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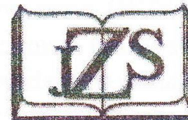
Studies on Antileutinizing Activity of Melissia Officinalis



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Abstract

The active Principle responsible for the antihormonal activity of M. officinalis is formed from phenolic precursors. For its formation, an oxidation step was found to be essential.As a test model, an isolated mous leydig cells were used for LH-induced testoserron realease.

The freeze-dried extract (FDE) of the plant leaves showed an LH- inhibition effect. The pre -incubated LH-hormone with FDE of M.officinalis in phosphate buffer pH 7.5 also showed a clear antihormonal effect, while the treatments of 20% oxidized FDE of Mel. officinalis showed relatively lesser effect than the above two mentioned treatments.. The most inhibition phase was associated with 20% oxidized Mel. officinalis (FDE) preincubated in phosphate buffer pH7.5.Oxidation of caffeic acid by the polyphenol oxidase, which has been isolated from Melissia and purified by ion exchange chromatography ,also leads to the formation of oligomers which exert also the LH inhibition to the same extent as the last mentioned treatment.So the addition of 25 µg of (20% oxidized M. officinalis ,preincubated in phosphate buffer) and the product formed by enzyme action,brought the LH-inhibtion to zero.

Keywords:- Melissia officinalis, antileutinizing compound, polyphenols

Introduction

An antigonadotropic activity of other plant such as Lithospermum lycopus leaves has been documented [1] . This activity has been shown to act against pregnant serum-gonadotropin. [1, 2, 3, 4].

It has been shown that gonadotropin action can be impaired or blocked by phenolic compounds with two or more hydroxyl groups [5]. Moreover, these data reveal that these compounds in order to excert their action, they must be oxidized to yield products which can bind to the gonadotropin and thus inactivate it [6].

In this study, the freeze-dried extract (FDE) of the M.officinalis leaves, the 20% of the oxidized plant extract, and the preincubated of the (FDE) leaves in phosphate buffer PH 7.5 showd a very clear antihormonal effect. Also the action of the immobilized enzyme polyphenol oxidase (ppo) on the caffeic acid showed an antihormoanal effect, this reveals that the enzyme (ppo) in this plant has an important role in the formation of oxidation products which exert the antihormoanal effect.

Materials and Methods

Healthy leaves were collected from M.officinalis plant grown naturally in the region of Shaklawa in Erbil City. The leaves were desiccated below 40°c, and ground in an electric mill through 40 meshes. The freeze-dried extracts {FDE)

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FDE) were prepared by mixing 1 part of the plant material with 9 parts of its equivalent of distilled-deionized water.

The mixtures were shaked for 2 hrs at 20°C and centrifuged at 2500Xg for 15 min. The debris was re-extracted twice and the supernatants were combined and lyophilized.

Oxidation with KMnO4

The lyophilized (FDE) was oxidized with $KMnO_4$[1]. The total Consumption of $KMnO_4$was determined by titration at an acid pH range and regarded as a 100% oxidation. The KI/ starch test was served as an indicator.

To obtain an oxidation grade of 20%, 1/5 of the total consumption was added to the test substances.

Enzyme isolation and purification

The enzyme polyphenol oxidase was extracted from leaves of M. officinalis plant, separated and purified by different methods using gel-filtration, ion-exchange chromatography as described in our previous publication[7]. The purified enzyme was immobilized, according to the methods used for preparation of peroxidase with little modifications [8].

Preparation of immobilized polyphenol oxidase(PPO)

Reagents for gel preparation:

Potassium phosphate buffer, 0.2M,pH 7.0

A. 30% acrylamide plus 0.8% methylene bisacrylamide in potassium phosphate buffer.

B.10% ammonium persulfate in potassium phosphate buffer.

C.N,N,N',N'-tetramethylethylenediamine (TMED).

Ammonium persulfate solution, 1.0 g/10mL of phosphate buffer (prepare just before use).

The purified (PPO), 0,5 mg/ml dissolved in glass distilled water

Caffeic acid solution: Prepared by dissolving 10mol/10mL in potassium phosphate buffer.

Gel preparation

To prepare the acrylamide gel, add the following to a 20-mL vial: 3.25 mL potassium phosphate buffer, 2.7mL of solution A (acrylamide- bisacrylamide)and 80µl of solution B(ammonium persulfate).Mix and add 2.0mL of 0.5mg/mL PPO solution. Add 10µl of reagent TEMED and mix with a vortex mixer .The solution should become opaque within a few minutes and completely polymerized within 20-30 minutes. With a spatula , transfer the gel to a vacuum filtration system to remove most of the solution. To wash the gel, transfer it to a test tube containing 5 mL of water. Break up the gel by aspirating with a Pasteur pipette. Centrifuge the gel mixture for 5 minutes at 1000-1200 rpm. Decant supernatant and add 10mL of water to the gel. Again, break up the gel by aspirating with a Pasteur pipette and centrifuge as before .Repeat this washing process two more times. The final centrifugation should be done for 5 minutes at 1200-1500rpm.Dry the gel by vacuum filtration for a few minutes and weigh on a balance .Approximately 2-3g of semi wet gel will be obtained.

Oxidation of caffeic acid with PPO immobilized enzyme

Weigh 2gm of immoblized ppo, add 5 mL of caffeic acid solution as substrate mix well and incubate for 10 minutes by 37°c.After incubation quench the reaction by pouring into the barrel of syringe with filter system. Use the plunger to force the solution through the filter in to a glass test tube (filtrate), which contains the product obtained after enzyme action and used for LH inhibition.

LH-assay

A Series of experiments were conduced with and without preincubation in phosphate buffer pH 7.5 for Mel.officinalis extract (FDE) , for oxidized product of the (FDE) and the oxidation product obtained by plyphenol oxidase . All four series were run with 50 ng/ul of the luteinizing hormnone (LH-hormone) in vitro. The LH-hormone was obtained from NH, Betheseda USA. As a test model, an isolated mouse Leydig cells were used for LH-induced testosterone release [9]. A control test with physiological saline,as well as a hormone standard of 50 ng/ul, were also conducted on the isolated mouse leydig cells.

Results and Discussion

Figure (1) shows the influences of different treatments, on the antigonadotropic activity. Thereby, the effect was examined to determine the extent at which the stimulated testosterone production was inhibited by M.offlcinalis extract. The extent of direct stimulation by 50 ng of LH hormone was regarded as a base line and designated 100% stimulation (Fig1,C_0). The reduction of stimulation effect was proportionally related to the concentrations of the added M. officinalis(FDE).

As seen from the(Fig1,C_3) (among treatments 1 microgram to 25 µg), the 25 µg of added M. officinalis (FDE) was the most effective treatment whereby stimulation effect has been reduced into 39% (Table 1). The treatments of preincubated Mel-officinalis in phosphate buffer pH 7.5 (Fig1,C_4,C_5,C_6) were more effective than the treatments of 20% oxidized Mel.officinalis at all concentration Levels(Fig1,C_7,C_8,C_9).

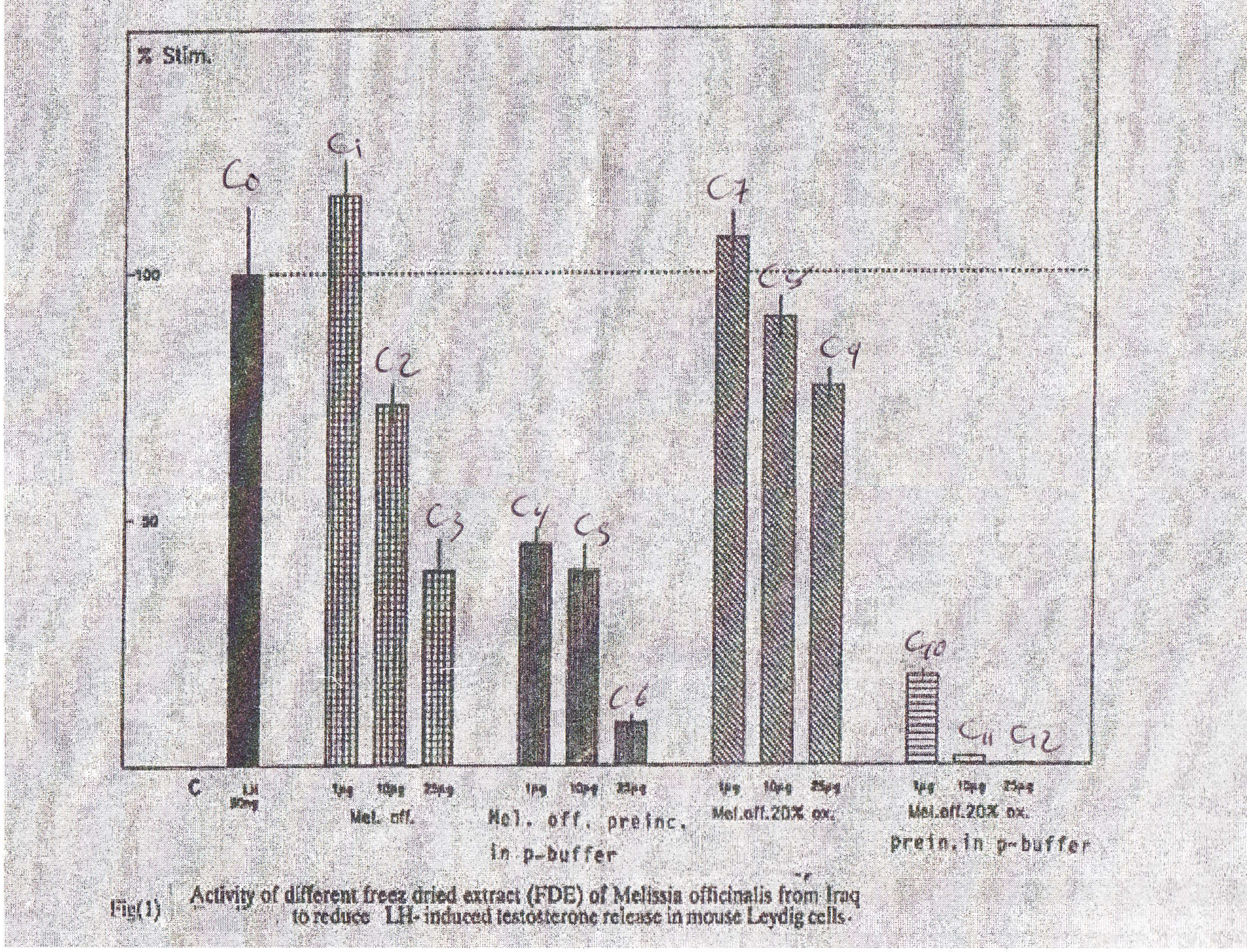
The most obvious reduction in the stimulation effect was seen with the concentrations of 10 and 25 µg of the M.officinalis, which were oxidized at 20% level and preincubated in phosphate buffer pH 7.5, where by the reduction of the stimulation has reached 98.2 and 100% respectively (Figl,C_{11},C_{12})& Table (1). These results reveal doubtless that LH should be also decreased – although it was not measured in the present work-because it is well known that the formation of testosterone is usually an LH – dependent process, hence the suppression of steroidogenesis (testosterone) depends upon adequate LH suppression [10]. The oxidation of caffeic acid by polyphenol oxidase leads to formation oligomers of caffeic acid (a relative molecular mass similar to caffeic acid tetramers) [11]. The activity of the oligomer fraction was used for its antihormonal effect. 100 microgram of the oligomer fraction was able to reduce the 100% stimulation, this indicates that the

activity of 25 microgram of 20% oxidized M. officinalis preincubated in phosphate buffer exceeds the effects of the oxidation product, which was produced by the enzyme action; this may reveals that to increase the activity of the antihormoanl effect, using the enzyme, many substrates ,which are present in plant extract- should be used to obtain very high antihormonal effect.

Table(1) Statistical analysis of different treatments on testosterone stimulation by leydig cells

Treatments	Mean ± Standard error mean	% standard deviation	%Stimulation ± Standard error mean
Control	0.2 ± 0.0	12.5	0.0 ± 0.0
LH 50 ng	5.8 ±0.1	2.2	100.0 ± 2.2
M. officinalis 1μg + LH 50ng	6.7 ± 0.4	6.0	116.0 ± 6.9
M. officinalis 10μg + LH 50ng	4.3 ±	5.6	73.4 ± 4.1
M. officinalis 25μg +LH 50 ng	2.4 ± 0.4	16.7	39.7 ± 6.6
M. officinalis 1μg +LH 50 ng with preincubation in P*	2.7 ± 0.2	7.4	45.0 ± 3.3
M. officinalis 10μg +LH 50 ng with preincubation in P*	2.4 ± 0.3	13.3	39.7 ± 5.3
M. officinalis 25μg +LH 50 ng with preincubation in P*	0.7 ± 0.1	19.7	8.9 ± 1.7
M. officinalis 20%ox. 1μg + LH 50 ng	6.2 ± 0.3	5.2	107.1 ± 5.5
M. officinalis 20%ox. 10μg + LH 50 ng	5.3 ± 0.2	4.5	91.1 ± 4.1
M. officinalis 20%ox. 25μg + LH 50 ng	4.5 ± 0.2	4.7	77.0 ± 3.6
M. officinalis 20%ox. 1μg + LH 50 ng with preincubation in P*.	1.2 ± 0.1	5.8	18.4 ± 1.1
M. officinalis 20%ox. 10μg + LH 50 ng with preincubation in P*.	0.3 ± 0.0	0.0	1.8 ± 0.0
M. officinalis 20%ox. 25μg + LH 50 ng with preincubation in P*	0.2 ± 0.0	7.1	0.2 ± 0.0
Oxidation product produced by enzyme action	0.2 ± 0.0	0.0	0.0 ± 0.0

*Phosphate Buffer**



Fig(1) Activity of different freez dried extract (FDE) of Melissia officinalis from Iraq to reduce LH- induced testosterone release in mouse Leydig cells.

C = Column Number
C_{12}*= The reduction of stimulation is 100%.
Co=Control

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دراسەتی نزم كردنەوەی هۆرمۆنی Leutinizing بە پوختەی رووەكی تورنجان

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پوختە

هەندێك ئاوێتە رۆلی گرنگیان هەیە لە نزمكردنەوەی هۆرمۆنی Leutinizing لە رووەكی تورنجان. ئەم جۆرە ئاوێتانەش بێگومان لە هەندێك Phenols Precursors دروست دەبن. پرۆسەی ئۆكساندن بۆ زیاكردنی چالاكی ئەم ئاوێتانە زۆر گرنگن و پێویستن.

(Freeze-dried) ی پوختەی ئەم رووەكە رێژەی هۆرمۆنی Leutinizing ناهێلێت. هەروەها داینەكردنی (حضن) ئەم هۆرمۆنە لەگەڵ پوختەی (Freeze-dried) ی رووەكە لە گیراوەی رێكخەری فۆسفات (pH 7.5) و پوختەی (Freeze- dried)ی ئۆكساو بە رێژەی ٢٠٪ بووە هۆی دابەزاندنی ئەم هۆرمۆنە، وە مامەڵەی داینە كردنی هۆرمۆن لەگەڵ پوختەی (Freeze- dried) و ٢٠٪ ئۆكساو كراو لە گیراوەی رێكخەری فۆسفات (pH 7.5) بوونە هۆی زیاترین دابەزاندنی هۆرمۆنەكە. هەروەها جیاواز كردنەوەی ئەنزیمی پۆلی فینۆل ئۆكسیدێز بە چەند جۆری جیاواز لە كرۆماتۆگرافیا وەك gel filtration و ion exchange ، وە بەكارهێنانی ئەم ئەنزیمە بۆ ئۆكساندكردنی Caffeic acid بووە هۆی دروست كردنی هەندێك ئاوێتەی ئۆكسیندراو كە رۆلێكی چالاكیان هەبوو لە دابەزاندنی هۆرمۆنەكە .

دراسة خفض هورمون اللوتنة باستخدام مستخلص نبات الترنجان

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الخلاصة

ان المركبات المسؤولة عن خفض مستوى هورمون اللوتنة في نبات الترنجان (M.officinalis) تتكون من أسلاف الفينولات . ولزيادة فعالية تلك المركبات فان أجراء عملية الاكسدة ضرورية لتلك المركبات. ان المستخلص المجفد (freeze-dried extract) لهذا النبات أظهر تثبيطاً لهورمون اللوتنة. هذا وقد وجد ان حضن هورمون اللوتنة مع المستخلص المجفد للنبات في محلول الفوسفات الداريء (pH 7.5) ، وكذلك مع المستخلص المجفد والمؤكسد بنسبة ٢٠% قد أظهرا ايضاً انخفاضاً واضحا لهورمون اللوتنة.
ومن بين المعاملات السابقة أظهرت معاملة حضن الهورمون مع المستخلص المجفد والمؤكسد بنسبة ٢٠% في المحلول الداريء (pH 7.5) أعلى تثبيط للهورمون. كما ان استخدام أنزيم ال immobilized Polyphenol oxidase - المنقى بطرق الكرموتوكرافيا المختلفة مثل gel filtrationوال ion exchange chromatography- لمساعدة تأكسد ال Caffeic acid ادى الى تكوين مواد مؤكسدة (oligomers) أظهرت فعلاً مثبطا ايضاً لهورمون اللوتنة ، حيث ان اضافة ٢٥ مايكروغرام من (مستخلص النبات المؤكسد ٢٠% والذي حضن في داريء الفوسفات) وكذلك استخدام المركب الناتج من فعل الانزيم اديا الى تثبيط هورمون اللوتنة كليا .

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