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The Role of Dill (Anethum greavelon) on Mammary Glands Performance During Lactation Period in Rats

A seminar submitted to the council of veterinary medicine at university of Diyala in partial fulfillment of requirement for the degree of Bachelors in surgery and veterinary medicine

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Acknowledgment

To candles that melted in pride …….

To illuminates every step in our path…..

To cringe every obstacle in front of us…….

They were messengers of science and ethics

Thank you all……..

Thanks to the dean of faculty of veterinary medicine, university of Diyala.

My deep gratitude to my advisor Dr. Muna M. ismail, head of the physiology and pharmacology department , college of veterinary medicine, Diyala university with my thanks and appreciation to each one assigning me to make this effort.

Dedication

To hit the message of the secretariat advised the nation……..

To the prophet of mercy and the light of the worlds……..

To the prophet Muhammad peace be upon him…..

To each of the illuminate mind his knowledge or other…thanks right answer puzzled saulah .

And I dedicate this to my research which says the two: (and lower to them Thewing of sub missing and humility through mercy and say the lord has decreased that you worship none small).

Diamonds which Ainser. Tender spring planting ethics in me and taught me always to improve good dad.

To Venus that do not wither ….spring tenderness of the words fail to describe and inhabit the waves of the sea to hear her name …mom.

I dedicate this humble effort to the alamamed God said to the messenger of Allah…praying God that lovgueni he loves and is pleased with all of you…..

Abstract

This investigation was designed to determine the effect of Dill alcoholic extract administration during the last trimester of gestation until the 11^{th} day of lactation on mammary glands .

Ten pregnant rats at 15^{th} day of gestation divided randomly into two group: control group received physiological normal saline daily from 15^{th} day of gestation till 11^{th} day of lactation. Treated group received alcoholic extract of dill daily in dose (0.017gm)from 15^{th} day of gestation till 11^{th} day of lactation.

At the 10^{th} day of lactation the litters were isolated from their mother overnight then reunited and allowed to suckling for 1hr then the mother and litters were weighted. They were anesthetized for blood collection and then sacrificed for mammary glands isolation from mother and stomachs of litters.

The results showed that Dill alcoholic extract produce relative increase in the mammary gland weight at the 11^{th} day of lactation; while the litter weight gain, litter stomach weight and prolactin hormone concentration recorded significant increase $p<0.01$ as compare to control group.

In conclusion, Dill alcoholic extract act as slightly mammogenic and highly lactogenic, probably by its indirect action on mammary gland.

Chapter one

Introduction

Dill is claimed to be a galactogogue (1). A galactogogue is an agent that promotes secretion and flow of milk(2). In several parts of the world, particularly in the developing countries with a heritage of folklore, herbal medicine has been practiced by practitioner of traditional medicine for enhancing the milk in lactating mother (1).

The effect of Dill alcoholic extract on mammary glands weight and milk production remain in a form of few unproved speculation; therefore this effort was made to throw light on the effect of Dill alcohol extract on following parameters at 11^{th} day of lactation:

1. mammary glands weight.
2. litter weight gain.
3. litter stomach weight.
4. prolactin estimation.

Chapter two

Review of literature

Anethum graveolens

Common name:*Dill*;

Order :*Apiales*;

Family :*Apiaceae*;

Genus :*Anethum*

Species: **Anethum graveolens**

Habitat : Dill is the native to south west Asia, Africa and Mediterranean region, now it is naturalized in many parts of Europe and USA. Dill prefer sun and sandy or loamy soil (3).

Description : Dill is an annual plant, growing up up 1.5 m in high. It has fern like ,pinnately divided, feathery leaves, about 0.3m long. Flower stems are stiff and hollow, flowers are born in large. Compound humbles they are small and numerous, yellow in color with tinny pestles yellow inward fruit a flat pod better in taste whole plant is strongly aromatic with taste similar to caraway.(3)**Medical use:** Dill is considered to have anti spasmodic , anticancer,

antibacterial, anti fungal, diuretic and carminative properties, dill oil is very useful in cases of hyperacidity, flatulence, indigestion and indigestion related diarrhea, its daily consumption promotes of blood boils and ulcer, combined with a fame oil, they can help ease swollen joint, seed are very helpful in treatment of bad breath and respiratory problem (such cold, influenza, its anti microbial activity can be helpful for lactation women, since it milk flow. Countries where its found: this plant is best found in the arctic circle which is regarded as most optimal place for the culture of dill through basically it originated from Russia European region (4).

Harvesting information: the arctic circle provides superb conditions for its cultivation. For the cultivation of the particular seed warm to hot summers are requires even a little less temperature can reduce the production. Hot sunshine is the foremost thing. Which it need to grow. These seeds work for three to ten years. Flowers head are bags from the supper part and left again in heat after getting loose from the stems. They are stored in conditions which are fully air light (3).

Compositions :

Anethum graveolens is known to contain: Dillanoside , psychotropic dillapiol and apoil, monterpenid ketodiols and glycosides, nitrate.

The bio actives of dill are not very well known at this moment in time, although it seems the essential oil component is fairly important and dill does have an appreciable nitrate content; while the essential oil fragment(found in seed and fruits)contain: carvone, dihydrocarvone, limonene, trepinene, phyellandrene, pinene, thymol and myristcin (5)

Table (1)Dill (Anithum graveolens) nutrition value:(5)

Principle	**Nutrient value**	**Vitamins**	**Nutrient value**	**Electrolytes**	**Nutrient value**
Energy	43kcal	Folate	150µg	Sodium	61mg
Carbohydrates	7g	Niacin	1.57mg	Potassium	738mg
Protein	3.46g	Pantothenic acid	0.39mg	Minerals	Nutrient value
Total fat	1.12g	Pyridoxine	0.185mg	Calcium	208mg
Cholesterol	0mg	Riboflavin	0.296mg	Copper	0.146mg
Dietary fiber	2.10g	Thiamin	0.058mg	Iron	6.59mg
		Vitamin A	7718IU	Magnesium	55mg
		Vitamine C	85mg	Phosphorus	66mg

Safety and toxicity:

Dill appear to interact with pregnancy, but currently there are no known toxic effects to the fetus or abortions induced from the supplement, while the recommended dose of Dill for is used as supplement is safe, there is toxicity associated with higher levels of dill and dosage where toxic effect are seen is low enough to feasibly be over consumed via food (6).

Mammary glands:

General view: mammary gland is one of the most important accessory glands of skin. It is distinguishing feature of mammals, and gets its name from mamma, the latin word of breast. The ultimate function of the mammary epithelium obviously is to produce milk, a secretion containing some substances that are synthesized only by this tissue(7).In rat and mouse they consist of flat sheets of tissue enveloping the body wall, in all female mammals except monotremes a nipple is present on each gland. Small nipples are present in males mammary gland of most

species except the mouse and rats, in whom the mammary duct end blindly (8).

The rate of mammary growth in rodents slows around 50-60days of age. Up to 40% of mammary growth take place during lactation in rats; in both mice and rats maximum mammary DNA content is reached by approximately day 10 of lactation. Additional development of mammary gland can induced by increasing milking frequency or suckling intensity.(9).

Mammary glands are accessory organ to female reproductive system, produce and secrete colostrums and milk to nourish the growing infants . the basic functional unit of mammary glands are glandular milk producing alveolar cells are surrounding by contractile myoepithelial cell, fibrous connective tissue and variable amount of adipose tissue (10).

Lactation: The synthesis and secretion of milk by female mammary glands. It is regulated by estrogen from mature follicles of the ovary and progesterone from corpus luteum and placenta and by prolactin hormone from anterior lobe of pituitary gland and oxytocin hormone from posterior lobe of pituitary gland(milk let-dawn) also response to autonomic nervous system reflex impulses (11).

Hormonal control on mammary gland growth and lactation

-puberty: estrogen,progesterone,glucocorticoid,insulin, growth hormone, prolactin.

-pregnancy:prolactin, progesterone, estrogen, glucocorticoid, insulin.

-lactation:prolactin, oxytocin, glucocorticoid.

Galactopoiesis: maintance of lactation, prolactin play important role in this process.(12)

Prolactin hormone :PLH lutotropin: Prolactin hormone release from anterior lobe of pituitary gland play important role in development of breast during pregnancy and also promote milk production. Prolactin hormone is controlled mainly by prolactin hormone inhibiting hormone from hypothalamus (dopamine).Prolactin inhibit gonadotropin hormones(FSH and LH).(12).

Chapter three

Materials and Methods

Preparation of dill alcoholic extract:

The seeds of dill were purchased from local market of Baghdad, the organic extract of seeds were prepared in pharma laboratory, college of veterinary medicine, Diyala university, by taking the 50gm of Dill and 300ml of ethanol as solvent by using soxhlet, the filtrate was evaporated at 70c^{o} .

Table (2):chemicals used in this study with their supplier and sources

Chemicals	Suppliers	Sources
Heparin	Leo pharmaceutica	Denmark
Ketamine	Holden	India
Xylazine	Xyla-ject	Egypt
Prolactin	Biomerieux	France

Table (3):instruments used in this study with their suppliers and sources

Instrument	Suppliers	Sources
Sensitive balance	Precisa	Swiss made
Soxhlet	Fine Tech	Korea
Centrifuge	Gallenkanp	England

Animals

a total number of 10 albino mature female rats were used in this investigation , they were fed ordinary pellet diet and had free access to food and water. The animals were kept at a temperature between 22-28c , the animals were housed as one male for two females in cages for mating , after that the pregnant animals were kept in cage individually until parturition and allowed to suckle their litters for the

first eleventh days of lactation at college of veterinary medicine /Diyala university . the light dark cycle was 12:12 , care was taken to avoid unnecessary stress as noise and cage crowding.

Experiment design

Ten female pregnant rats at last trimester of gestation were divided into two equal groups:

A .treated group: rats of this group were administrated dill alcoholic extract at dose (0.017 gm /kg bw) orally daily from beginning of last week of gestation until the 11^{th} day of lactation.

B. control group: rats of this group were administrated normal saline from beginning of last week of gestation until the 11^{th} day of lactation.

Parameters used in this investigation:

All following parameters were collected at 11^{th} day of lactation.

1.mammary gland weight as percent to body weight: after recorded of mother weight the mothers were sacrificed and mammary glands isolated.

Mgw ×100/bw

2.litters weight gain: at 10 day of lactation the litters were isolated from their mothers overnight, at the end of isolation the mother and young were reunited and the litters permitted to nurse for 1hr , after the nursing period the litters were weighted .

3.litter stomach weight : the litters were sacrificed and stomach isolated for weighted after anesthetized by ketamine xylazine.

4.prolactin hormone estimation: after weighted the mothers and before sacrificed the blood samples were collected for prolactin hormone estimation.

Blood collection:

After deep anesthesia by ketamine xylazine blood samples were obtained via cardiac puncture from each anesthetic rats control and treated using disposable syringe washed with heparin . samples were centrifuged at 3000 rpm for 15 min and then plasma samples were store in deep freezer till used. Each supernatant plasma was used for some biochemical determined such as total protein, globulin, triglycerides , cholesterol, glucose and some ions and hormones such calcium and prolactine. All these estimation were carried out at 11^{th} day of lactation for both control and treated rats.

Tissue sampling:

After longitudinal abdominal opining of animals the mammary glands tissues was then carefully dissected from the overlying skin, from each rat. All tissues samples were carried out between10:00am to12:00am to prevent post mortom changes.

Statistical analysis: the data were analyzed by T-test (13).

Chapter four

Results and Discussions

1.mammary glands weight %: table (4) revealed the presence of un significant $p>0.01$ increase in mammary glands size in treated group as compare to control.

This may be attributed that Dill alcoholic extract slightly increase the receptors for estrogen and progesterone hormones which they are responsible for ducts and alveoli of mammary glands respectively(14),resulted in slightly increase in its size.

2.litters weight gain: the effect of Dill alcoholic extract on litter weight gain of treated rats are shown in table (4). A significant increase $p<0.01$ was observed in treated rat as compare to control. This result may be interpreted that the increase in mammary gland weight mean increasing the mass of mammary tissues (15) and as a result increase the site of milk synthesis(mammary cells) and extraction from blood stream of main milk constituents such as protein, lipids, carbohydrate, minerals and water(16). Thus the litter weight gain may be had come indirectly from elevation of milk quality and or quantity which lead to increase growth rate of newborns.

3.stomach weight: the results in table (4).explain that dill alcoholic extract treated lead to a significant increase $p<0.01$ in stomach weight at eleventh day of lactation as compare to control. The weight of stomach(which reflect the milk obtained from nursing mother) is used as an index of lactation performance(17). After isolation and reuniting the mother and young, during this interval any milk present in stomachs of newborns was digested and mammary glands of the mother became turgid with milk. This mean that Dill alcoholic extract increase milk production

and reflected by increase milk obtaining.

4.prolactin hormone: the data pertaining to the prolactin concentration are depicted in table (4). Prolactin level at the eleventh day of lactation was significantly increase ($p<0.01$) higher in dill alcoholic extract treated group as compare to control.

The sites responsible for the release of prolactin from pituitary gland are number of lactophores (acidophil) in the anterior lobe of pituitary gland, they respond to hypothalamic prolactin releasing hormone or to prolactin inhibiting hormone (dopamine)which increase or decrease its pituitary secretion respectively (18). Thus , dill alcoholic extract possibly either increases the number of lactophores or their secretory activity of and then stimulate the release of prolactin from these cells.or it may inhibit the dopamine and increase the prolactin hormone releasing hormone.

Table (4):effect of Dill alcoholic extract administration daily and orally (0.017gm) from the last week of gestation until 11th day of lactation on mammary glands weight, litter weight gain, stomach weight and prolactin value.

Parameters	**Control**	**Treated**
Mammary glands%	5.88±0.899	6.63±0.929 **a**
Litter weight gain	13.68±0.849	18.00±0.363 **A**
Stomach weight	0.416±0.036	0.582±0.051 **A**
Prolactin hormone	0.8±0.31	1.5±0.18 **A**

Capital litters denote significant differences $p<0.01$ between control and treated group.

Small litters denote un significant differences $p>0.01$ between control and treated group.

Chapter five

Conclusions and Recommendations

Conclusions :

1.Dill alcoholic extract is slightly mammaogenic herb through slightly increase in mammary gland weight.

2.Dill alcoholic extract is highly lactogenic herb though significantly increase in litter weight gain and litter stomach weight.

Recommendations:

1.study the effect of Dill aqueous extract and compare with Dill alcoholic extract results of this study.

2.survay other herbs that are traditionally named as galactogogues.

3.study the various active ingredients of Dill to find the exactly galactogogual part.

4.apply this study on farm animals.

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جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية الطب البيطري

دور المستخلص الكحولي لنبات الشبنت في كفاءة الغدد اللبنية خلال فترة الرضاعة في الجرذان

بحث مقدم إلى مجلس كلية الطب البيطري جامعة ديالى كأحد متطلبات نيل شهادة البكالوريوس في الجراحة والطب البيطري

من قبل الطالبة

نور محمود أحمد

بأشراف

د.منى محمد إسماعيل

2014

الخلاصة

استهدفت هذه الدراسة التعرف على دور المستخلص الكحولي لنبات الشبنت نمو وتطور الغدد اللبنية خلال فترة الرضاعة والذي ينعكس على كمية الحليب وأوزان المواليد.

تم استخدام عشرة حيوانات أنثى جرذ حامل قسمت عشوائيا إلى مجموعتين : مجموعة سيطرة 5 حيوانات جرعة يوميا بمحلول الملحي الفسلجي منذ بداية الثلث الأخير من الحمل ولغاية اليوم الحادي عشر من الرضاعة، ومجموعة معاملة 5 حيوانات جرعت يوميا بمستخلص الكحولي لنبات الشبنت بجرعة 0.017 غم يوميا ومنذ بداية الثلث الأخير من الحمل ولغاية اليوم الحادي عش من الرضاعة . وقد تم اعتماد المعايير التالية في تقييم كفاءة الغدد اللبنية:

1.أوزان الغدد اللبنية نسبة إلى وزن الأمهات 2 .أوزان المواليد

3.أوزان المعدة للمواليد 4.مستوى هرمون الحليب (البرولاكتين)

تم فصل الأم عن المواليد في اليوم العاشر من الرضاعة لمدة 12 ساعة لتجويع المواليد من جهة ولتجميع الحليب في الغدد اللبنية للام من جهة ثانية وفي اليوم التالي أعيدت المواليد للام وتركت لترضع لمدة ساعة ، بعدها تم تخدير الحيوانات الأمهات والمواليد و وسجلت أوزانها ثم تم جمع عينات الدم من الأمهات لغرض تقييم هرمون الحليب و قتل الأمهات والمواليد وعزل الغدد اللبنية و المعدة على التوالي وتم تسجيل الأوزان.

فقد أظهرت النتائج إن المستخلص الكحولي لنبات الشبنت أدى إلى زيادة غير معنوية في أوزان الغدد اللبنية لمجموعة المعاملة مقارنة مع مجموعة السيطرة في حين سجلت أوزان المواليد وأوزان المعدة لمواليد مجموعة المعاملة و مستوى هرمون البرولاكتين زيادة معنوية على مستوى 0.01 مقارنة مع مجموعة السيطرة.

يستنتج من هذه الدراسة ان نبات الشبنت ادى الى تحسين كفاءة الغدد اللبنية خلال فترة الرضاعة .