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Gene Expression Associated with Comparative study between Lepidium sativum extract and hormonal treatment of ovarian inactivity in crossbreed cows

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Abstract

This research aimed to study the effect of Lepidium sativum (LS) seed extract and to compare between it and GnRH in treating inactive ovaries in cows by gene expression, the study was conducted on 27 lactating anestrus crossbreed cows, aged between 3-5 years in Al-Thahab Al-Abiad village / Abu-Ghriab/ Baghdad province during June 2020-July 2021. These animals were suffered from ovarian inactivity after 60-90 days postpartum. These dairy cows were randomly separated in to three equal groups. (9 cows on each). The 1st group treated by injected IM with 0.021mg/animal GnRH (5ml), the 2nd group treated with Lepidium sativum 25mg/orally/ for 10days (250mg/animal), the 3rd group left without treatment. Reproductive parameters included animal response, period of response, services number /conceptions, rate of pregnancy and days open. The results revealed that animal response in the 1st, 2nd and 3rd groups was significantly increase for the 1st (GnRH treated group) and 2nd (LS treated group) in compare with the 3rd group (control) , the duration of response in the three groups recording superior significantly ($P<0.01$) for treatment groups (G1 , G2) when compare with the control group (G3) and for G1 compared with G2, Pregnancy rate and days open in the 1st, 2nd and 3rd group was high significant in treatment groups in comparative with control group. We concluded that LS seed extract was effective in treating ovarian inactivity in cows close to GnRH.

Keyword: inactive ovaries, cow, *Lepidium sativum*, GnRH.

Introduction

Inactive ovaries, a reproductive problem in which the ovaries are small, smooth, and related with losses structures in ovary (1). So, the cow lacks signs of estrous and when examined by rectal palpation for diagnosis found small size ovaries with smooth surface and sometime found small follicle present (2). For this reason, the activity of ovary insufficient to produce gonadotropin hormone, this hormone necessary to induced follicle development, also sometime failure of ovary to respond to gonadotropin, and this condition association dairy cow with highly yield occasionally in heifer in first calving (3). In this field multiple hormones used to estrous induction when the cow diagnosis anestrous, the commune hormones used include (GnRH) gonadotropin releasing hormone, (FSH) follicle stimulating hormone, (LH) luteinizing hormone and (E2) estradiol hormone (4 and 5). Some recent studies also encourage the use of progesterone to treatment the inactive ovaries in cow (6; 3). On the other hand, the use of medicinal plants has contributed to the treatment of many conditions related to public health and reproduction in worldwide (7 and 8). The *Lepidium sativum* (LS), usually identified as cress garden, is a yearly concrescence, comestible, the herb fast growing which belongs to Brassicaceae family (9). The LS seeds contained multiple of nutrient component protein, carbohydrate, fat, phosphorous, potassium, magnesium, sulfur, calcium sodium, copper, iron, zinc and manganese, other contained the non-essential amino acid (glutamic acid, aspartic acid, serine, glycine, tyrosine, alanine and proline also contains essential amino acid (arginine, histidine, threonine, phenyl alanine, methionine, leucine, isoleucine, and lysine) (10). The LS herb extract used to treatment of different human aliment such as man seminal weakness (11), fracture healing (12), liver disorders (13). The herb extract has high inhibitory effect agent different pathogen organisms such as *Escherichia coli, Staphylococcus aureus, Proteus vulgaris and Klebsiella pneumonae* (14). In female LS seed extract used in different breeding aliment such as inducing abortion (15) , used contraceptive (16) and increasing milk production (17),. But to date no study investigates the effects of seed extract to LS herb on ovarian activity. So, this study aimed to compare between effect of seed extract to LS herb and hormonal treatments to inactive ovaries in cows

Materials and methods

Ethical approval: The study has been accepted by the Depart. of Surgery and Therio., College of Vet. Med., Uni, of Baghdad Iraq.

Study period and location: This study was conducted on 27 lactating anestrus crossbreed cows, their age between 3-5 years old in Al-Thahab Al-Abiad village / Abu-Ghriab/ Baghdad province during June 2020-July 2021.

Animals: collected Blood was purified using chloroform phenol (18). Using Roche's 1st Strand cDNA Synthesis Kit for RT-PCR (AMV), 0.5-1.5 µg of total RNA was used to synthesize single-strand cDNA and a random primer. (19)

PCR gene expression: The cycle RT consists of ten minutes of annealing at 25 degrees Celsius, sixty minutes of cDNA synthesis at 42 degrees Celsius, and five minutes of inactivation at 99 degrees Celsius. I had Electrophoresis done with DNase with a commercial kit (Promega, USA). These animals were suffered from ovarian inactivity after 60-90 days postpartum and diagnosed by rectal palpation and ultrasonography. These cows divided randomly into three equal groups (9 cows on each). The 1st group treated by GnRH (Receptal®- Ceva) injected IM with 0.021 mg/animal (5ml), the 2nd group treated with Lepidium sativum (The seeds were ground by an electric mixer into a fine powder, and then the powder was kept in a clean container in the refrigerator at a temperature of 4°C until used in the experiment , then the seeds powder were mixed with distal water at the time of treatment (suspension) in dose 25 mg/orally/ for 10days (250mg/animal), while the 3rd group considered as control group (without treatment).The reproductive parameters include animal response, the time it takes of response, services number per conception, rate of pregnancy and days open were recorded (4).

Statistical analysis: The statistical analysis includes the mean, the standard error, the Chi-Square, the T test and variance analysis were used according to (18).

Results and discussion

The results are shown in table- 1 explain the number of animal response were 7 (77.7%), 6 (66.6%) and 3 (33.3%) in the 1st, 2nd and 3rd group respectively with a highly significant different in ($P<0.01$) for the 1st (GnRH treated group) and 2nd (LS treated group) in compare with the 3rd group (control), The duration of response recorded 6.36±1.24, 13.72±2.45 and 87.2±0.1in the 1st, 2nd and 3rd group respectively, recording a highly significant differences ($P<0.01$) for the treatment groups (G1, G2) when compare with control group (G3) and for G1 compared with G2, the No. of services/conception showed no significant differences ($P<0.01$) in the three groups respectively 1.32±0.7 , 1.25±0.3 and 1.5±0.0. Pregnancy rate (table 2) was 85.7%, 83.3% and 66.6% in the three groups respectively with a highly significant difference in ($P<0.01$) for the 1st and 2nd group when compare with the 3rd group, and days open recorded 106.35±5.47, 114.26±6.52 and 187.40±0.0 in the 1st, 2nd and 3rd group respectively which was high significantly in ($P<0.01$) in treatment groups than the control group. **In conclusion**: the use of LS seed extract was effective in treated ovarian inactivity in lactating anestrus crossbreed cows close to GnRH. The clinical roles of UHPLC-QTOF-MS were further investigated. TCGA data showed that expression of UHPLC-QTOF-MS in treatment by Lepidium sativum was significantly higher than control (Fig. 1)

The results of table 1 recorded a highly significant different in ($P<0.01$) in animal response for the 1st (GnRH treated group) and 2nd (LS treated group) in compare with the 3rd group (control) and this agreed with (20), (7) who used a different herbal extract for treatment of inactive ovaries in cows achieving 80% response rate and it agreed with (21) when used LS seed supplement with intake food , observed the herb lead to increase food intake and increase

secretion of gonadotropins hormone in rabbit When the gonadotropin mediated through LS seeds activated the estrogen to resulting increase of FSH and LH hormone secretion. Also, it agreed with (22, 23 and 24) who recorded 50%; 66.6% and 50% respectively to response to GnRH treatment of ovarian inactivity in cows and buffaloes, the result studies explain the systemic administration of GnRH hormone causes to increase FSH and LH levels in plasma in cows (2, 4), then these hormones stimulated development of follicles in the ovary and induction of estrous signs in female (23, 24 and 5, 6). The duration of response recording a highly significant differences ($P<0.01$) for the treatment groups (G1, G2) when compare with control group (G3) and for G1 compared with G2 and these results agreed with (22) , (25) and with (26), (21), the number of services/conception showed no significant differences in the three groups respectively and this agreed with (22 , 26). Pregnancy rate (table 2) recording a highly significant difference in ($P<0.01$) for the 1st and 2nd group when compare with the 3rd group, and days open recorded high significantly ($P<0.01$) in treatment groups than the control group, Increased expression of UHPLC-QTOF-MS Metabolite (Fig. 1, 2) these results agree with (27; 28)

Table - 1. Show the groups used in study, the number of cow response, and the time required to response and number of services per conception in lactating anestrus crossbreed cows.

Groups	No. of animals	Type of treatment	Animals' response No.	%	Duration of response M±SE	No. of services/ conception s
G1: GnRH	9	GnRH (Receptal)® 0.021mg/animal / IM	7 **a**	77.7	6.36±1.29 **c**	1.32±0.7 **a**
G2: Lepidium sativum	9	Lepidium sativum (LS) 5mg/orally/ daily for 10days (250mg/animal)	6 **a**	66.6	13.70±2.45 **b**	1.25±0.3 **a**
G3: control	9	Without treatment (control)	3 **b**	33.3	87.2±0.1 **a**	1.5±0.0 **a**
Total	27	----------	Treated 13/18 Control 3/9	72.2 33.3		

Different small letters mean the significant differences in (P<0.01) among groups (vertical).

Table-2. **Showed the type of treatment, animals' response, pregnancy rate and days open in lactating anestrus crossbreed cows.**

Groups	Type of the treatment	No. Animals response %		Pregnancy rate in cow %		Days open M±SE
G1: GnRH	GnRH (Receptal)® 0.021mg/animal / IM	7/9	77.7	6/7 **a**	85.7	106.35±5.47 **b**
G2: Lepidium sativum	Lepidium sativum (LS) 5mg/orally/ daily for 10days (250mg/animal)	6/9	66.6	5/6 **a**	83.3	114.26±6.52 **b**
G3: control	Without treatment (control)	3/9	33.3	2/3 **b**	66.6	187.40±0.0 **a**
Total	----------	T: 13/18 C: 3/9		T: 11/13 C: 2/3	84.6 66.6	

Different small letters mean the significant differences in (P<0.01) among groups (vertical).

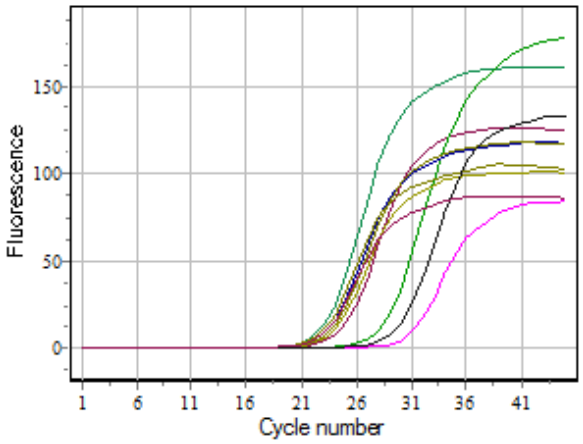

Figure 1: FAM channel fluorescence is determined by the cycle number.

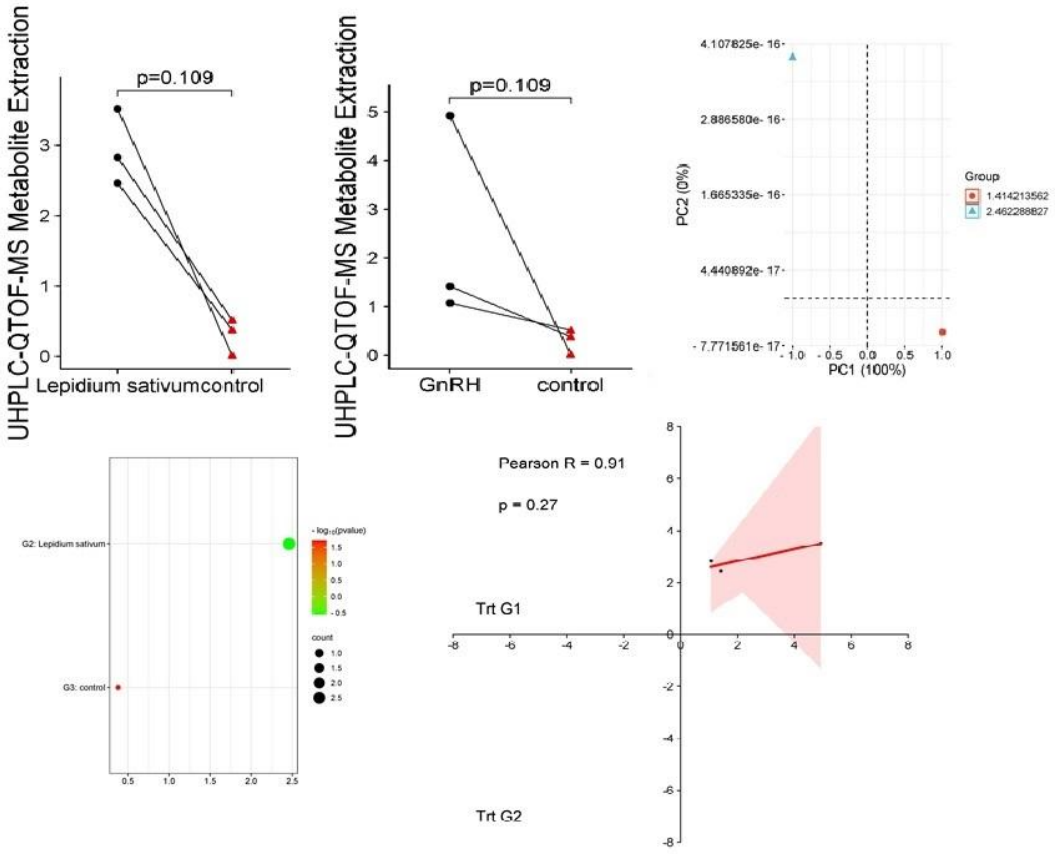

Figure 2: The gene expression of UHPLC-QTOF-MS Metabolite Extraction in cows

Conclusion

The use of LS seed extract was effective in treated ovarian inactivity in lactating anestrus crossbreed cows and it is similar to the effect of hormonal therapy with fewer negative aspects that may occur during hormone treatment.

Acknowledgments

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Novelty Statement

This study introduces an innovative approach to obtaining Gene Expression Associated with a Comparative study between Lepidium sativum extract and hormonal treatment of ovarian inactivity in crossbreed cows with prolificacy in improving reproductive efficiency among different cow

Conflicts Of Interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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التعبير الجيني المرتبط بدراسة مقارنة بين مستخلص نبات الرشاد والعلاج الهرموني خمول المبايض في الأبقار الهجينة

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الخلاصة

هدف هذا البحث إلى دراسة تأثير مستخلص بذور نبات الرشاد ومقارنته مع هرمون المحفز للقند في علاج خمول المبايض في الأبقار عن طريق التعبير الجيني، أجريت الدراسة على 27 بقرة هجينة مرضعة تراوحت أعمارها بين 3-5 سنوات في قرية الذهب الأبيض (أبو غريب) خلال الفترة من يونيو 2020 إلى يوليو 2021. عانت هذه الحيوانات من خمول المبايض بعد 60-90 يومًا من الولادة. تم تقسيم هذه الأبقار إلى ثلاث مجموعات .المجموعة الأولى عولجت عن طريق الحقن العضلي لهرمون المحفز للقند بـ 0.021 ملغ / حيوان ، المجموعة الثانية عولجت نبات الرشاد بـ 25 ملغ / عن طريق الفم / لمدة 10 أيام (250 ملغ / حيوان)، المجموعة الثالثة تركت بدون علاج. وشملت المعايير الإنجابية استجابة الحيوان، وفترة الاستجابة، ومعدل الحمل والأيام المفتوحة. أظهرت النتائج أن استجابة الحيوانات كانت 7 (77.7٪) و 6 (66.6٪) و 3 (33.3٪) في المجموعة الأولى والثانية والثالثة على التوالي مع زيادة معنوية ($P <0.01$) للمجموعة الأولى والثانية بالمقارنة مع المجموعة الثالثة وسجلت مدة الاستجابة 6.36 ± 1.24 و 13.72 ± 2.45 و 87.2 ± 0.1 في المجموعات الثلاث على التوالي مسجلة تفوقًا معنويًا ($P <0.01$) لمجموعات العلاج (G1 و G2) عند مقارنتها بمجموعة السيطرة (G3) وبالنسبة لـ G1 مقارنة بـ G2، وكان معدل الحمل 85.7٪ و 83.3٪ و 66.6٪ وسجلت أيام المفتوحة 106.35 ± 5.47 و 114.26 ± 6.52 و 187.40±0.0 في المجموعة الأولى والثانية والثالثة على التوالي والتي كانت ذات دلالة إحصائية عالية ($P<0.01$) في مجموعات العلاج بالمقارنة مع مجموعة السيطرة. ونستنتج إلى أن مستخلص بذور LS كان فعالاً في علاج خمول المبايض في الأبقار.

الكلمات المفتاحية: خمول المبايض ، البقرة، بذور الرشاد، هرمون المحفز القند.