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Hesperidin improves fertility in female mice infected by*Brucella melitensis*

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ABSTRACT

The present study was designed to improve fertility in female mice caused by virulent strain of *Brucella melitensis*. Sixty female mice were divided into 5 groups equally: Group1 giving normal saline 0.3 ml orally serve as control negative. Group2 served as control positive infected with a virulent strain of *B. melitensis*($1x10^8$) CFU/ ml I/P. Group 3 administrated hesperidin 10mg /kg B.W. group 4 infected with *B. melitensis*and treated with hesperidin 10mg/kg B.W. Group 5 treated with hesperidin 10mg /kg B.W. and infected with *B. melitensis*($1x10^8$). At thirty and sixty days of the experiment (half and end of the experiment), six mice from each group were sacrificed to determine serum hormones (FSH, LH, estrogen, and progesterone). The results revealed that hesperidin reduces estrogen-progesterone levels and increases levels of FSH and LH in comparison with an infected group (G2) serum levels of estrogen, progesterone was high and FSH and LH were low levels. This study concluded that hesperidin improves fertility because of act as anti-cancer, anti-oxidant anti-inflammatory action that giving protect of female reproductive organs of female mice in comparison with infected mice with *B. melitensis*which caused a different change of hormonal levels that lead to cause infertility.

Keywords: Hesperidin, brucellosis, infertility and reproductive hormones.

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INTRODUCTION

The bioflavonoid hesperidin with increasing its concentration in citrus fruits like as lemon and orange, also derivative plant beverages, like as olive oil and tea used in conventional medicines commonly, the beneficial function in cardiovascular, anti-inflammation anti-oxidant diabetes type II. Recently determinates that Hesperidin more beneficial effect for skin care including recovery of wound, anti-skin cancer, anti-inflammatory, skin lightening and anti-microbial (Garg *et al*., 2001; Mao-Qiang *et al*., 2019).

Mao-Qiang *et al*., (2019), mentioned the mechanism of hesperidin advantage the skin function attributable to anti-oxidant features of hesperidin, stimulate the proliferation of epidermal , production of lipid, differentiation and inhibit pathways of MAPK- depended signals.Benefits A bioflavonoid hesperidin, have been well appreciated on human health. Many studies have detected that systematic administrations of hesperidin exhibit advantage for a different disease, including, Cancer, Alzheimer's, diabetes and cardiovascular (Ahmadi and Shadboorestan 2016; Sugasawa *et al*., 2018). Garg *et al*(2001) recorded that Hesperidin has diverse pharmacological actions, such as anti-carcinogenic, anti-viral, antifungal, anti-bacterial, antiulcer, anticancer activity, anti-inflammatory antioxidant, analgesic and anticarcinogenic. Also, hesperidin has antiproliferative effect versus MCF-7 with programe cell death function on pancreatic and colon cancer (Park *et al*., 2008; Natarajan *et al*., 2011). Sahu *et al*., (2013), and Jangra *et al*., (2015) mentioned that hesperidin protects the tissues from infectious and toxic agent that caused oxidative stress and free radical-scavenging action.

Brucellosis it's a contagious and infectious disease caused by intracellular facultative bacteria named brucella one of the many zoonotic disease in the world that infected humansand animals,causingabortion of animals as a resulting of reproductive system disease, infection with brucella needed fast medical treatment including vaccine and other treatment (Hull and Schumaker, 2018; Vitry *et al*., 2020). Species of brucella are non-motile gram-negative (ve-) cocobacilli intracellular facultative dose not form toxin and spore,most kind of its spescies infected human because its zoonoticsuch as brucella melitensis infected goat and sheep, aboutus infect cattle, suis in swin and canis for dog (Brown *et al*., 2018; Jiang *et al*., 2020). Brucellosis transmited to human during dairy product and drinking milk from infected animals (Pecket *al*., 2017).

Głowacka *et al*., (2018), reported brucellosis transmitted during milk from infected goat. Silva *et al*., (2011) mentioned that infection with brucella causes oxidative stress in human and high range in wild and domestic animals with public problem of health in world and economic loss due reproductive diseases. Megid *et al*., (2010), also reported that brcella cause infertility for both male and females infection increase mortality rate in kids and lambs, with orchitis, epididymitis, seminal vesiculitis in male and stillbirth, weak offspring birth, and abortion in females. these organism affects most internal organs of the host particularly male and female reproductive system that lead to infertility and abortion (D'Anastasio *et al*., 2011). *Brucella* have two line of defense mechanisms against phagocytic cell ROS killing, the first line is catalase, superoxide dismutase which act to detoxify phagocytic ROS and the second line including enzyme used to (Hornback and Roop,2006) *Brucella* resistance oxidative damage of ROS generated in phagosome of macrophages following phagocytosis (Roop *et al*.,2004). after bacterial replication, Brucella mutated into rough strain which cause cytotoxic effect on macrophage and they release from these infected cell and favors their dissemination (Pei *et al*., 2014), however, *Brucella* lipoprotein cause inhibited adaptive immune response via stimulated T cells apoptosis (Velasquez *et al*., 2012). Few studies reported about female reproductive system in female mice (Faez *et al*., 2013).

Materials and Methods

Determination therapeutic dose of hesperidin

Therapeutic concentration dose of hesperidin was 10 mg / kgB.W according to (Felipe *et al*., 2015).

Bacterial isolate

Virulent *Brucella melitensis*isolate obtained from Al-Nahdha Veterinary Laboratories /Baghdad, biochemical tests and growth were performed for isolation, confirmation and diagnosis according to (Quinn *et al.*, 2004).

Determination of challenge dose of B. melitensis

Activation of bacteria and bacterial counting doneaccording to (Quinn *et al.*, 2004). The challenge dose was 1×10^8 CFU/ml (Milles and Misra, 1983).

Determination of serum hormonal values of FSH and LH, Estrogen and Progesterone

Levels of Estrogen, Progesterone, FSH and LH hormone in mice sera were assessed by using coulter kit obtained from Immunotech/Czech Republic (Kit). The result was reported as (Pg/ml). The test was carried out according to the assay protocol of manufacture

Experimental Design

Including sixty (BALB/ C Strain) female mice used in this study, aged 8-10 weeks and weighed 27-30 gm. were divide into five groups equally, every group, treated in the following.

*__Group 1__(n=12): Administrated normal saline oral route 0.3 ml and served as a control group.

*__Group 2__ (n=12): Infected with ($1x10^8$ CFU/ml) of virulent *B. melitensis.*

*__Group 3__(n=12): Hesperidin giving orally (10 mg /kg B.W) for thirty days.

*__Group4__ (n=12): infected with ($1x10^8$ CFU/ml) of virulent *B. melitensis*, after 30 days treated Hesperidin (10mg /kg B.W) for 30 days also.

*__Group5__ (n=12): Hesperidin giving orally (10 mg /kg B.W) for thirty days and infected with virulent *B. melitensis*. The period of this study was sixty days after first thirty days six female mice from each group were sacrificed and serum blood were collected to determine the levels of for estimation of hormonal level (L.H, F.S.H, estrogen and progesterone) in each group. Also, after the sixty days (end of experiment) the process of blood sample collecting and measuring levels of reproductive hormones were done.

Statistical Analysis

Data were represented as means + SE. One-way analysis of variance according to (Snedecor and Cochran, 1989), by using (One-way ANOVA) SPSS program, the valeus of statistical significance was set at ($P < 0.05$).

Ethical approval

Approved the present study by the ethical and research committee- Baghdad University, Ministry of High Education and Scientific Research. College of Veterinary Medicine.

Results and discussion

Levels of reproductive hormones at day 30 post infection:

The results in table (1) revealed that the means of serum FSH, LH, Significant decrease in the infected group (G2). (7.9±0.340, 4.9±0.280) respectively in comparison with control group (G1) (15.1±0.620, 8.1±0.400) respectively. While serum values of FSH and LH was increase in groups treated with hesperidin (G3, G4 and G5) (14.2 ± 0.290, 7.7 ± 0.330), (11.3±0.550, 5.8±0.870) and (14.5±0.640 ,7.9±0.120) respectively. Also, the serum levels of estrogen and progesterone were increase in group infected (G2) (72.6± 0.20,61.9±0.11) respectively, in comparison with control group (G1) (35.3 ± 0.11, 34.7 ± 0.20) respectively. While the levels of estrogen and progesterone were decrease in all groups treated with hesperidin (G3, G4, and G4) (39.5± 0.60, 35.1± 0.80), (49.7± 0.90,43.7± 0.40) and (40.1± 0.66,34.7±0.90) respectively (table:1).

Table 1: Values of serum levels of hormones in immunized animals at thirty days post infection.

Time group	**FSH (Pg/ml)**	**LH (Pg/ml)**	**Estrogen (Pg/ml)**	**Progesterone (Pg/ml)**
G1	15.1 ± 0.620 **A**	8.1 ± 0.400 A	35.3 ± 0.11 **E**	34.7 ± 0.20 **D**
G2	7.9 ± 0.340 **D**	4.9 ± 0.280**D**	72. 6 ± 0.20**A**	61.9 ± 0.11 A
G3	14.2 ± 0.290 **B**	7.7 ± 0.330 **B**	39.5 ± 0.60 **D**	35.1 ± 0.80 **C**
G4	11.3 ± 0.550 **C**	5.8 ±0.870 C	49.7 ± 0. 90 **B**	43.7 ± 0.40 B
G5	14.5 ± 0.640 **AB**	7.9 ± 0.120 AB	40.1 ± 0.66 C	34.7 ±0.90 **D**

Different capital letter means significant (*P*≤ 0.05).

Levels of reproductive hormones at day 30 post infection:

The results in table (2) revealed that the means of serum FSH, LH, Significant decrease in the infected group (G2). (6.2 ± 0.970, 5.7 ± 0.420) respectively in comparison with control group (G1) (15.5± 0.330, 8.9±0.500) respectively. While the serum levels of FSH and LH was increase in all groups treated with hesperidin (G3, G4 and G5) (15.1 ± 0.240, 7.3 ± 0.110), (14.2± 0.120, 6.9±0.500) and (14.6±0.700,7.9±0.900) respectively. Also, the serum levels of estrogen and progesterone were increase in group infected (G2) (90.7± 0.880, 74.1 ± 0.50) respectively, in comparison with control group (G1) (37.8 ± 0.70, 35.9 ± 0.44) respectively. While the levels of estrogen and progesterone were decrease in all groups treated with hesperidin (G3, G4, and G4) (34.7±0.70, 33.0±0.87), (36.9±1.20,36.6±0.67) and (33.8±0.75, 35.9±0.20) respectively (table:2).

Table 2: Values of serum levels of hormones in immunized animals at sixty days post infection.

Time group	FSH (Pg/ml).	LH (Pg/ml).	Estrogen (Pg/ml).	Progesterone (Pg/ml).
G1	15.5 ± 0.330**A**	8.9 ± 0.500**A**	37.8 ± 0.70 **B**	35.9 ± 0.44 **B**

G2	6.2 ± 0.970**C**	5.7 ± 0.420**E**	90. 7 ± 0.880 **A**	74.1 ± 0.50 **A**
G3	15.1 ± 0.240**AB**	7.3 ± 0.110**C**	34.7 ± 0.70 **B**	33.0 ± 0.87 D
G4	14.2 ± 0.120**B**	6.9 ±0.500 D	36.9 ± 1. 20 C	36.6 ± 0.67 **B**
G5	14.6 ± 0.700**B**	7.9 ± 0.900 **B**	33.8 ± 0.75 C	35.9 ±0.20 C

Different capital letter means significant ($P \leq 0.05$).

Discussion

The present finding revealed that serum levels of FSH and LH at thirty and sixty days of the experiment were decreasing in the infected animals with Brucella melitensis (G2) in comparison with groups treated with hesperidin were increased (G3, G4, and G5) and control group (G1). Also, the serum levels of estrogen and progesterone at 30 and 60 days increased in the infected group (G2) in comparison with all groups (G3, G4, and G5) treated with hesperidin and negative control group (G1). Results may reveal that infection by B. melitensis causes oxidative stress that leads to caused lipid peroxidation and damage to the nuclear cell DNA this result with an agreement with Alkhafajy (2017), who reported that infected animals by Brucella melitensis cause oxidative stress associated with infertility in female and male mice with severe damage to the reproductive organ ovary and uterus, these pathogen cause reproductive disorder, due to degenerative changes occur in the hypothalamus, pituitary, and gonads that lead to a decline in the concentration of sex hormone particularly estrogen and progesterone which essential hormone in the reproductive process.Goto *et al*., 1993 and Shoorei et al., 2017 mentioned thatproduction of free radicals causes decreasing follicular development. Also, ROS influence on the growth and maturation of the follicles, it may be a main caused of poor oocyte quality and may thereby decrease the developmental competence of oocytes in vivo and in vitro.

Also, Agarwal *et al*., (2012) and Zanganeh*et al*., (2017) record oxidation act as important actors in the pathogenesis of subfertility in both sex females and males. The adverse action of oxidative stress on females' reproductive function and on oocytes. Agarwal and Said, 2005 record that the variance between antioxidants and pro-oxidants will cause reproductive diseases such as unexplained infertility and endometriosis. An abnormal rise in reproductive hormones estrogen and progesterone may associated with a defect in the normal development of ovarian follicular; this idea was in agreement with an investigation of Othman *et al*., (2016) who stated that infection by intracellular facultative bacteria in non-pregnant does may lead to infertility resulting from pathological changes in the reproductive organs as well as an imbalance levels in reproductive hormones. Also these results in agreement with Hall (2015) mentioned Brucella infection cause damage of pituitary gland in the anterior lobe a that responsible for production LH and FSH hormones,brucellosis induced damage in the ovary, since LH and FSH hormone are glycoprotein in nature because of the effect of GNRH that secreted from hypothalamus, under hormonal of ovarian regulation, progesterone and estradiol (mechanism feedback negative and positive). Kataria *et al*., (2010), mentioned that brucella causes depletion of anti-oxidant and have the ability to increase the production of free radical that has role in pathogenesis, severe oxidative damage to the organs. Infertility, abortion in female reproductive system, and damage to the internal organs are the most features of Brucella infection with reproductive disturbance (D'Anastasio *et al*., 2011; Silva *et al*., 2011).

The lowering levels of estrogen and progesterone with an increase in FSH and LH hormone in all groups treated with hesperidin because of the anti-oxidant, anti-fibrotic, anti-inflammatory anti-bacterial, and bactericidal effect of hesperidin and these results in agreement with the Jones et al (2004) and Mao-Qiang *et al*., (2019) who mentioned the important role and feature of hesperidin exhibits different pharmacological and biological characteristic's like as vitamin-like activity and will reduce permeability (vitamin P), of the capillary, fragility, and leakiness. hesperidin also acts as antioxidant, anti-inflammatory, anticarcinogenic, anti-bacterial, lipid-lowering, antihypertensive, vasoprotective, and protect against ischemia-reperfusion tissue damage. hesperidin is natural flavonoid have several features used in medical treatment because of their action of antioxidant effect that lowering peroxidation of lipid in biological membranes and thus act as a potential therapeutic factor (Amic *et al*., 2003). Also, the result in agreement with Kim *et al*., (2019), who recorded treatment by hesperetin wouldimprove the in vitro development of aging porcine oocytes and expression of mRNA in some cytoplasmic maturation marker genes, such as morphogenetic protein of bone and growth prolifrationfactor. Anti-oxidant supplement such as hesperidin induce maturity and growth of follicles by lowering oxidative stress and reducing peroxidation of lipid that protect tissue from damage with promote development of follicles, embryonal development and improve fertilization rate (Kim *et al*., 2019). Also, agreement with Khedr, (2015) recorded that hesperidin, due to their antioxidant activity, provides the protection of fertility against reactive oxygen species induced by CP I ovaries of rats. Treatment by Bioflovonoid hesperidin showing increase of follicular stimulating hormone receptor to mRNA and increase levels of estrogen and progesterone, also its essential for ovarian function and fertility (Shoorei *et al*., 2019).

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