Tikrit Journal for Agricultural Sciences Vol. (18) No.(4) – 2018
ISSN-1813-1646

Turmeric Oil Effect on the Reproductive Efficacy of Female Albino Rats Treated with Iron Overload

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Key words:
Tumeric oil, Iron overload, Glutathione, Malondialdehyde.

Article History

Received: 05/12/2017
Accepted: 24/01/2018
Available online:
30/12/2018

ABSTRACT

The present study was designed to investigate the protective role of turmeric oil on reproductive performing of female rat exposed to iron overload. Thirty immature female rats aged (21) day were utilized in this study and distributed into three equal groups includes control grp , group treated with ferrous sulfate (100mg|kg) and group treated with ferrous sulfate 100mg|kg and with turmeric oil (20 mg|kg) at half an hour interval, each group of (10) rats treated by oral administration for 14 day. Administration of iron for immature female rat caused significant increase in malondialdehyde level, Hb concentration, PCV and iron serum level accompanied by significant decrease in body weight, uterus weight, ovaries weight, numbers of mature follicles, diameters of mature follicles, estrogen and glutathione concentration compared with control group while the treatment with iron overload and turmeric oil showed a significant increase in body weight, number of mature ovarian follicles, estrogen, glutathione concentration and significant decrease in malondialdehyde level, Hb concentration and iron serum level compared with iron overload group.

التأثير الوقائي لزيت الكركم على الكفاءة التناسلية في إناث الجرذان البيض المعاملة بفرط الحديد

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الخلاصة

اجريت هذه الدراسة لمعرفة تأثير فرط الحديد والدور الوقائي لزيت الكركم في الجرذان المعاملة بفرط الحديد على عملية تكوين الجريبات المبيضية في الاناث غير البالغة حيث تم استخدام (30) من اناث الجرذان غير البالغة بعمر (21) يوما وقسمت الى ثلاث مجاميع متساوية تضمنت مجموعة السيطرة السليمة ومجموعة فرط الحديد (تم تجريعها بكبريتات الحديدوز 100 ملغم/كغم من وزن الجسم) ومجموعة المعاملة بزيت الكركم وفرط الحديد (جرعت بكبريتات الحديدوز 100 ملغم/كغم من وزن الجسم ومن ثم زيت الكركم بجرعة (20) ملغم/كغم من وزن الجسم بعد نصف ساعه) كل مجموعة مكونة من 10 جرذان وعوملت الحيوانات يوميا ولمدة 14 يوما وبوساطة التغذية الأنبوبية عن طريق الفم وقد تم تحضير المحاليل آنيا. أحدثت معاملة إناث الجرذان غير البالغة بالحديد زيادة معنوية في مستوى المالون ثنائي الديهايد ، تركيز الهيموكلوبين، حجم خلايا الدم المرصوصة ومستوى الحديد رافقه انخفاض معنوي في وزن الجسم، وزن الرحم، وزن المبيضين، أعداد الجريبات المبيضية الناضجة، معدل أقطار الجريبات الناضجة ومستوى هرمون الاستروجين ومستوى الكلوتاثايون الكلية مقارنة مع مجموعة السيطرة في حين أظهرت المعاملة بالحديد وزيت الكركم معا زيادة معنوية في وزن الجسم, أعداد الجريبات المبيضية الناضجة، مستوى هرمون الاستروجين ومستوى الكلوتاثايون مع انخفاض معنوي في مستوى المالون ثنائي الديهايد، تركيز الهيموكلوبين ومستوى الحديد مقارنة مع مجموعة الحديد .

الكلمات المفتاحية:
التأثير الوقائي، زيت الكركم، الكفاءة التناسلية، الجرذان البيض، فرط الحديد.

الاستلام: 2017/12/5
القبول: 2018/1/24

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Introduction:

Term iron overload represent a condition result from increase total iron store in the body which cause impaired in organs function (Piperno,1998; Dogar *et al.*, 2013). Iron is take up from food in duodenum and superior part of jejunum. Utmost food have two essential types of iron: ferrous iron protoporphyrin from the red meat and ferric iron from grain and vegetable (Conrad *et al.*, 1991). Living beings have developed mechanism to keep iron homeostasis, comprising the planned direction of iron ingestion, reusing iron and utilization of iron stored. In any case, in spite of these mechanisms, creatures have a restricted capacity to discharge abundance iron, likely because of absence of developmental powers for this ability (Sanchez *et al.*, 2011). The iron is lost from body through uncontrolled losses of the blood normally gastrointestinal, additionally huge iron lost via menstrual bleeding in ladies of kid bearing age, kidney epithelial cells, skin and by excretions for instance sweat, tears and gastrointestinal discharges (Troadec *et al.*, 2011).

Sterility in female patients with beta thalassemia gives off an impression of being started via direct and indirect effect of iron overload. The dysfunction of female reproductive axis resulted by increased oxidative stress (as a result of prooxidants- antioxidants imbalance) and iron deposition effect (Roussou *et al.*, 2013) .

Turmeric is acknowledged as the (brilliant flavor) in addition to the (zest of life). In India turmeric has been used as a therapeutic plant, and held hallowed from timeworn . Curcuma L-Zingiberaceace genus contain numerous benefit, therapeutic with cultural value (Shrishail *et al.*, 2013; Hossen *et al.*, 2017). Turmeric oil and its ingredients have evidenced to be antiviral, antibacterial and antifungal (Chattopadhyaya *et al.*, 2004; Farkhondeh and Samarghandian, 2016). Curcumin used to treat different infections and can be used as food addition as a result of its little price, the chemoprotection, restorative and pharmacological actions for turmeric at invivo and invitro made its valuable medicine(Vijay Kumar *et al.*, 2011; Jaiswal *et al.*, 2016) . Curcumin displays strong cancer prevention agent action, equivalent to vitamin C and E and its confirmed antioxidant properties and anti- inflammatory has been appeared to be an effective scrounger of a variety of reactive oxygen species including nitrogen dioxide radicals, hydroxyl radicals and superoxide anion radicals (Fatima and Mahmood, 2007; Lee *et al.*, 2017).

Objective of the study: This study was conducted to know the effect of iron overload and the role of turmeric oil on the process of the development of ovarian follicles on immature female rats and to assess the effects of turmeric oil on glutathione and malondialdehyde level.

Materials and methods:

Chemicals:

1. Ferrous Sulfate

$FeSo_4$ (British Drug Houses, Eng.) melted in (0.01N) hydrochloric acid.

Animal were given orally $FeSo_4$ (100 milligram/kilogram B.W.) once a day (Bodiga and Krishnapillai, 2007).

2. Turmeric oil 20 mg/kg of body weight in a single daily dose (Hastak *et al.*, 1997).

Experimental design: Thirty immature female rats *Rattus norvegicus* at the age of 21 days were used in this experiment . female rats were gained from SDI, Iraq. Rats were housed in wire-floored cages under standard laboratory conditions of 12 h/12 h light/dark, 25±20C with free access to food and water. All animals were acclimatized to laboratory condition for a week before commencement of experiment.

The female rats were distributed into three major groups each group consist of 10 female rats at age twenty one day and treated for (14 day):1) Control group: treated with (0.01N) hydrochloric acid, 2) group treated with $FeSO_4$, 3) group treated with $Feso_4$ and after half an hour given turmeric oil. Animals were killed at the end of experiment and the ovaries were taken and weighed, then put in 10% neutral buffered formalin solution for fixation and then left in 70% alcohol and prepared for histological sectioning (Bancroft and Stevens, 1987) .

Preparations for histological study: Ovaries were taken for each experimental group directly after dissection and histologically processed . Paraffin sections 5µm were prepared and stained with hematoxylin and eosin stain according to Bancroft & Stevens. The histological sections of each ovary were examined and the number of mature ovarian follicles was recorded and the diameters of mature ovarian follicles in each section were measured using the ocular micrometer (Bancroft and Stevens, 1987).

Blood Sampling: The blood was gathered from each female rat into a clean centrifuge tube straight after sacrificing. The blood was centrifuged to discrete the serum for fifteen minutes. The isolated serum was put away at -20C for consequent determination of biochemical parameters such as, glutathione, malondialdelyde (MDA), iron serum level and estrogen. hemoglobin (HB) and mean corpuscular volume (MCV) were analyzed using an automated hematology analyzer , Sysmex Corporation Ltd., Germany).

Statistical analysis: All data were examined using SPSS and Microsoft Excel XP system. ANOVA test were used to assess the variables between grps. The mean were compared using the test of DMR (Brunning and Kintz, 1977).

Results:

1. **Body weight, weight of ovaries and uterus, numbers of mature ovarian follicles and their diameters and estrogen level.**

   The treatment with $Feso_4$ reveal a significant decrease in body weight, uterine weight, weight of ovaries, numbers of mature ovarian follicles and their diameter rate and estrogen level compared with control group. Treatment with iron and turmeric oil significantly increased P <0.05) in body weight and numbers of mature ovarian follicles and their diameter rate and estrogen level with no significant differences in weight of the uterus and ovaries compared to iron overload group table (1).

2. **Malondialdelyde (MDA) and Glutathione (GSH) Level**s

   The results showed significant increase of glutathione levels in group treated with $Feso_4$ and turmeric oil compared with control grp and $Feso_4$ group as well as a significant increase (p <0.05) in the level of Malondialdelyde $Feso_4$ group compared with $Feso_4$ and turmeric oil group and control grp table (2).

3. **Serum Iron Level**, **Packed Cell Volume (PCV) and Hemoglobin Concentration (Hb)**

   Results indicates a significant increase in the concentration of hemoglobin **(Hb)** and packed cell volume **(PCV)** in the $Feso_4$ group compared with the group treated with $Feso_4$ and turmeric oil together and the control grp ,in addition to significant increase of serum iron concentration in $Feso_4$ group and the group treated with $FeSO_4$ and turmeric oil compared to the control group table (2).

Table (1): Protective effect of Tumeric oil on body weight, ovarian weight, uterine weight, mature follicles numbers and mature follicle diameter in various animals groups.

Variables \ Groups	Control	$Feso_4$	$Feso_4$ and Turmeric oil
Body weight (gm)	79.65± 1.27 a	47.22 ± 1.71 c	68.12 ± 1.93 b
Ovarian weight (gm)	0.88 ± 0.091 a	0.70 ± 0.065 b	0.76± 0.071 b
Uterine weight (gm)	3.50 ± .182 a	1.93 ± 1.51 b	2.33 ± 1.92 b
Number of mature follicles	2.49 ± 0.066 a	0.94 ± 0.038 b	2.29 ± 0.066 a
Diameter of mature follicle (µm)	293.50±3.68 a	201.33±1.55 b	298.18±1.48 a
Estrogen (Pg/ ml)	53.62 ± 1.94 c	40.11 ± 1.35 b	60.93 ± 1.89 a

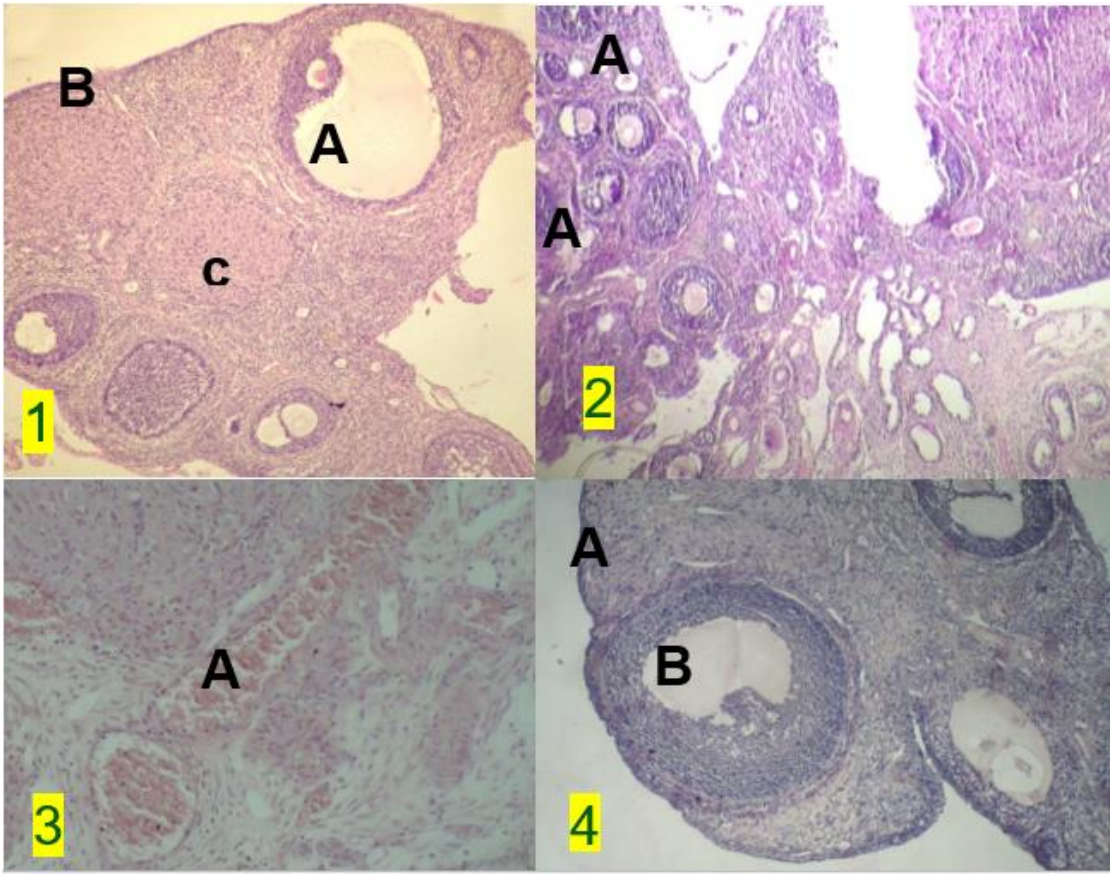
Table (2): Protective effect of Tumeric oil on Malondialdelyde, Glutathione, Hb, PCV and the level serum iron in various animals groups.

Variables \ Groups	Control	Iron overload	Iron overload & Turmeric oil
MDA (µMOL/L)	1.660±0.629 b	2.920±0.529 a	1.403±0.444 c
GSH (mg/dl)	6.319±0.068 b	6.006±0.057 c	6.965±0.062 a
Hb (gm/100ml)	11.883±0.386 b	14.026±0.534 a	12.132±0.382 b
PCV%	35.434±0.063 b	42.389±0.032 a	35.222±0.060 b
Serum iron (µg/100ml)	165.722±0.032 c	183.087±0.065 a	180.962±0.041 a

4. Histological Finding

Results of histological examination of female rats ovaries of control group demonstrated normal follicles and germinal epithelium (Picture 1).

Picture (2) shows ovary of female rats in $FeSO_4$ group exhibited several harmful histological changes, many atretic follicles, and germinal epithelium degeneration, While Picture (3) reveals congested veins in the medulla of female rat ovary. The ovaries female rats treated with $Feso_4$ and Turmeric oil showed mature follicle and different stages of developmental follicles (Picture 4).



(Picture1): The ovary of control group reveals normal mature follicles in the cortex (A), germinal epithelium (B) and medulla (C) . (Picture 2) shows degeneration and atretic follicles A in the cortex of ovary of female rats treated with $FeSO_4$. (Picture 3) showed congested veins (A) in medulla of ovary $Feso_4$ group. (Picture 4) Ovary of female rat administered Turmeric oil and $Feso_4$ shows germinal epithelium (A) and mature follicles (B) . (H&E 100X).

Discussion:

The administration of iron with turmeric oil separately in this study caused significant normalization of the body weight in simultaneousness with Al-Sultan (2003) who watched that administration of turmeric equal to 5gram/kilogram caused body weights increased. Several studies supposed that the increase in body weight perhaps because of turmeric antioxidant activity that promote protein production through enzymatic system. Other articles revealed that the turmeric oil had capability to enhance GIT besides the lipase enzyme that secrete from pancreas (Platel and Srinivasan, 2000).

Iron overload group in the present investigation showed numerous injurious histological changes congestive veins with significant increase in the number of atretic follicles and the number of primary follicles, secondary and mature follicles decrease significantly. Addison to decrease in ovarian and uterus weight these findings similar with the study of Elbetiha and AL-Hamood (1997). Diminished sulpha-hydryle groups is a pointer to increase oxidative stress in rat given potassium dichromate (Elbetieha and Al-Hamood,1997; Shabana *et al.*, 2017). Administration of Iron with turmeric oil presented obvious recovery in the histologic state compared with Iron overload group these noticed might be resulted from raised concentration of estrogen hormone that has important role in increasing endometrium thickness through increased the height and number of its cells, besides, increase straighted tubular glands number of endomaterium and increase their development (Platel and Srinivasan, 2000; Lee *et al.*, 2017) . Estrogen acts in a feedback mechanism, inducing the production of FSH that stimulates the development of the

immature ovarian follicles, which increases the production of estrogen from the theca cells of ovary (Shabana *et al.*, 2017).

The Data of our results showed significant increase of Hb, serum iron level, and PCV in $FeSO_4$ group compared with control group, these finding agree with studies of Karmakar *et al.*, (2000) and Abu-Taweel *et al.*, (2013). Exposure to Iron overload can cause toxic effect on many tissues, however the first to be influenced is blood, as reactive oxygen species combined with RBCs membrane then transferred to the liver. RBCs are the most common pointers of oxidative stress because of their cell membranes sensitivity and the sensitivity of its enzymes to free radicals (Halliwell and Gutteridge,2007). The administration of iron with turmeric oil returned the blood parameters mean values propose the antioxidant activity and protective effect and iron chelated properties of these substances the result are in accordance with (Abu-Taweel *et al.*, 2013).

Many studies have revealed that herbal plants extracts with protective effects against oxidative stress induced reproductive damages are due to the presence of antioxidant agents (Abu-Taweel *et al.*, 2013). The present investigation demonstrates that the administration of turmeric oil restores the control values of oxidative stress markers. This study provides evidence that the antioxidative properties of turmeric oil may contribute to its ability to restore the level of glutathione and to reduce the MDA concentration. The antioxidant activity of turmeric might be attributed to curcumin the main active ingredient in Turmeric (Shabana *et al.*, 2017).

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