tandem with androgens, and affecting androgen metabolic activity. Furthermore, many studies have found that administration of aqueous extracts of medicinal herbs causes a considerable drop in progesterone levels, which is consistent with our findings (Adewale *etal*.,2014). In addition, the decline of Estrogen in present study indicate the inhibited effectiveness of Silymarin, whereas the progesterone raised on the gonadotrophs cells in pituitary gland. This finding akin with (yama *etal*.,2011) that describe the pituitary gonadal axis effectiveness with medicine herbs for maintenance of reproductive hormones.

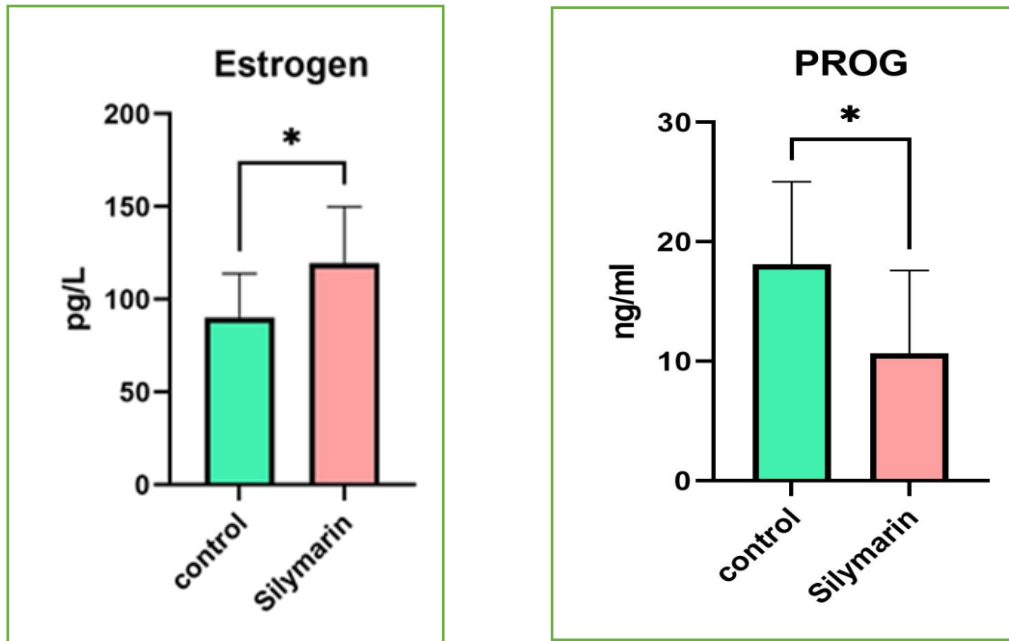


Figure 2: Role of orally intubated 200 mg/kg/day of silymarin on serum progesterone and estrogen level during lactation period on the female rats

Conclusion

The current study provides further evidence of Silymarin's participation in several physiological properties and the amount of sex hormones in serum, as well as an elevation in mRNA for prolactin hormone gene expression in the pituitary gland.

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