

The Role of Dill (*Anethum graveolon*) Alcoholic Extract on Mammary Glands Performance and Some Plasma Biochemical Parameters During Lactation Period in Rats

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Abstract

This investigation was designed to determine the effect of Dill alcoholic extract administration during the last trimester of gestation until the 11th day of lactation on milk quantity and quality. Ten pregnant rats at 15th day of gestation divided randomly into two groups: control group received physiological normal saline daily from 15th day of gestation till 11th day of lactation. Treated group received alcoholic extract of dill daily in dose (0.017g) from 15th day of gestation till 11th day of lactation. At the 10th 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 estimated some plasma milk related component like plasma glucose, triglycerides, cholesterol, total protein, albumin, globulin and calcium which they reflect on milk quality then the mothers and litters were sacrificed for mammary glands isolation and stomachs of litters in order to estimate the milk quantity. The results showed that Dill alcoholic extract produce relative increase in the mammary gland weight at the 11th day of lactation; while the litter weight gain, litter stomach weight and prolactin hormone concentration recorded significant increase $p < 0.01$ as compared to control.

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group. The results of biochemical study revealed that Dill alcoholic extract caused a significant increase in plasma globulin, triglycerides and calcium at eleventh day of lactation while plasma total protein, albumin, cholesterol and glucose showed un significantly increase in treated group as compare to control.

In conclusion, Dill alcoholic extract act as slightly mammogenic and highly lactogenic, probably by its indirect action on mammary gland and the Dill alcoholic extract increase the quality of milk through the enhancement the extraction of some essential milk component from plasma.

Key words: Dill, milk quantity and quality, mammary gland, prolactin hormone, milk component, rats.

دور المستخلص الكحولي لنبات الشبنت في كفاءة الغدد اللبنية وعلى بعض المعايير البايوكيميائية بلازما الدم خلال فترة الرضاعة في الجرذان

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الخلاصة

استهدفت هذه الدراسة التعرف على دور المستخلص الكحولي الخام لنبات الشبنت في نمو وتطور الغدد اللبنية خلال فترة الرضاعة والذي ينعكس على اوزان الغدد اللبنية و كمية الحليب وأوزان المواليد بالاضافة على بعض مكونات بلازما الدم والذي يعكس محتويات الحليب وبالتالي نوعية الحليب. تم استخدام عشرة حيوانات أنثى جرد حامل قسمت عشوائيا إلى مجموعتين : مجموعة سيطرة 5 حيوانات جرعت يوميا بمحلول الملح 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 عشر 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)

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5. البروتين الكلي 6. الألبومين 7. الكلوبولين 8. الكلوكرز 9. الكولسترول 10. الكلسيريدات الثلاثية 11. الكالسيوم
تم فصل الأم عن المواليد في اليوم العاشر من الرضاعة لمدة 12 ساعة لتجويد المواليد من جهة ولتجميع الحليب في الغدد اللبنية للام من جهة ثانية وفي اليوم التالي أعيدت المواليد للام وتركت ترضع لمدة ساعة ، بعدها تم تخدير الحيوانات الأمهات والمواليد و سجلت أوزانها ثم تم جمع عينات الدم من الأمهات لغرض تقييم هرمون الحليب والمعايير البايوكيميائية الأخرى وقتل 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 بينما أظهرت نتائج الفحوصات البايوكيميائية إن المستخلص الكحولي لنبات الشبنت أدى إلى زيادة معنوية في كل من الكلوبولين والكلسيريدات الثلاثية والكالسيوم في حين شهد كل من البروتين الكلي والألبومين زيادة غير معنوية بينما أظهرت النتائج ان هناك انخفاض غير معنوي في الكولسترول والألبومين . يستنتج من هذه الدراسة ان نبات الشبنت ادى الى تحسين كفاءة الغدد اللبنية خلال فترة الرضاعة في استخلاص مكونات بلازما الدم بصورة غير مباشرة ربما من خلال تحفيز الهرمونات وبالتالي زيادة محتويات الحليب من البروتينات والدهون والسكريات والأملاح والذي ينعكس ايجابيا على نوعية وكمية الحليب.

الكلمات المفتاحية: الشبنت، نوعية وكمية الحليب، الغدد اللبنية، هرمون البرولاكتين، مكونات الحليب، الجرذان

Introduction

Dill is claimed to be a galactagogue . A galactagogue is an agent that promotes secretion and flow of milk(1). 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 (2). Dill is a food product appear to have some traditional medicine usage for gastrointestinal health, urinary, heart and feminine health related to lactation(2). Many plant and herbs are known to be galactoguge , among these plant is Dill which has been employed as folk remedy to increase milk production since ancient centuries(3). 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 11th day of lactation: mammary glands weight, litter weight gain, litter stomach weight, prolactin hormone, plasma glucose, triglycerides,

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cholesterol, total protein, globulin, albumin and calcium. *Anethum graveolens*, Common name: *Dill*; Order: *Apiales*; Family: *Apiaceae*; Genus: *Anethum*, Species: ***Anethum graveolens***
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).

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 myristicin (5). 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 (6). The rate of mammary growth in rodents slows around 50-60 days 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. (6).

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 (7).

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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-down) also response to autonomic nervous system reflex impulses (8).

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: maintenance of lactation, prolactin play important role in this process.

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 inhibiting hormone from hypothalamus (dopamine). Prolactin inhibit gonadotropin hormones (FSH and LH). (8,9).

Equilibrium between blood and milk:

1. milk and blood have the same osmotic pressure (6.6 atmospheres), however they are not in equilibrium. Milk contain 90x as much sugar, 9x as much fat, 1/2 as much protein, 1/7 as much Na, 5x as much K, 13x as much Ca and 10x as much as blood plasma.

2. lactose in milk account for about 50% of osmotic pressure of milk, and Na, K and Cl account for the rest (10).

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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° .

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

Parameters used in this investigation:All following parameters were collected at 11th 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.

$$\text{Mgw} \times 100/\text{Bw}$$

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- 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.
- litter stomach weight: the litters were sacrificed and stomach isolated for weighted after anesthetized by ketamine xylazine.
- prolactin hormone estimation: after weighted the mothers and before sacrificed the blood samples were collected for prolactin hormone estimation, Plasma total protein, plasma albumin, plasma globulin, plasma glucose, plasma cholesterol, plasma triglycerides and plasma calcium.

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, albumin, triglycerides, cholesterol, glucose and some ions and hormones such calcium and prolactine. All these estimation were carried out at 11th day of lactation for both control and treated rats.

Tissue sampling: After longitudinal abdominal opening of animals the mammary glands tissues was then carefully dissected from the overlying skin, from each rat. All tissues samples were carried out between 10:00am to 12:00am to prevent post mortom changes.

Statistical analysis: the data were analyzed by T-test (11).

Results and Discussions

- mammry glands weight %: table (1) 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 (6,12), resulted in slightly increase in its size.

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3. litter weight gain: the effect of Dill alcoholic extract on litter weight gain of treated rats are shown in table (1). 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 (13) 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 (10). 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.
4. Stomach weight: the results in table (1). 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 (14). 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.
5. prolactin hormone: the data pertaining to the prolactin concentration are depicted in table (1). 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 (15,16). 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.

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Table (1): effect of Dill alcoholic extract administration daily and orally (0.017 gm) 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 letters denote significant differences $p < 0.01$ between control and treated group.

1. plasma total protein: Plasma total protein levels in control and treated rats are represented in table (2). The results showed that Dill alcoholic extract caused a significant increase $p < 0.01$ in plasma total protein of treated group at 11th day of lactation. Mammary glands are unique organs that can synthesis milk proteins in ribosomes on rough endoplasmic reticulum of their alveoli. The elevation of plasma proteins at eleventh day of lactation can be accounted for by the transfer of protein from extravascular sources. Experimental evidence indicated that intravascular and extravascular protein are in state of dynamic equilibrium (17); a decrease in the concentration of one compartment results in shifting of protein from the other compartment. Therefore tissue proteins of muscle and liver are often degrade in addition to enhanced intestinal absorption to provide for maintains of plasma protein level.
2. plasma albumin: the results in table (2) explain that there was un significant increase in its concentration of treated group as compare to control. The plasma proteins occupy a central and dominant position in metabolism of protein, because of their intimate

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3. relation to metabolism in the liver as well as their interaction with other tissues like mammary gland (17) so the un significant increase may be due to that Dill alcohol extract increase extraction of albumin by mammary gland.
4. plasma globulin: the effect of Dill alcoholic extract on plasma globulin concentration of treated rats are shown in table (2). A significant increase $p < 0.01$ was observed in plasma at eleventh day of lactation in treated rats. Plasma globulin are divided into three major types: alfa, beta and gamma globulin (17); the alfa and beta globulin which synthesis by the liver perform divers function in the circulation, such as transporting other substances and transporting of protein itself from one part of the body to another, on the other hand gamma globulin are synthesized by lymphocytes, plasma cell and transition cell lymph node, spleen and bone marrow (17). In general, an increase in globulin concentration may be due to that Dill alcoholic extract increase antibody titer which play important role in protecting the newborn against infection.
5. plasma glucose: the data pertaining to plasma glucose concentration of control and treated rats are dedicated in table (2) showed un significant decrease in treated group. The main milk carbohydrate is lactose, which is formed from glucose and galactose under the influence of lactose synthetase, lactose synthesis is dependant on milk protein synthesis, particularly lacoalbumin, which is the major component of lactose synthetase (18) so that the slightly reduction may be due to that dill alcoholic extract increase extraction of glucose by mammary gland.
6. plasma cholesterol: values of total plasma cholesterol level of treated group at eleventh day of lactation are illustrated in table (2) un significant decrease. Cholesterol is different from triglycerides in that its basic structure is sterol nucleus (degradation of fatty acids) other than fatty acids. Cholesterol is present in all tissue cells and their plasma membrane for their fluidity (18) so the reduction may comes from its corporation for synthesis of steroid hormone or due to its extraction by mammary glands.

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7. plasma triglycerides: the present study demonstrated elevation in plasma triglycerides concentration at 11th day of lactation table(2). The lipids of metabolic significance in the mammalian organism include triglycerides (neutral fat) which is biologically important and represent the major milk fat constituents (18). The alveolar cells of mammary tissues can synthesis short and medium chain fatty acids of milk triglycerides, mean while , most long chain fatty acids are derived from dietary fat, and transported in the blood to mammary tissue as triglycerol; so the significant increase in its concentration may be due to that Dill alcoholic extract enhanced intestinal absorption of triglycerides ; on the other hand may due to elevation of thyroxin hormone during lactation which is one of the necessary hormone for maintance of lactation through its positive effect on milk fat content and milk quality in addition to its lipolysis effect on adipose tissue .
8. plasma calcium: table (2) illustrated the concentration of plasma calcium in treated and control rats which appear increased significantly in treated group. Milk contain many minerals, including calcium ion(9,10) thus the elevation in its concentration may be due to that Dill alcoholic extract enhance intestinal absorption of calcium, or the Dill alcoholic extract inter act with parathormone hormone which elevates plasma calcium by increasing bone resorption, intestinal absorption and kidney re absorption of calcium

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Table (2): The Effect of Dill alcoholic extract 0.017gm from the last week of gestation till 11th day of some plasma biochemical parameters during lactation in rats

Parameters	Control	Treated
Total protein	6.15±0.111	6.821±0.229 a
Albumin	4.55±0.123	4.753±0.167 a
Globulin	1.60±0.071	2.068±0.092 A
Glucose	102.71±1.123	102.52±4.96 a
Cholesterol	128.49±3.98	128.08±6.97 a
Triglycerides	101.39±5.01	129.86±7.01 A
Calcium	5.50±0.39	8.33±0.8 A

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

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.
3. Dill alcoholic extract enhanced the ability of mammary gland in extraction of essential component from plasma for milk synthesis and finally increase milk quality.

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