

Effect of water extract of broccoli plant (*Brassica oleracea*) on some hormones of female white rats exposed to oxidative stress with hydrogen peroxide

تأثير المستخلص المائي لنبات البروكلي (*Brassica*

oleracea) على بعض الهرمونات لإناث الجرذان البيض المعرضة

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Abstract

The effect of the water extract of broccoli *Brassica oleracea* on some adult female rats hormones was studied to reduce the oxidativestress developed by hydrogen peroxide. The rats age from (3-4 months) and weights from (100-200 g), The experimeat period was from 2016/12 / 18 until 18/18/2017 .Twenty five rats was divided in toFive groups

(Five rats in each group) as follows:- The negative group (C-) was given water and food only group was the positive group (C +) was given food and H_2O_2 (0.5%) in drinking water (group 3 to group 5) were given food and H_2O_2 (0.5%) in drinking water as well as of the broccoli extract (600,500,400 mg / kg) daily and for 30 days, respectively, results showed that the crude extract contained dclcosides, flavonoids, phenolic compounds, carbohydrates, and tannins, as well as resins. When the alkaloids were detected when the Mayer and Marx detectors were found, broccoli contained alkaloids, while the extract did not contain the serotonin, sterols and triterphenoid. The incidence of elevated progesterone concentration, prolactin, and follicle stimulating hormone ($P \leq 0.05$), female white rats subjected to oxidative stress induced by hydrogen peroxide compared to the control group sound, ($P \leq 0.05$), while the results of the current study and statistical data showed no significant differences ($P > 0.05$) for estrogen compared to the control group.

Keywords: Broccoli, hydrogen peroxide, female hormones

الخلاصة

دُرُس تأثير المستخلص المائي لنبات البروكلي *Brassica oleracea* على بعض الهرمونات من خلال التقليل من الاجهاد التأكسدي والمستحدث بوساطة بيروكسيد الهيدروجين في إناث الجرذان البيض البالغة التي تراوحت اعمارها 3-4 أشهر واوزانها (100-200غم)، للمدة من 2016/12/18 لغاية 2017/1/18 إذ قسمت إلى (25جرذ) إلى 5 مجاميع ، تضمنت كل مجموعة 5جرذان وكالاتي:

المجموعة الاولى وهي مجموعة السيطرة السالبة (C-) أعطيت الماء والغذاء فقط، المجموعة الثانية هي مجموعة السيطرة الموجبة (C+) اعطيت H_2O_2 0.5% في ماء الشرب الاعتيادي والغذاء، (المجموعة الثالثة ولغاية المجموعة الخامسة) اعطيت الغذاء و H_2O_2 0.5% في ماء الشرب الاعتيادي فضلاً عن إعطاءها المستخلص المائي لنبات البروكلي وبالتراكيز (600,500,400 ملغم/كغم) على التوالي، يوميا ولمدة 30يوم، اظهرت النتائج مايتي:

احتواء المستخلص الخام على الكلايكوسيدات، والفلافونيدات، ومركبات فينولية، والكاربوهيدرات، و التانينات، فضلاً عن الراتنجات، حين تم الكشف عن القلويدات عند اجراء كشف ماير وماركس تبين أن البروكلي يحتوي على القلويدات، بينما لا يحتوي هذا المستخلص على الصابونيات والستيرولات والترايتيربينويد، وأظهرت نتائج الدراسة الحالية حدوث ارتفاع في تركيز هرمون البروجستيرون، والبرولاكتين، و الهرمون المحفز للجريبات وبمعنوية ($P \leq 0.05$) لإنات الجرذان البيض المعرضة للإجهاد التأكسدي المستحث بوساطة بيروكسيد الهيدروجين مقارنة بمجموعة السيطرة السليمة، وحدث انخفاضاً بتركيز الهرمون اللوتيني مقارنة مع مجموعة السيطرة السليمة وبمعنوية ($P \leq 0.05$)، بينما أظهرت نتائج الدراسة الحالية ومن خلال البيانات الإحصائية عدم وجود فروق معنوية ($P > 0.05$) لهرمون الاستروجين مقارنة مع مجموعة السيطرة السليمة.

الكلمات المفتاحية:- البروكلي، بيروكسيد الهيدروجين، الهرمونات الانثوية.

Introduction

The broccoli is a dark, dark green plant with a white carnation, but it has soft flowers and hard-boiled vegetables. It is a winter vegetable belonging to the crusader or cabbage family and is spread throughout the Mediterranean basin. (Boras et al., 2004). Broccoli is one of the top 20 foods in terms of the (INDI) index, which measures vitamins, minerals, calories, and broccoli. The broccoli is a source of vitamin C, vitamin K and minerals, including selenium, Potassium, Magnesium, Manganese Giniz, iron, zinc, thiamine, niacin, riboflavin, carotene, folic acid according to (USDA, 2015). Broccoli is a bioactive plant. Studies show that a diet rich in cruciferous vegetables, such as broccoli, cauliflower, cabbage, Brussels sprouts, cauliflower, Can significantly reduce the risk of certain forms of cancer (Kohlmeier & Su, 1997). Studies have shown that broccoli works as an antioxidant and a free-radical crusher and can be used as a dietary supplement to reduce oxidative stress because it contains flavonoids and phenolic compounds in the methanolic extract of the plant (Bhagat et al., 2012). Le et al. (2003) noted that broccoli possessed a DIM

compound. This compound has anti-androgen properties that can be used as a testosterone for human androgen androgen antagonist when treating prostate cancer. Broccoli has an important role in the body as it regulates Estrogene because it contains DIM and I3C, which have an effect on the concentrations of estrogen metabolites. They reduce the metabolite of 16-Dihydroxyestrone (16-DHE) and have a detrimental effect on breast cancer. (DIM and I3C) increases the concentration of the body's non-harmful estrogen (DH-2) (Rajoriaetal., 2011). And between (Jeffery and Keck 2008) that eating five or more servings of broccoli every week has a protective effect on bladder cancer.

Materials and methods

The plant used in the research and the method of preparing the broccoli extract:-The water extract of the plant samples is prepared by taking 100 g of dried powder mixed with 1000 ml distilled water using the electric mixer and left for 24 hours at room temperature. The mixture is then filtered using layers of medical gauze and then centrifuged at 3000 cycles per minute for 10 minutes after that the extract was filtered using filtration papers to obtain a clear solution. The extract is dried using the incubator at 38 ° C and then kept in the refrigerator until use (Al-Badri, 2013)

Experiminet Design:-First group: (G1) First control group (negative): given water and food only. Second group (G2): Second control group infected (positive): Hydrogen peroxide was given at 0.5% concentration with normal drinking water and prepared daily (G3, G4, and G5). Hydrogen peroxide was given 0.5% with normal drinking water and 0.2 mL of broccoli extract was extracted at a concentration of 400, 500 and 600 mg / kg of body weight)

respectively orally via tubular feeding and fed daily for 30 days.

Estimate the level of sex hormones:-Determination of the level of sex hormones Following the steps accompanying the pre-set analysis of the ELISA manufacturer's instructions, Hormonal concentration (LH, FSH) was assessed in the serum following the method attached to their prepared analysis kit and based on ELISA (Lenton et al., 1982), and progesterone and estrogen by (Tietz,1994)and milk hormone (Maddox et al., 1991)

Results and discussion

Specific data for active compounds in broccoli:-

An empirical study was conducted to detect the active compounds in the raw extract of the broccoli plant. The results showed a clear variation in the ingredients of the extract as in Table (1).The results of the study showed that the raw extract contains clicosides, flavonoids and phenolic compounds. These substances have many biological functions and act as antioxidants against free radicals. These results are consistent with (Villarreal-Garcia & Jacobo-Velazquez 2016) and contain carbohydrates. (Abdel Magiedetal., 2016). Tannins, which are of great importance, are a source of energy consumed by plants in the process of bio-metabolism and protect plants from fungi and harmful insects. This study is compatible with Delimontetal., 2017), as well as resins These findings are consistent with (Thongsook& Barrett 2005).While the alkaloids were detected with three reagents when conducting the Drankdrov test, it was found that broccoli did not contain alkaloids. This was not consistent with (Talreja& Moon (2014). When the Mayer and Marx tests revealed that broccoli contained alkaloids, (Talreja Moon, 2014), while this study does not contain soap and sterols. This study is

consistent with (Talreja & Moon 2014) and triterphenoid. This study was consistent with (Martelancetal., 2007). **Prolactin hormone:** -The results of the present study showed a significant increase in the hormone prolactin ($P \leq 0.05$) for female white rats exposed to oxidative stress induced by hydrogen peroxide compared with the control group sound as in Figure (1). The reason for the rise of milk hormone (prolactin) to low levels of progesterone Which inhibits the production of a hormone inhibitor of milk from the hypothalamus leading to the production of milk hormone from the pituitary gland (Akidi, 2009). The presence of effective oxygen, which is produced by the oxidative stress caused by hydrogen peroxide as it inhibits the effectiveness of the neurotransmitter dopamine due to a defect in the pituitary gland and lead to the production of milk hormone in large quantities, and in a study conducted on female infertility, Important causes of infertility are due to high levels of prolactin (Ajibola et al., 2012). This inhibits the ovarian cycle and then prevents the secretion of follicle stimulating hormone (FSH) and gonadotropin hormones. The results of the treatment with the water extract of the broccoli produces a rise in the levels of prolactin compared to the control group, and also in comparison to the control group exposed to oxidative stress Induced by hydrogen peroxide in the group that was injected with 600mg / kg of the water extract. These results were consistent with Eniola et al., 2012). The study was conducted on a group of non-pregnant women found that the high milk hormone may lead to infertility and the reason for the rise to lower levels of progesterone and then not the occurrence of ovarian cycle, as the hormone progesterone a major role in regular menstruation and the occurrence of changes during pregnancy (Berkson, 2016), while significantly decreased in the group of 400mg / kg and 500, due to the flavonoids

contained in the broccoli (Feucht et al., 2016), which activates the neurotransmitter dopamine, which in turn inhibits the production of milk hormone (YE et al., 2010 where both Fitzgerald and Dinan (2008) reported a N The neurotransmitter Dopamine is of great importance as being a major inhibitor of secretion of the milk hormone.

LH hormone:-The results of the present study showed that there was a decrease in the concentration of LH compared with the control group and ($P \leq 0.05$) as in Fig. (2). This study was consistent with Eniola et al., 2012, due to the low levels of LH In the levels of the milk hormone or as a result of damage to the functions of the pituitary due to some diseases (Mancini et al., 2008) as well as a shortage of antioxidants that remove the free radicals caused by hydrogen peroxide (Goud et al., 2008). As for the treatment with the water extract of broccoli, we note a rise in comparison with the control group exposed to oxidative stress by hydrogen peroxide. This is due to the fact that broccoli contains substances such as vitamin E, which plays an important role in the process of pituitary changes. These changes lead to increased concentration of lutein (Hamad, 2013).

Follicle stimulating hormone FSH:-The results of the present study showed an increase in the concentration of follicle stimulating hormone in female rats exposed to oxidative stress by hydrogen peroxide compared with the control group and ($P \leq 0.05$) as in Figure 3. This study is consistent with (Mieroetal .,2004) The reason for the increase in follicle stimulating hormone is that the free radicals affect the ovary and produce the hormone. It is responsible for the weak egg and it affects the reproductive system because it acts as signaling molecules for a number of diseases and infections that affect the body (Raietal., 2015). As for the treatment with the water extract of

broccoli, we note a decrease in the levels of follicle stimulating hormone in the concentration of 500mg / kg in the water extract compared with the control group of female rats exposed to oxidative stress with hydrogen peroxide. This study agreed with(Meissner et a., 2005) Maca, which is one of the types of broccoli for men, and there was a decrease in levels of hormone stimulating follicles and this is because the broccoli contains flavonoids contained in broccoli and works to reduce sex hormones also contain antioxidants and some vitamins such as vitamin E (Kootietal., 2015). In the other concentrations (600,400mg/kg), the statistical results showed no significant differences. This study was consistent with Brooks etal (2008). The study was conducted on women by giving Maca, which is a broccoli. Significant differences.

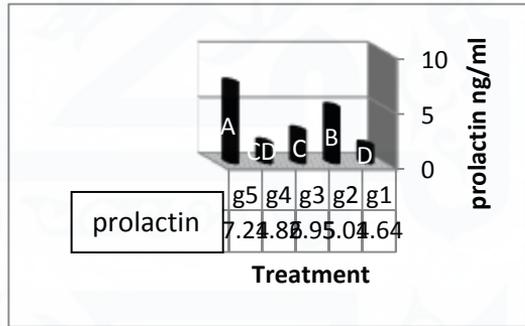
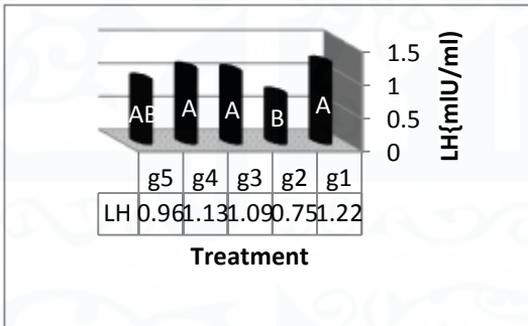
Estrogen hormone:- The results of the current study and statistical data showed no significant differences ($P>0.05$) in estrogen between the group of rats exposed to oxidative stress induced by hydrogen peroxide and the control group sound as in Figure (6). As for treatment with water extract, we note that there was an increase in comparison with the negative and positive control groups of rats exposed to oxidative stress in the group of rats, which was 400mg /kg. This result coincided with (Krishnamoorthyetal .,2013). The broccoli contains vitamin E and C (Porter, 2012) or may be due to the flavonoids contained in broccoli that have been detected in the specific qualitative data that activate genes responsible for estrogen production (Jarryetal., 2003) In the group of rats with a concentration of 600mg / kg, there was no significant difference in statistical results, while estrogen concentration decreased in the group of rats with a concentration of 500mg / kg. This result was consistent with(Gavaler ,1998) The hormone to the hormonal imbalance of these groups

due to the hypothalamus affected the oxidative stress caused by hydrogen peroxide and thus adversely affect the production of estrogen.

Progesterone:-As shown in Figure 5, the results of the study showed that the progesterone concentration of ($P<0.05$), for females of white rats exposed to oxidative stress induced by hydrogen peroxide compared to the control group was good. This study agreed with (Al-Azemi ,2012) Progesterone is due to lower levels of estrogen and thus reduces the risk of pregnancy, which reduces fertility as well as the role played by free radicals on the ovary in reducing the number of follicles in the ovary (Shkolnik et al., 2011) The results of the treated groups of broccoli showed a decrease in progesterone concentration. The group with 600 mg / kg of alcohol extract was the lowest and the results were consistent with the researcher (Saleh, 2015).The study was conducted on the female white rats through the dosage of broccoli juice, which led to the effects of positive reduced the number of objects or zero effectiveness or both and there was a significant decrease in the rate of body weight and ovaries and fatty pillows as well as a significant increase in weight of the uterus, Studies on the use of water and alcohol extract of broccoli in this area, may be attributed to the antioxidants contained in the broccoli plant, which works to fight free radicals and prevent their formation, such as active compounds, such as flavonoids, which is a group of polyphenols and Some vitamins, including vitamin E, which have a sweeping effect of free radicals as well as their ability to inhibit the damage induced by the process of oxidation, including hydrogen peroxide.

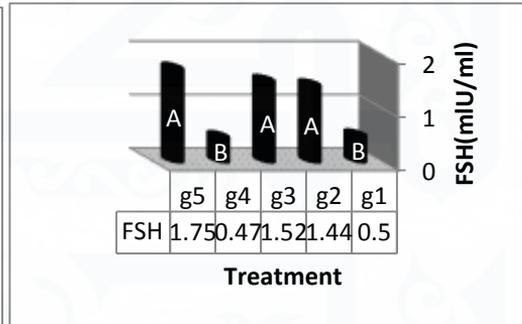
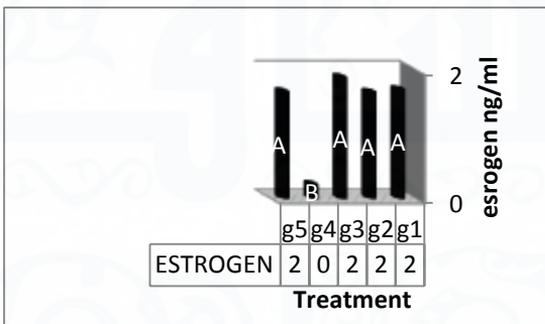
Table (1) Specific data for active compounds in broccoli

Result of detection of raw extract of broccoli plant	Detection Name	General Statements
+	Molch revealed	Carbohydrate detection
+	Detection of sodium hydroxide	Detection of clicosides
-	Drankdorff revealed	Detection of alkaloids
++	Meyer revealed	
+	Marx revealed	
-	Foam detection	Detection of saphphones
++	Detection of resins	Detection of resins
-	Skalkowski revealed	Detecting sterols
+	Detection of flavonoids and flavonols	Detection of flavonoids
++	Detection of iron chloride	Detection of phenols and phenolic compounds
-	Detection of triterphenoid	Detection of triterphenoid



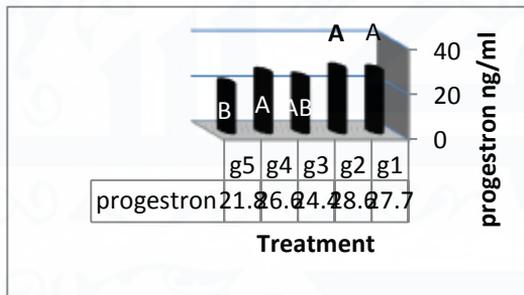
FIG(2) Effect of aqueous extract of Broccoli for (30 days) on LH levels in female rats exposed to hydrogen peroxide (0.5%) in drinking water

FIG(1) Effect of aqueous extract of Broccoli for (30 days) on prolactin levels in female rats exposed to hydrogen Peroxide (0.5%) in drinking water



FIG(4) Effect of aqueous extract of Broccoli for (30 days) on estrogen levels in female rats exposed to hydrogen peroxide (0.5%) in drinking water

FIG(3) Effect of aqueous extract of Broccoli for (30 days) on FSH levels in female rats exposed to hydrogen peroxide (0.05%) in drinking water



FIG(5) Effect of aqueous extract of Broccoli for (30 days) on progesterone levels in female rats exposed to hydrogen peroxide (0.05%) in drinking water

Estrogen	Progesteron	PRL	LH	FSH	standar
Mean ± s.d	Mean ± s.d	Mean ± s.d	Mean ± s.d	Mean ± s.d	aggregates
1.69± 0.27 A	27.73±1.91 A	1.64± 0.46D	1.22± 0.31 A	0.5± 0.09B	G1
1.65 ± 0.23A	28.56±4.14 A	5.04± 0.27 B	0.75 ± 0.18 B	1.44 ± 0.25 A	G2
1.88 ±1.38 A	24.41 ±3.13 AB	2.95± 0.97C	1.09 ± 0.19 A	1.52 ± 0.26 A	G3
0.22 ±0.24 B	26.56 ±3.49 A	1.86± 0.50CD	1.13 ± 0.11 A	0.47 ± 0.09 B	G4
1.66 ± 0.24 A	21.87 ±1.46 B	7.24±1.66A	0.96 ± 0.11 AB	1.75 ± 0.45 A	G5

Table (2) The rate of female hormones measured in different treatments

_Values represent the mean ± standard deviation.

_The vertically different letters mean a significant difference at a significant level (p <0.05) .

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