# THE CHANGES IN SOME PLASMA COMPONENTS DURING LACTATION PEROID UNDER EFFECT OF ANISEED OIL IN RATS

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## ABSTRACT

This study was designed to determine the effects of aniseed oil administration during the last trimester of gestation on some plasma biochemical constituents changes at the first and eleventh days of lactation in rats including: Total proteins, triglycerides, total cholesterol, glucose and some ions calcium, phosphorus, magnesium, sodium. Twenty pregnant rats, at the 15$^{th}$ day of pregnancy, were randomly divided into two equal groups: control group (10 rats) received tap water and treated group (10 rats) received aniseed oil (0.043 g kg$^{-1}$ B.w) orally daily during the last week of gestation. After parturition the litters were set (6 pups mother$^{-1}$) the mother were anesthetized and the blood samples were collected for estimation of some biochemical constituents in the plasma then the mothers allowed to lactated their litters for eleventh day of lactation and blood samples were collected also for estimation of some plasma parameters which mentioned above, The results of biochemical study revealed that aniseed oil lead to a significant reduction at the first day of lactation in all parameters with exception of plasma, Na ion concentration increased in the treated group at the first and eleventh day of lactation. In conclusion, aniseed oil enhanced extraction of milk constituents from plasma which lead to increase milk quality.

**Key words:** aniseed oil, lactation, milk components, rat.

## INTRODUCTION

Milk is composed of water, protein, fat, carbohydrate (lactose), minerals and vitamins. Milk is secreted as a complex mixture of these components. The first secretion to be collected from the gland is called colostrum, which is produced upon delivery of newborn, is important to the offspring and colostroum may have 1.5 times as much fat and up to 40 times as much protein as normal milk. Most of this protein is globulins, which are antibodies necessary for passive immunity against disease until the young develops its own immunity (Mourad *et al.*, 2014). It contains lymphocytes and monocytes that protect against exposure to infection. It also contains a greatly increased amount of protein and high levels of vitamins A, E, carotene and riboflavin. However, lactose, vitamin D and iron are all low

compared to normal milk. The composition of the colostrum secretion gradually changes to that of mature milk (Mather, 2000). A galactagogue is an agent that promotes secretion and flow of milk (Ballard and Morrow, 2013). In several parts of the world, particularly in the developing countries with a heritage of folklore, herbal medicine has been practiced by the practitioners of traditional medicine, for enhancing the milk secretion in lactating mothers and many plants and herbs are known to be galactagogues, among these, is the plant aniseed, which has been employed as a folk remedy to increase milk production since ancient centuries (Westfall, 2003). The galactogogual role of crude extract of anise seeds was proved by Al-Saadi (1997) who concluded that aniseed extract administration (0.152 g $kg^{-1}$ B.wt during the last trimester of gestation in rats) has a positive effect on rat's mammary gland development and performance. On the other hand, Al-Jubori (1999) found that anise extract stimulates mammary gland growth when given at prepuberal time. This effect was extended both at postpubertal time and during lactation. However, the effects of anise oil on certain plasma milk-related biochemical parameters remain in a form of few unproved speculations. Therefore, this effort was made to throw light on the effects of anise oil on: Some biochemical parameters in plasma at the first and eleventh day of lactation including: a. The plasma concentrations of total proteins, triglycerides, total cholesterol and glucose. b. The plasma concentrations of Ca, P, Mg and Na ions.

## MATERIALS AND METHODS

Experimental design: Twenty, Norway albino female rats, at last trimester of gestation, were used in this experiment their average weight ranged between 220 - 270 g. The aniseed oil was given orogastrically to the experimental rats and in a dose of 0.043 g $kg^{-1}$ B.wt daily for one week (last week of gestation); control rats received the same volume of tap water as a placebo, under similar condition. They were fed ordinary pellet diet. The animals were kept at a temperature between 23-28 °C. The animals were housed as one male for each five females in wire meshed stainless steel cages (56, 40, 17) for mating, after that the pregnant animals kept in cages (33, 15, 13) individually until parturition and others allowed to suckle their litters for the first eleventh days of lactation, at the Iraqi Center for Cancer and Medical Genetic Researches in Baghdad. The light and dark cycle was 12:12 hr. Animal had free access to food and water. Care was taken to avoid unnecessary stress as noise and cage crowding.

**Preparation of aniseed oil:** The seeds of anise were purchased from the local market and authorized in the "Iraqi National Herbarium". The seeds of herb were cleaned and ground in a grinder and pressed by mechanical hydraulic press (H. Fisher and Co. norf, Germany) without heating under pressure of 400 b. The yield was 80 ml of aniseed oil from 5 kg of aniseeds.

**Blood sampling:** After deep anesthesia by diethyl ether (BDH Chemicals Ltd, England), blood samples were obtained via cardiac puncture from each anaesthetized rat (control and experimental) using disposable syringes washed with heparin (Leo pharmaceutica products, Denmark). Samples were centrifuged (Gallenkanp, England) at 3000 rpm for 15minutes, and then plasma samples were stored in deep freeze till use. Each supernatant plasma was used for some biochemical determination such as total proteins, triglycerides, cholesterol, glucose; some ions such as Ca, P, Mg, Na. All of these estimations were carried out at 1$^{st}$ day and 11$^{th}$ days of lactation for both control and experimental rats. All blood plasma biochemical parameters were determined photometrically using test kits (Bio diagnostic Co).

**Statistical analysis:** Statistical analysis of data was performed on the basis of either one way or two-way analysis of variance (ANOVA), depending on the experimental design at each time. Specific group differences were determined using least significant difference (LSD) test (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

**Plasma total proteins concentration:** Plasma total proteins levels in control and aniseed oil treated rats are presented in table 1. The results showed that aniseed oil caused a significant decrease ($p<0.05$) in level of plasma total proteins at the first day of lactation as compared to control. On the other hand, there was a significant increase ($p<0.05$) in plasma total proteins of treated group at eleventh day of lactation. Whereas in the control group there was no significant change in the plasma total protein with time. The plasma proteins occupy a central and dominant position in the metabolism of proteins, because of their intimate relation to metabolism in the liver, as well as their interaction with other tissues like mammary gland beside mammary glands are unique organs that can synthesis milk proteins in ribosome on the RER of their secretory epithelial cell. They also can derive essential amino acids from blood to be incorporated in the process of protein biosynthesis (Ballard and Morrow, 2013). So the reduction of plasma protein at the

first day of lactation may be attributed largely to that anise can increase the ability of mammary gland to supply itself by the essential elements (probably by enhancing the transport mechanism from blood to tissue) that support the increased milk protein yields. This process is achieved by increasing extraction efficiency of essential amino acids which were linearly related to their arterial concentration (Mackle *et al.*, 2000). Yet, it has been mentioned that crude anise seeds extract increase DNA and RNA concentration in the mammary gland during lactation (Al-Saadi, 1997). An increased DNA and RNA concentrations indirectly reflects enhancement in the process of protein synthesis to match the increased demand of milk and secretion in the lactating mothers (Westfall, 2003). While 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 proteins are in a state of dynamic equilibrium and 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 maintenance of plasma protein level (Dandotiya *et al.*, 2013).

**Plasma triglycerides concentration:** The results in table 1 explain that aniseed oil treatment lead to a significant decrease ($p<0.05$) in plasma triglycerides concentration at the first day of lactation as compared to the control. While there were no significant increases ($p>0.05$) at eleventh day of lactation between the two groups. Furthermore, there was a significant increase ($p<0.05$) in plasma triglycerides concentrations with time in both treated and control rats. The lipids of metabolic significance in the mammalian organism include triglycerides (neutral fat) which are biologically important and represent the major milk fat constituents (Ganter *et al.*, 2015). The alveolar cells of mammary tissue can synthesis short and medium chain fatty acids of milk triglycerides. Meanwhile, most of the long chain fatty acids are derived from dietary fat and transported in the blood to the mammary tissues as triglycerol in chylomicrons (Mourad *et al.*, 2014).

The reduction of plasma triglycerides at the first day of lactation in the treated rats can be attributed to that aniseed oil tends to increase triacylglycerol extraction by the mammary gland which correlates with their respective arteriovnous differences (Ismail *et al.*, 2016). Thyroxin is one of the necessary hormone for maintenance of lactation through its positive effect on milk fat content and milk quantity in addition, thyroxin causes lipolysis in adipose tissue (by direct effect on

adenyl cyclase and through increase sensitization to other factors, which hydrolyze lipids, like catacholomines, growth hormone and glucocorticoides) which results in increased plasma triglycerides (Bazzano *et al*., 2016). Therefore, the increased triglycerides at the eleventh day of lactation can be related to enhanced thyroxin activity during lactation.

**Plasma total cholesterol concentration:** Values of plasma total cholesterol level of treated and control groups at first and eleventh day of lactation, are illustrated in table 1. Plasma cholesterol level decreased significantly ($p<0.05$) in the treated animals at the first day of lactation as compared to control. While its levels increased significantly in the treated group at the eleventh day of lactation as compared to control. With time, the total plasma cholesterol levels of control rats showed a significant decrease, but did not change significantly in the treated group. Cholesterol is different from triglycerides in that its basic structure is a sterol nucleus (degradation of fatty acids) other than fatty acids (Ganter *et al.*, 2015). Cholesterol is present in all tissue cells and their plasma membranes for their fluidity (Vandar *et al*., 1998). A number of in vivo and in vitro studies indicated that cholesterol serves as a direct or indirect precursor in the synthesis of spectrum of steroid hormones including ovarian, testicular and adrenocorical steroid hormones (Guyton, 1996). So, the reduction of total cholesterol at the first day of lactation in treated rats could have come as a result of lowering action of estradiol, probably due to an action of hormone on lipoproteins associated with cholesterol in the circulation (Ismail *et al.*, 2016). The reduction of cholesterol could be also due to an increase in its extraction by the mammary cells for their metabolism and plasma membrane synthesis which undergo high proliferate activity (Budzynska *et al.*, 2012). At the eleventh day of lactation, the total cholesterol concentration was elevated. This also could be due to the elevation of thyroxin, which causes lipolysis in the adipose tissue and as a result, increases releasing of fatty acids and cholesterol to the blood stream (Ganter *et al.*, 2015).

**Plasma glucose concentration:** The data pertaining to plasma glucose concentration of control and aniseed oil treated rats are depicted in table 1. Plasma glucose concentration showed a significant decrease ($p<0.05$) in treated group as compared to the control rats at the first day of lactation. Values of eleventh day of lactation of treated rats were significantly higher than those of control rats. No significant decrease ($p>0.05$) were observed between the first and eleventh day of

lactation in plasma glucose level of treated rats, while were significantly decreased at the eleventh day of lactation in the control group. The main milk carbohydrate is lactose, which is formed from glucose and galactose under the influences of lactose synthatase, lactose synthesis is dependent on milk protein synthesis, particularly-lactalbumin, which is the major component of lactose synthase (Shojaii and Fard, 2012). So that, the reduction of plasma glucose concentration at the first day of lactation (accompanied by reduction of plasma total proteins) may be explained largely by that aniseed oil can increase the ability of mammary glands to supply itself by essential elements that support milk synthesis by extraction of amino acids and glucose from blood circulation. The elevation of glucose at the eleventh day of lactation however, could be accounted for by the effect of various diabetogenic hormones which are associated with lactation such as, thyroid hormones, glucocorticoids and growth hormone (Chard *et al.*, 2016). The principle hyperglycemic effect of thyroid hormone is to increase absorption of glucose from the intestine, but the hormone also cause (probably through liberation of epinephrine) some degree of hepatic glycogen depletion whereas the major diabetogenic effect of glucocorticoid is an increase in protein catabolism with increased gluconeogenesis in the liver and (with growth hormone) decrease in peripheral glucose utilization that may be due to inhibition of glucose phosphrylation (McKee and McKee, 1996).

**Plasma calcium ion concentration:** The effect of anise oil administration during the last trimester on plasma Ca concentration is shown in table 2. In particular, there was a marked decrease ($p<0.05$) in plasma Ca concentration in treated rats as compared to control at the first day of lactation. On the other hand, it increased significantly ($p<0.05$) at the eleventh day of lactation in the treated group when compared with the control. Furthermore, its concentration increased significantly ($p<0.05$) at the eleventh day vs. the first day of lactation in the treated group. Its level decreased significantly ($p<0.05$) with time in the control group. Milk contains many minerals, including Ca ion, which causes a considerable loss that occurs in the milk of lactating animals (De Santiago *et al.*, 2002). Total plasma Ca concentration is an affected by total plasma protein concentration particularly the protein bound Ca (Stamp *et al.*, 2000). Thus, the reduction of plasma Ca concentration at the first day of lactation can be due to, either the reduction of plasma total protein (recorded in this study), or due to an increase in its extraction from the circulation by the mammary gland. During lactation, extraction of plasma

Ca by mammary gland continuous. This necessities the presence of an effective mechanism to keep the supply of sufficient amounts of Ca to the plasma. Such mechanism could occur probably under the effect of parathyroid hormone (parathormone) which elevates plasma Ca by increasing bone resorption, intestinal absorption and kidney reabsorption of Ca (Strewler, 2000). The elevation of plasma Ca in treated rats at the eleventh day of lactation may also come from the increased prolactin hormones, This hormone stimulate the activity of 1 hydroxylase enzyme which lead to increase 1,25 Dihydroxycholichalcepherol. Latter, in turn enhances bone resorption, intestinal absorption and renal reabsorption of Ca leading finally to an increase in plasma Ca concentration (Alshammari *et al.*, 2017).

**Plasma phosphorus ion concentration:** At the first day of lactation plasma P ion concentration decreased significantly ($p<0.05$) in treated group table 2. Upon the administration of 0.043 g $Kg^{-1}$ B.wt, during the last trimester of gestation, there was a significant decrease ($p<0.05$) in the in plasma P concentration of treated group at the first day of lactation as compared to control. Meanwhile, there was a significant increased ($p<0.05$) in its value at the eleventh day of lactation in comparison with the control group. Besides, its concentration increased significantly ($p<0.05$) in the treated group at the eleventh day vs. the first day of lactation. Its level decreased significantly ($p<0.05$) with time in the control group. P is an important constituent of bone, nucleic acids, phospholipids, nucleotides and milk. The latter is the route for a considerable loss of P from blood (Ganong, 1996). The reduction of P at the first day of lactation may be due to an increase in its extraction by the mammary gland to be added to milk constituents and used in mammary cell plasma membrane synthesis. The plasma P concentration is under the control of parathormone, calcitonin and vitamin D. However, there is a certain degree of physico-chemical control, which depends on the solubility equilibrium between skeletal P and extracellular fluid P that bathes bone crystals. These bone crystals are described as having labile and stable fraction. The labile crystals readily exchange P with plasma, rapidly sequester foreign ion and make a net contribution of P and other ions by means of strictly physico-chemical process (Alshammari *et al.*, 2017). Therefore, may elevate plasma P ion concentration at the eleventh day of lactation. Therefore, this mechanism, in one way or another could be related to the increased plasma P of lactating rats.

**Plasma magnesium ion concentration:** Table 2 illustrate the concentration of plasma Mg ion in aniseed oil treated and control rats. There was a non-significant decrease ($p>0.05$) in plasma Mg ion level of treated rats at the first day of lactation as compared to the control. However, it increased significantly ($p<0.05$) in treated group as compared to the control group at the eleventh day of lactation. Moreover, its level decreased ($p<0.05$) with time in the control group while it increased ($p<0.05$) with time in the treated group. Another major cation in the body is Mg, which has a considerable amount been lost in the milk (Ganong, 1996). The synthesis of all proteins, nucleic acids, nucleotides, lipids and carbohydrates require the presence of Mg ion, so, the decrease in Mg ion at the first day of lactation in treated rats may result from enhanced mammary cell extraction of Mg for synthesis of various milk constituents. Although there is no specific hormone that controls Mg levels, previous evidence suggested that hypomagnesaemia stimulates release of parathormone (Haenel and Mayfield, 2000 ; Udelsman, 2002). Thus, the elevation of Mg ion at the eleventh day of lactation may be due to that aniseed oil stimulates parathormone secretion leading to increase Mg level.

**Plasma sodium ion concentration:** The results in table 2 explain that Na ion concentration at the first day of lactation in treated rats increased significantly ($p<0.05$) as compared to the control. Statistical differences were absent at eleventh day of lactation between treated and control rats. Furthermore, Na ion concentration increased with time in the control, but not in the treated group. Na ion is the major cation in the extracellular fluid and it is important in the maintenance of osmotic pressure of body fluids (Granner, 2000). Although Na ion excretion though the kidney is controlled by aldosterone hormone from adrenal cortex (Ganong, 1995; Guyton, 1996). Yet, and according to Chard *et al.*, (2016), hyponatremia may be occurring with hyperglycemia (in which extracellular fluid osmolarity is increased by glucose) and the response is Na excretion to avoid creating hyperosmolarity. So the reduction of glucose at the first day of lactation (recorded in this study) in addition to the body fluid loss during lactation, lead to increase Na ion concentration to keep the normal blood osmolarity, or may be the elevation of Na ion concentration is due to that aniseed oil stimulates aldosterone secretion and as a result the Na ion reabsorption is increased through the kidney.

**Table 1. The changes in some plasma biochemical components concentration during lactation period under effect of aniseed oil in rats**

| Groups / Parameters | Control 1st day of lactation | Treatment 1st day of lactation | Control 11th day of lactation | Treatment 11th day of lactation | LSD values |
|---|---|---|---|---|---|
| Plasma Total protein (gm $dl^{-1}$) | 6.3±0.130 Aa | 4.9±0.063 Ba | 3.7±0.083 Bb | 4.5±0.070 Aa | 0.741 |
| Plasma tiglycerides (mg $dl^{-1}$) | 47 ± 0.83 Ab | 39 ± 2.00 Bb | 66 ± 0.77 Aa | 71 ± 0.90 Aa | 5.205 |
| Plasma cholesterol (mg $dl^{-1}$) | 85 ± 1.707 Aa | 74 ±1.371 Ba | 63 ±1.54 Bb | 71 ±1.54 Aa | 5.292 |
| Plasma glucose (mg $dl^{-1}$) | 105 ± 1.00 Aa | 96 ± 0.632 Ba | 86 ± 1.301 Bb | 96 ± 0.661 Aa | 5.453 |

Values are expressed as mean ± SE. n=5 group.

Capital letters denote significant differences, ($p<0.05$) between control and treatment groups.

Small letters denote within group differences, ($p<0.05$) at first day vs. eleventh day of lactation.

**Table 2. The changes in some plasma ions concentration during lactation period under effect of aniseed oil in rats**

| Groups / Parameters | Control 1st day of lactation | Treatment 1st day of lactation | Control 11th day of lactation | Treatment 11th day of lactation | LSD values |
|---|---|---|---|---|---|
| Plasma calcium ion concentration (mg $dl^{-1}$) | 10.5 ± 0.13 Aa | 8.5 ± 0.04 Ba | 7.9 ± 0.22 Ab | 10.9 ± 0.17 Bb | 2.123 |
| Plasma hosphorous ion concentration (mg $dl^{-1}$) | 5.0 ± 0.09 Aa | 1.1 ±0.15 Bb | 4.1 ± 0.17 Bb | 5.0 ± 0.14 Aa | 0.811 |
| Plasma magnesium ion concentration (mg $dl^{-1}$) | 1.9 ± 0.11 Aa | 1.6 ± 0.09 Ab | 1.4 ± 0.70 Bb | 2.2 ± 0.99 Aa | 0.4 |
| Plasma sodium ion concentration (Equ $L^{-1}$) | 142± 0.75 Bb | 154 ± 0.60 Aa | 152 ± 0.78 Aa | 153 ± 0.54 Aa | 0.881 |

Values are expressed as mean ± SE. n=5 $group^{-1}$.

Capital letters denote significant differences, ($p<0.05$) between control and treatment groups.

Small letters denote within group differences, ($p<0.05$) at first day vs. eleventh day of lactation.

## CONCLUSSION

Aniseed oil enhances the ability of mammary gland in extraction of essential components from plasma for milk synthesis.

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# التغيرات في بعض مكونات البلازما خلال فترة الرضاعة تحت تأثير زيت بذور نبات الينسون في الجرذان

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**المستخلص**

استهدفت هذه الدراسة تحديد بعض التأثيرات التي يمكن أن يؤديها تناول زيت بذور نبات الينسون، في الثلث الأخير من فترة الحمل، في بعض المعايير الكيموحيوية لبلازما الدم في اليوم الأول والحادي عشر من الرضاعة بوصفها مؤشراً لكفاءتها في تصنيع وإفراز اللبن في إناث الجرذان. تضمنت المعايير الكيموحيوية قياس كل من: تركيز البروتين الكلي، والكلسيريدات الثلاثية، والكولسترول والكلوكوز؛ وبعض أيونات بلازما الدم، مثل: الكالسيوم، والفسفور، والمغنيسيوم والصوديوم. استخدمت 20 انثى حاملاً في اليوم الخامس عشر من حملها وقسمت عشوائياً إلى مجموعتين ضمت الأولى (السيطرة) عشر إناث حاملة وضمت الثانية (المعالجة) عشر إناث حاملة تناولت يوميا زيت بذور نبات الينسون (0.043 غم كغم$^{-1}$ من وزن الجسم) في الثلث الأخير من فترة الحمل. بعد الولادة مباشرة تم تخدير الامهات لغرض جمع عينات الدم وتم تحديد المواليد بواقع (6 جراء أم$^{-1}$) وتركت ترضع مواليدها لغاية اليوم الحادي عشر من الرضاعة عندها ايضا تم تخدير الامهات وجمع عينات الدم لغرض اجراء الفحوصات الكيموحيوية المذكورة اعلاه. أظهرت نتائج هذه التجربة بان زيت بذور نبات الينسون ادى الى انخفاض معنوي في تركيز كل من البروتين الكلي، والدهون الثلاثية، والكولستول والكلوكوز وتركيز بعض أيونات بلازما الدم (الكالسيوم والفسفور والمغنيسيوم) اما ايون الصوديوم فقد ارتفع معنويا في اليوم الأول والحادي عشر من الرضاعة، في حين شهد اليوم الحادي عشرمن الرضاعة زيادة في كل المعايير المذكورة أعلاه. نستنتج من هذه ألدراسة بأن زيت بذور نبات الينسون عمل على زيادة كفاءة الغدد اللبنية في استخلاص المواد الأولية المكونة للبن من الدم في اليوم الأول من الرضاعة وبالتالي ادى الى تحسين نوعية الحليب.

**الكلمات المفتاحية:** زيت اليانسون، فترة الرضاعة، مكونات الحليب، الفئران.