

THE HISTOLOGICAL AND BIOCHEMICAL STUDY OF THE EFFECT OF PURE FLAX SEED LIGNIN ON THE MAMMARY GLAND IN FEMAL RABBITS

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ABSTRACT

This study was carried out to investigate the effect that result from taking 40 mg /kg \BW of purified lignan from the seed of flax *Linum usitatissimum L.* in the growth and development of mammary glands during virgin , pregnancy and lactating stages for 14 days. Thirty (30) rabbits were used in this experiment divided into six groups, two groups at maturity (virgin) stage, two groups at pregnancy stages and two groups at lactating stages (5 rabbits\each group). The following studies were decided: histological, histochemical, biochemical and hormonal studies for each group. Microscopic examination of mammary gland in the virgin rabbits that treated with pure flax lignan showed more growth in the size of lobules and alveoli. In pregnant, the treated groups showed more branching of alveoli with more flattened epithelium. In lactating treated group showed the lobules were expanded and contained more branching alveoli with discontinuous flattened epithelium. The PAS staining reaction of histological sections of mammary gland from treated groups and control were showed positive reactions in all groups.

The biochemical studies showed a significant increase ($p<0.05$) in the serum reduced glutathione concentration with decrease in the malondialdehyde concentration in the three stages that treated with pure flax lignan compared with control. The hormonal study indicated a noticeable increase in prolactin hormones with significant decreased in estrogen in all groups of treated animals for the three physiological stages (virgin, pregnant and lactating) compared with their controls.

INTRODUCTION

Milk has an important role in human feeding by direct consumption or via food industry, so that an increase in milk production has attracted world wide interested by scientific researches.(1). Low supply of milk is one of the most common reasons given for discontinuing breast feeding. Galactagogues are medications or substances believed to assist initiation, maintenance and augmentation of maternal milk production.(٢). Flaxseed (FS) is the richest known source of the phytoestrogen

lignan precursors, in particular secoisolariciresinol diglucoside (SDG; Ref. 12). On fermentation by the bacterial flora in the colon of mammals, SDG is converted to the mammalian lignans enterodiol (ED) and enterolactone (EL; Ref. 13). These biphenolic compounds have chemical structures that closely resemble that of endogenous 17- β estradiol and possess biphasic agonistic (estrogenic) and antagonistic (antiestrogenic) activities in vitro (3) and in vivo (4). Therefore, this study was designed to cast a light on the capability of purified lignan on mammary gland development in three stages (virgin, pregnant and lactating rabbits).

MATERIAL AND METHODS

The average body weight of animals were ranged between 750-1500 gm, 4 months old at the beginning of the study. They were housed individually in plastic cages and kept at a temperature between 20 -24 ° C (room temperature). The light/dark cycle was 12/12d-h in Kerbala university \Education collage for pure sciences\animal house.

Thirty (30) adult female rabbits were divided into 6 groups for three stages (virgin, pregnant and lactating) two groups in each stage (5 rabbits\ each group), in each stage one of the groups gave 40mg/kg/day of pure flax lignan and considered as treatment group where the other group considered as control for (14) days.

Flaxseeds were collected from the local market. Firstly, cleaning flax from derbies which include other plants seeds, some parts of vegetarian of flaxseed and dust, Secondly grinding flaxseeds properly by a grinder machine eventually obtained on a homogenized powder that was ready for extraction. The extraction of Crude Lignan was described by (5), while the purification of lignan was carried out by column chromatography (purification) which was described by (6)

For histological study of rabbit tissue, the rabbits were anaesthetized by chloroform and killed. Immediately after death the mammary gland was excised and preserved in fixative solution till the preparation of histological sections. Several tissue sections were prepared according to (7).

Fasting blood sample were drawn via intra-cardiac puncture, blood was kept into epindrof tube without EDTA, held for not more than four hours before serum collection by centrifugation 3000 rpm for 15 minutes and frozen at -20C⁰ until analysis. Serum samples were used for measurements of glutathione (GSH), malondialdehyde (MDA) concentrations and hormonal concentration.

RESULTS

Histological sections (HE stained sections) of mammary gland of virgin controls showed small lobules scattered among huge amount of adipose and connective tissues, On the other hand, experimental rabbit's mammary gland arrayed relatively

very large lobules, which were seen fully packed by a large size and widely dilated alveoli (figure 1 and 2). In pregnant mammary tissue that treated with pure flax lignan revealed an increase in branching of alveoli with more flattened luminal epithelium (Figures 3,4), while treated lactating mammary tissue revealed greatly dilated lobules containing more branching and more dilated alveoli with flattened epithelium (figures 5,6). PAS stained sections of mammary gland of control and treated groups demonstrated histological features similar to those stained with HE stain. Likewise, experimental rabbit's mammary glands gave identical features of experimental rabbit's mammary gland stained by HE stain, except the appearance of strong positive PAS stain in the homogenous part of milk secretion within acinar and ductal lumens in all treated groups (figures 7,8,9,10,11,12) in virgins, pregnant and lactating rabbits respectively.

The serum MDA concentration was decreased significantly ($p < 0.05$) and increase significantly ($p < 0.05$) in GSH concentration level in all groups that treated with spearmint comparing to control (table 1 and 2) respectively.

The hormonal levels of prolactine were increased in treated animals in all treated groups in compared with controls, while estrogen hormone level significant decreased in treated animal with non significant change in progesterone level (table 3)

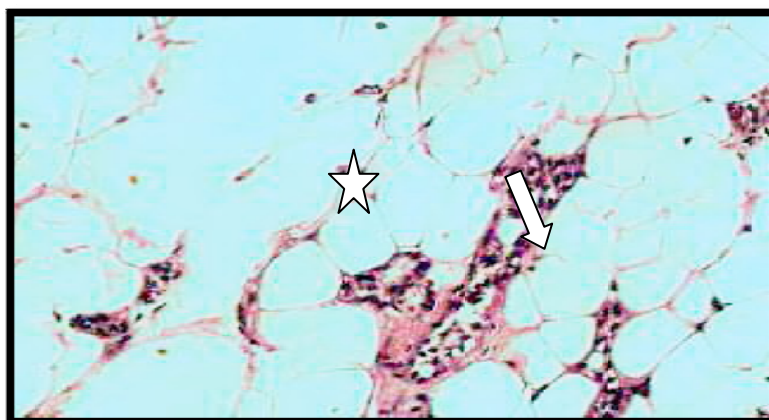
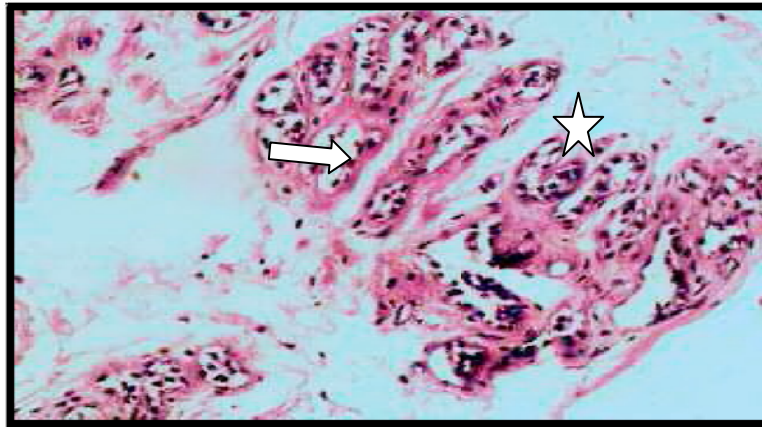
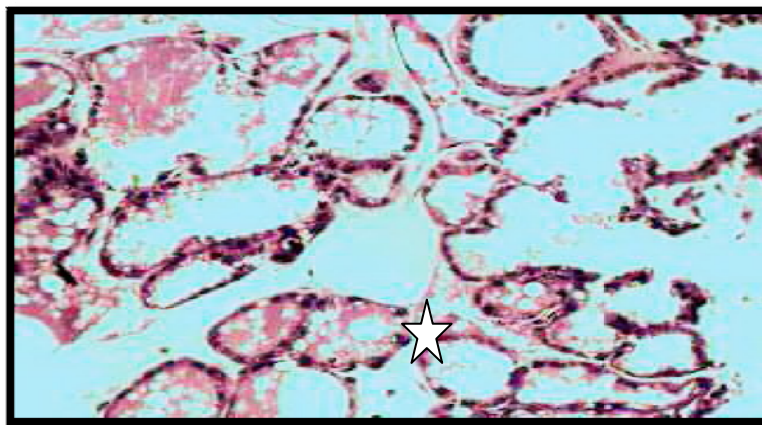


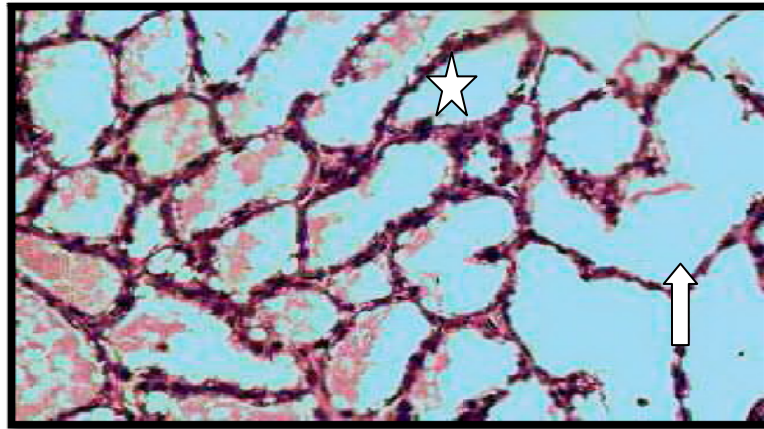
Figure (1) section of mammary glands of virgin in control group. Showe small lobules (arrow) scattered among huge amount of adipose tissue (star) (H & E) (200X).



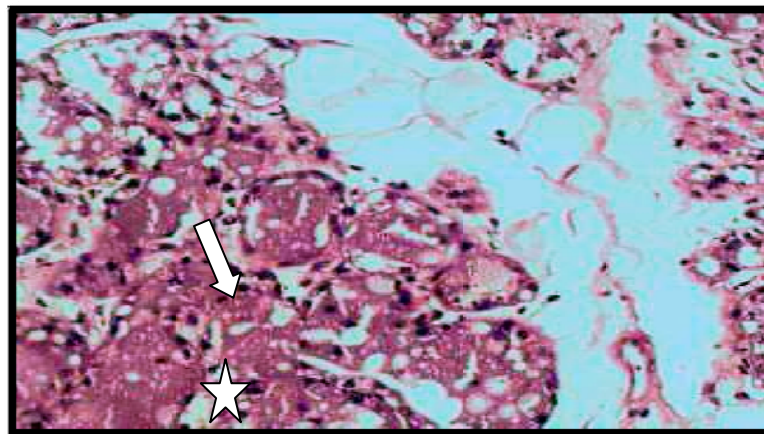
Figure(2) Section of mammary gland of virgin treated with pure flax lignan show large size of lobules (arrow) packed by alveolar ducts(star) (H & E stain) (200X).



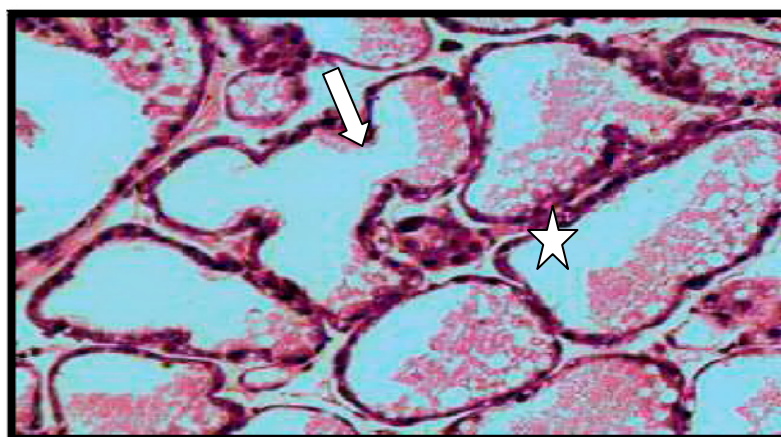
Figure(3) Section of mammary gland of pregnant in control group show large size of alveoli (arrow) some of these alveoli filed with secretory product(star) (H & E stain) (200X).



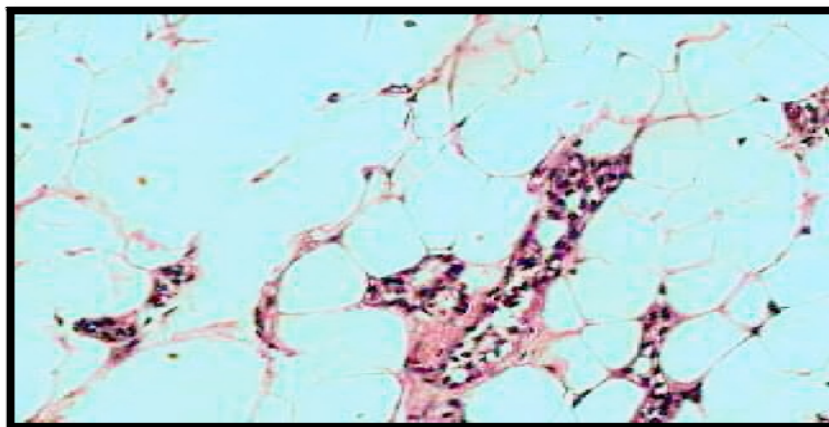
Figure(4) Section of mammary gland of pregnant rabbit treated with pure flax lignan show branching alveoli (star) with flattened epithelium (arrow)(H & E stain) (200X).



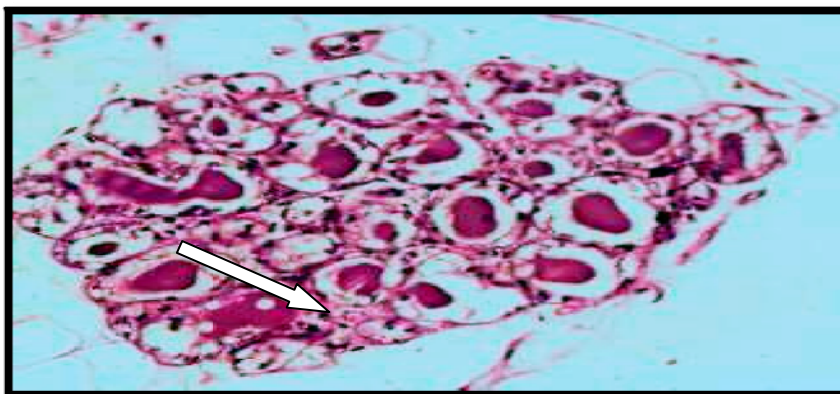
Figure(5) Section of mammary gland lactating rabbit in control group show dilated alveoli (arrow) with secretory products (star)(H a& E stain) (200X).



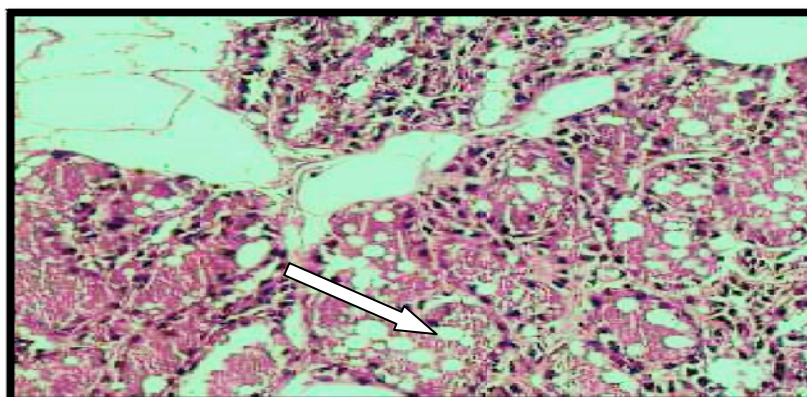
Figure(6). Section mammary gland of lactating rabbit treated with pure flax lignan show the alveoli were more dilated (star)and more branched (arrow)than other groups (H & E stain) (200X).



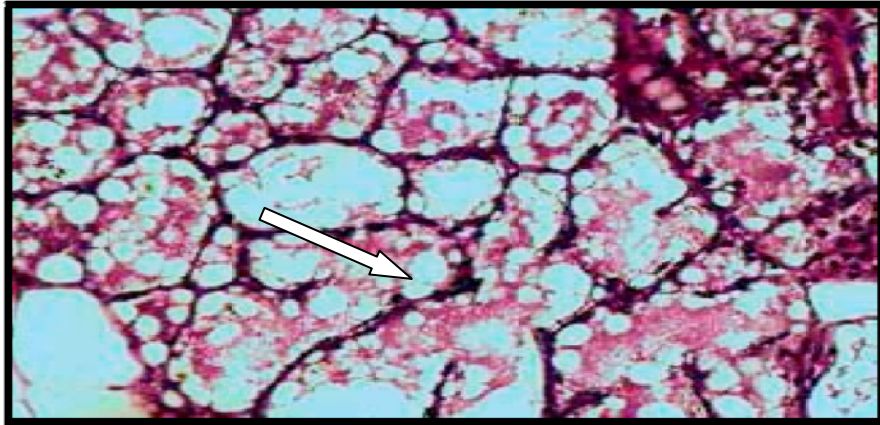
Figure(7) Section of mammary gland of virgin rabbit in control group show negative reaction to the PAS stain (200X).



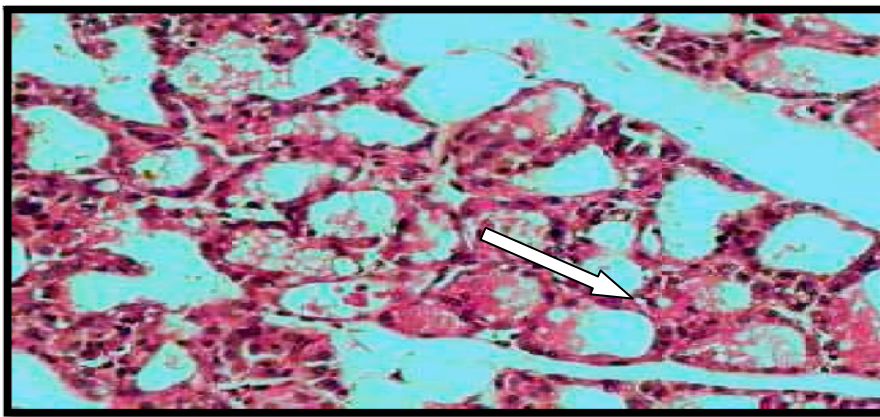
Figure(8) Section of mammary gland of virgin rabbits treated with pure flax lignan show positive reaction to the PAS stain (arrow) (200X).



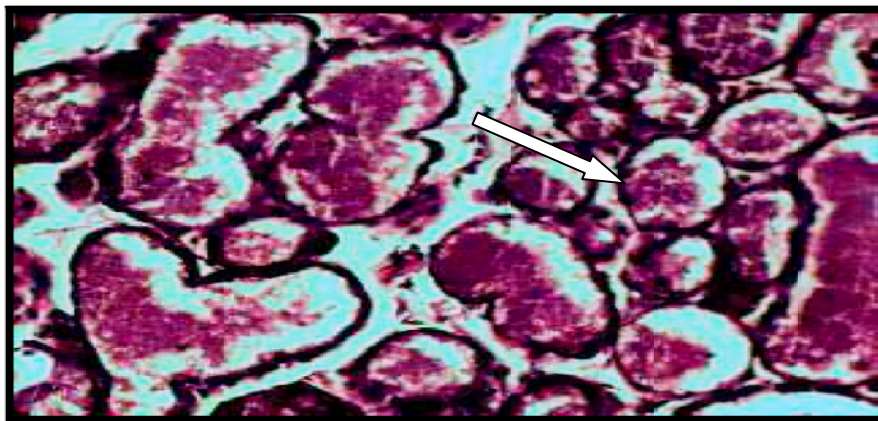
Figure(9) Section of mammary gland of pregnant rabbit in control group show positive reaction to the PAS stain (arrow) (200X).



Figure(10) Section of mammary gland of pregnant rabbit treated with pure flax lignan show positive reaction to the PAS stain (200X).



Figure(11)Section of mammary gland of lactating rabbit in control group show positive reaction to the PAS stain (arrow) (200X)



Figure(12) Section of mammary gland of lactating rabbit treated with pure flax lignan show positive reaction to the PAS stain (arrow) (200X)

Table (1) Serum Malondialdehyde concentration (mg/dl) in female rabbits treated with pure flax lignan for two weeks in different physiological stages

Stages Group	Virgin	Pregnant	Lactation
Control	0.65 A a ±0.52	0.99 A b ±0.33	0.87 A b ±0.44
Pure lignan	0.42 B a ±0.23	0.67 B b ±0.29	0.58 B b ±0.41

Values are expressed as mean ± SE. N= 5/ group. Small letters denote difference within group p<0.05. Capital letters denote difference between groups p<0.05.

Table (2) Serum reduced glutathione concentration (µmol/L) in female rabbits treated with pure flax lignan for two weeks in different physiological stages.

Stages Group	Virgin	Pregnant	Lactation
Control	28.0 B a ±0.32	20.0 B b ±0.41	22.07 B b ±0.22
Pure lignan	40.29 A b ±0.40	32.33 A a ±0.29	33.30 A a ±0.25

Values are expressed as mean ± SE. N= 5/ group. Small letters denote difference within group p<0.05. Capital letters denote difference between groups p<0.05

Table (3) The effect of effective dose of Pure flax lignan on main value to serum hormones level in adult female rats for two weeks in three physiological stages

stages Hormones	Virgin		Pregnant		Lactating	
	Control	Pure lignan	Control	Pure Lignan	Control	Pure lignan
Estrogen ng\ml	99.7 ±0.27 A	70.80 ±0.35 B	139.20 ±4.5 A	113.79 ±6.77 B	127.40 ±1.3 A	100.59 ±3.80 b
Progesteron ng\ml	1.50 ±0.30 B	5.50 ±0.10 A	10.42 ±0.40 B	23.10 ±0.60 A	14.84 ±0.19 B	24 ±0.31 A
Prolactin ng\ml	4.33 ±0.21 A	4.10 ±0.11 A	12.97 ±0.25 A	13.00 ±0.26 A	14.55 ±0.47 A	13.56 ±0.46 A

Values are expressed as mean ± SE. N= 5/ group. Capital letters denote difference between groups $p < 0.05$.

DISCUSSION

The present study showed that the pure flax lignin influence mammary gland structures which caused an increase in the size of lobules that packed with branching dilated alveoli in virgin, gestation and lactation period when compared with the control . These histological features indicate that the control mammary glands revealed features of a resting mammary gland, which match the age and physiology of this group (8) , while the pure flax lignin treated groups showed that the pure lignan had ability to induce mammogenesis in the mammary glands of virgin ,pregnant and lactating rabbits in addition to estrogen , progesterone and prolactine., this might be due to increase in estrogen level that found in experiments treated groups (table 3).

In previous studies, (9, 10) the exposure of rats to 10% flax seed or it's equivalent level of lignan during early life, especially suckling, enhanced mammary gland differentiation. The mechanism through which flax seed or its major lignin facilitates mammary gland differentiation remains unclear. The isoflavone genistein has been reported to upregulate epidermal growth factor receptor (EGFR) and it's ligand, transforming growth factor (TGF)- α in terminal end buds of rat mammary glands (11). This enhanced EGFR-signaling cascade was shown to be modulated by an estrogen-receptor (ER)-based mechanism (12). Because lignans and genistein share similarities in chemical structure and biological activities (13, 14), both phytoestrogens may enhance mammary gland differentiation through similar mechanisms, So that the flax seed and its lignan participated in an estrogenic pathway via ER and EGFR signaling to mediate mammary gland morphogenesis. The

development of mammary glands involves a complex mammary epithelial-stromal communication that is coordinated through autocrine and paracrine regulations of a myriad of hormones and growth factors (15). This results were in agreement with (16) and with other previous studies used anther plants such as Anise (17) , Cresson (18) Harmal and Borage (19) and spearmint and barley (20)

Our study also showed that there was a significant decrease in the level of lipid peroxidation end product MDA and an increase in GSH level , , this reduction in lipid peroxidation level and increased in GSH may be attributed to the inbuilt scavenging and reducing property of the lignan. Phytoestrogens may exhibit a wide range of biologic activities not necessarily linked to ERs, including inhibition of lipid peroxidation, and activity of enzyme systems including especially lipoxygenase (21) The flaxseed lignan secoisolariciresinol diglucoside (SDG) and mammalian lignans enterodiol (ED) and enterolactone (EL) were previously shown to be effective antioxidants against DNA damage and lipid peroxidation. Plant lignan antioxidant activity was attributed to the 3-methoxy-4-hydroxyl, Benzylic hydrogen abstraction and potential resonance stabilization of phenoxyl radicals in an aqueous environment likely contributed to the antioxidant activity of the mammalian lignans.(22)

In hormonal study we found that the lignin caused a significant increase ($p<0.05$) in prolactin hormone while estrogen level showed a significant decrease ($p<0.05$) in treated animals(23) . The exact mechanism by which flaxseed and its component plant lignans secoisolariciresinol and matairesinol or the mammalian lignan products enterolactone and enterodiol lower the concentrations of estrone sulfate and 17_β-estradiol is not known. However, enterolactone and secoisolariciresinol have been shown to inhibit aromatase activity in vitro (24). Aromatase is a principal regulator of estrogen biosynthesis in humans, including the conversions of androstenedione to estrone and testosterone to 17_β-estradiol (25) Inhibition of aromatase by enterolactone or secoisolariciresinol would decrease the amount of 17_β-estradiol formed from testosterone, thereby explaining the decrease in 17_β-estradiol concentrations found in this study. This results were in agreement with (26)

دراسة نسجية وكيموحيوية لتأثير اللكنان المنقى من بذور الكتان على الغدد اللبنية لأنثى

الأرانب

وفاق جبوري البازي

سيناء جبوري البازي

قسم علوم الحياة ، كلية التربية للعلوم الصرفة ، جامعة كربلاء ، كربلاء ، العراق.

الخلاصة

أجريت هذه الدراسة بهدف تسليط الضوء على التأثيرات التي يمكن أن يؤديها تناول ٤٠ ملغم / كغم من وزن الحيوان من اللكنان المنقى المستخلص (Lignan) من بذور نبات الكتان (Linum) usitatissimum L. في زيادة ونمو وتطور الغدد اللبنية أثناء مراحل (البلوغ "العذارى" والحمل والرضاعة)

لمدة ١٤ يوم. استخدم (٣٠) ارنب في هذه التجربة قسمت إلى ست مجاميع، مجموعتين في مرحلة البلوغ ومجموعتين في مرحلة الحمل بالإضافة إلى مجموعتين في مرحلة الرضاعة (٥ ارنب لكل مجموعة)، ثم أجريت عليها دراسات نسجية وكيميائية نسيجية كيموحيوية بالإضافة إلى القياسات النسجية لكل مجموعة. بينت النتائج المعطيات التالية: أظهرت المقاطع النسجية إن الحيوانات العذاري المجرعة باللكنان المنقى زيادة في حجم وعدد الفصيصات. أما في مرحلة الحوامل فقد لوحظ وجود تفرعات في الأسناخ مع تسطح في البطانة الثلاثية بالإضافة الى توسع في النبيبات الفصيضية. أما عند معاملة مجاميع المرضعات فان الفصيصات أظهرت توسعا أكثر مع تفرع واضح في الاسناخ. من ناحية اخرى، بينت المقاطع النسجية المصبوغة بصبغة الـ PAS لمجاميع الحيوانات المعاملة باللكنان المنقى لجميع و مجاميع السيطرة إفران موجب للصبغة الـ PAS. اما الدراسات الكيموحيوية فأظهرت أن المجاميع المعاملة باللكنان ارتفاعا معنويا ($p < 0.05$) في مستوى الكلوتاثيون مع انخفاض معنوي في مستوى المألون داي الديهايد في مصل الأرنب للمجاميع المعاملة الثلاث مقارنة بالسيطرة. أوضحت نتائج الدراسات الهرمونية زيادة معنوية ($p < 0.05$) في مستوى هرمون البرولاكتين مع انخفاض معنوي في مستوى هرمون الاستروجين للمجاميع المعاملة مقارنة مع مجاميع السيطرة.

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