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The Role of Polyphenol Oxidase(PPO) in Antiprolactin Activity

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Abstract

The role of polyphenol oxidase which has been extracted from Mellssia officinalis and purified by gel filtration and ion exchange chromatography was tested on phenolic compounds such as: chlorogenic acid and catechol to decrease the level of prolactin. Similarly the effect of 100mg of freeze-dried extract of the plant and 100mg of 20% oxidation products of freeze-dried extract have been studied. The most effective treatment, was the enzyme treatment, which had reduced the prolactin level from 3.372 ng/ml to 1.7644 ng/ml .This reveals that such products, which were formed by the enzyme action on the substrates catechol and chlorogenic acid could be used to reduce the prolactin by individuals who suffer from hyperprolactinemia ,It is well known that hyperprolactinemia reduces libido sexual impotence and infertility in men.

Keywords:- polyphenol oxidase; antiprolactin acivity;Melissia officinalis

Introduction

As observed in our work [1], that a decades of generation by mountaineer in north Iraq used the plant M. officinalis because they believed that it helps in being fertile: for this reason we have met some people who has taken this plant and admitted in beneficial action by having pregnancy after along period of infertility.

Literature survey shows that this has a noticeable effect in decreasing prolactin hormone [2]. It is believed that this decrease is due to phenolic plant constituents and their oxidation products [3]. It is well documented that hyperprolactinemia is involved in the fertility of the amenorrheic women. Reduction of the prolactin level in the blood of these patients with bromocriptin has shown stimulation of Ovulation [4]. Antiprolactin effect of plant extracts have reported by NOBEL et.al. for *lithospermum ruderle* DOUGL [5]. The effect of aqueous plant extracts of M.officinalis leaves and the oxidation step of aqueous extract by treatment with $KMnO_4$, as well as oxidation of diphenols, which formed through the action of purified polyphenol oxidase activity were used to detect their effect on the level of prolactin. Literature survey indicates no work has been done about separation of the enzyme ppo in plants such as *lithospermum ruderle* and M.officinalis which possess antiprolactin activity. To show and to prove the role of enzyme on the formation of compounds in M. officinalis, the enzyme has been separated and their products have been tested to show the anti – prolactin activity.

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Materials and Methods

Plant preparation of aqueous extract:

Leaves from plant of own cultivation in July in region Shaklawa in Erbil City were desiccated quickly below 40°C, and ground in a mill. Freeze dried extracts (FDE) were prepared from powdered leaves under conditions shaking with ten fold amount of water (2h, 20 °C) and centrifuged at 2500g. The supernatant was lypholized. 100mg of lypholized extract were injected per rat.

2-

Oxidation with $KMnO_4$

The total consumption of $KMnO_4$ was determined by titration at an acid pH and defined as 100% oxidation.

KI/starch served as indicator to obtain an oxidation grade 20%,1/5 of total consumption was added to the test substance (FDE was the test substance).

Enzyme preparation

The enzyme polyphenol oxidase (PPO) was extracted, separated and purified by deferent methods in our previous publication [1], using calcium acetate, gel filtration on Sephadex G-75,and chromatography on DEAE Sephrose CL-6B.The fractions obtained from the ion exchange chromatography , including peaks 1,2 and 3 have been used for oxidation of diphenols.

Oxidation of diphenoles using PPO enzyme

10mg of purified enzyme from M.officinalis [1] was added to a mixture of 20ml catechol (10mM) and 20ml of chlorogenic acid (10mM) and incubated at 35 °C. The enzyme was separated from the mixture by Amicon Micro-Ultrafiltration System 8MC and ultimately freeze dried.

Animal Used

Male Wistar rates (310-510g) were used in the experiment,five rats had been used in each group. The animals were kept in air-conditioned animal room and have been injected itraperitonialy with:

1- 100mg of the polyphenols (produced by the enzyme action on the both substrate chlorogenic acid and catechol) dissolved in 1ml of normal saline(T1).

2-In the all treated groups compared to that 100mg of the FDE dissolved in 1ml saline(T2).

3-100mg of 20% oxidized FDE dissolved in 1ml saline(T3) .

Blood samples have been collected intracardially, after 16 hrs to the i.p. injection. The control animals have been treated only with 1ml of normal saline. Through out 16 hrs there were no abnormal symptoms of control.

Hormone analysis: Statistical

Serum prolactin was measured in duplicate by RIA with list supplied by Iraqi Radiopharmaceutical atomic energy Commission (IRAQ-Baghdad). The statistical analysis had been used least significant design (LSD) at level of significant 0.01.[6]

Results & discussion

The enzyme PPO used for oxidation of diphenols has been separated and the purification steps have been shown as in table(1).

For other details and elution profile see our previous work [1].

According to the hormone analysis it seems that there are

Table (1) Purification steps of PPO from Melissa officinals

Purification steps	Vol. ml	Activity unit\ml	mg\ml	Specific activity unit\mg	Total activity	% recovery	Fold purification
1-extraction from acetone powder	40	140	64.8	2.16	5600	100	1
2-calium acetate treatment	34	128	36	3.55	4352	77.4	1.64
3-gel filtration on sephadex G-75	54	77.4	12.6	5.99	4180	74.6	2.8
4- ion exchange chromatography on DEAE-sepharose CL-6B							
peak 1	75	25	0.28	89.3	1875	33.44	41.3
peak 2	43	21	0.25	84	903	16.14	39.0
peak 3	35	24	0.29	82.7	840	15.4	38.0
peak 4	18	30	0.4	75	540	9.63	34.0

Significant differences between most of the treatments except T2 & T3. Table(2) & fig.(1) show the mean of each treatment; it's obvious that treatment (T1) in which the animals had been injected with the polyphenols, which produced by the enzyme action has a potential effect in reducing the level of blood prolactin significantly, in other words treatment(T1) causes a maximum decrease of prolactin in animal's blood comparing it with other treatments (T2,T3 &C); hence the preparation of such compounds by the action of PPO enzyme open a new field for lowering of blood prolactine. The present work proves that the oxidation of plant phenols to other active compounds (polyphenols) by the enzyme PPO has a very important role in decreasing the blood prolactin levels specially in individual ,who suffer from hyperprolactinemia, which suppresses the secretion of gonadotropic hormones (7).

Table (2) applies a multiple comparison procedure to determine means which are significantly different from each other. The bottom half of the table(2) shows the estimated difference between each pair of means. An asterisk has been placed next to 5 pairs , indicating that these pairs show statistically significant differences at the 99.0% confidence level.

Table(2) Mean of treatments and multiple range tests:

Treatment	No. of rats/group	Mean prolactin ng/ml
Control(C)	5	3.372
Animals injected with polyphenols produced by enzyme action(T1)	5	1.7644
Animals treated with FDE(T2)	5	2.4988
Animals treated with FDE which has been oxidized with 20% $KMnO_4$ (T3)	5	2.21
Contrast	Difference	+/- Limits LSD
C-T1	*1.6076	0.404151
C-T2	*0.8732	0.404151
C-T3	*1.162	0.404151
T1-T2	*-0.7344	0.404151
T1-T3	*-0.4456	0.404151
T3-T2	0.2888	0.404151

*denote a statistically significant difference.

It seems that the polyphenols possess different properties in different plants, for example among many types of tea, green tea contains the most polyphenols and has five times black tea's in vitro polyphenol antioxidant potency enough to rival vitamin E's (8); green tea polyphenols also prevent dental caries (9) ; while in the present work it seems that the leaves of M. .officinalis contain polyphenol compounds, Which decrease the prolactin hormon.

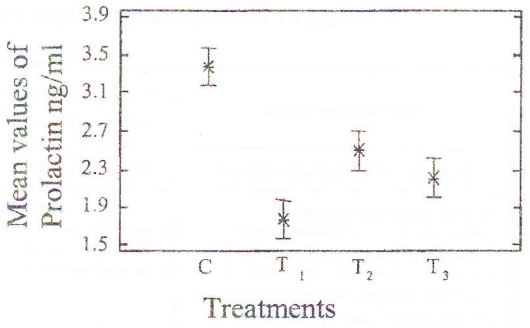

Fig (1) The effect of the studied treatments on blood prolactin level.

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References

[1] Sabir, D.M., putros B.Y:*J.Zankoy Sulaimani (JZS)*,part A, . 2000,**3**(1),1-13.

[2] Sourgenes H., WINTERHOFF H.G., and Kemper F.H. *plant med.*1988, 45(**2**), 78-860.

[3] Winterhoff, H., Gumbinger H. and Sourgens H., *planta medica*, 1988, **2**,101-106.

[4] Goodman and Gilmans: pharmacological basis of therapeutic (1986), seventh ed., p. 1374.

[5] Milton, J.S., and Jesse C. Arnold: Introduction to probability and statistics,1995.535.

[6] Nobel R.I., Plunkett E.R. and Graham R.C.B. *J.Endocriniology*1954, **10**,212.

[7] Dawn B.Marks, Biochemistry, 1994, 222, Harwal puplishing, ISBN 0-683-05597-b.

[8] Walsh G.P. ,*lancet* 1997 ,***349*** , 735

[9] Sakanaka S. ,Mujo K, *Polyphenols Actualitie's* . 1998,**18**,18-21.

رۆلی ئەنزیمی پۆلیفینۆل ئۆکسیدێز لە نزمکردنەوەی ڕێژەی هۆرمۆنی پرۆلاکتین

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پوختە

لەم توێژینەوەیەدا دراسەتی هۆکاری ئەنزیمی پۆلی فینۆل ئۆکسیدێزی جیاکراو لە ڕووەکی تورنجان پالأو کراوە بە چەند جۆری جیاواز لە کرۆمۆتۆگرافیا وەك (Ion exchange, gel filtration) لە سەر دروست کردنی پێکهاتەی پۆلی فینۆل کرا وە بینرا ئەم پێکهاتانە دەبنە هۆی کەم کردنەوەی چالأکی هۆرمۆنی پرۆلاکتین.

سێ جۆری جیاواز لە مامەڵەکردن یان هۆکار بە کارهێنرا، دراسەی کاری هەر یەکێکیان لەسەر نزم کردنەوەی ڕێژەی هۆرمۆنی پرۆلاکتین، ئەم هۆکارانەش بریتی بوون لە :

١- دەرئەنجامی کاری ئەنزیم لەسەر هەردوو ماددەی بنەڕەتی ترشی کلۆرۆجینیك و کاتیکۆل.

٢- دەرئەنجامی (١٠٠) میلیگرام لەو بەشە پالأوتەی ئەم ڕووەکە کە بە شێوەی ووشکە بەستەڵەك ئامادەکراوە (Freeze- dried).

لە دواییدا لێکۆڵینەوە لەسەر هۆکاری (١٠٠) ملیگرام لە پالأوتەی ئۆکسێنراوی ٢٠٪ ی ئەم ڕووەکە کرا.

لەئەنجامی ئامارکردن دا دەرکەوت کە پێکهاتووی مامەڵەکراوی یەکەم کە لەسەرەوە ئاماژەی پێکرا ڕۆڵێکی زیاتری هەیە بۆ نزمکردنەوەی ڕێژەی هۆرمۆنی پرۆلاکتین لە خوێنی ئەو ئاژەڵانەی کە لە ژێر تاقیکردنەوەدا بوون وە بوە هۆی نزم کردنەوەی هۆرمونی پرولاکتین لە ٣,٣٧٢ نانۆگرام/ مل بۆ ١,٧٦٤٤ نانۆگرام/ مل. ئەمەو ئەم شێوە دەرئەنجامانە سەرەتایی یەکە بۆ ئامادەکردنی ئەم پێکهاتووانەی کە بە هۆکاری ئەم ئەنزیمە دروست دەبن.

وەك ئاشکرایە کە بەرزبوونەوەی ڕێژەی پرۆلاکتین لە خوێندا یەکێکە لە هۆیەکانی نەزۆکی لە ئافرەت و پیاودا.

دور أنزيم البوليفينول اوكسيديز في خفض مستوى البرولاكتين

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الخلاصة

تم في هذا البحث دراسة دور انزيم الـ Polyphenol oxidase المستخلص والمنقى بطرق الكروماتوكرافيا المختلفة مثل gel filtration و Ion exchange chromatography- في نبات الترنجان *Melissia officinalis* في انتاج مركبات Polyphenols التي تحد من نشاط هورمون البرولاكتين(Prolactin). استخدمت في هذا البحث ثلاث معاملات مختلفة، ودرست تأثير كل منها على خفض مستوى هورمون البرولاكتين ، وكانت المعاملات كالأتي:

أولها ناتجة من فعل الانزيم على الركيزتين Chlorogenic acid و Catechol وثانيها ناتجة من تأثير ١٠٠ملغم من المستخلص النباتي المجفد(Freez-dried). وأخيرا تم دراسة تأثير ١٠٠ ملغم من المستخلص النباتي المؤكسد ٢٠% . وقد أظهرت النتائج الاحصائية ان للمركبات الناتجة من تأثير الانزيم على الركيزتين اكبر دور لخفض مستوى هورمون البرولاكتين في دم الحيوانات الخاضعة لتلك التجارب، حيث ادى الناتج المتكون من فعل الانزيم الى خفض مستوى هورمون البرولاكتين من ٣,٣٧٢ نانوغرام / مل الى ١,٧٦٤٤ نانو غرام / مل. . هذا وان مثل هذه النتائج ستفتح بابا لتحضير مركبات ناتجة من فعل ذلك الانزيم لخفض مستوى البرولاكتين، حيث كما هو معروف ان ارتفاع مستوى البرولاكتين في الإنسان هو من أحد أسباب العقم عند النساء والرجال على حد سواء.

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