The effect of Marjoram and Cocoa on oxidative stress caused by ovariectomy in rats

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Abstract

It has known that postmenopausal life affects aoxidative status for bodies. Ovariectomy in rats was the same effect on postmenopausal for several organs. In the present study it can be hypothesized that dietary high in antioxidant such as marjoram and cocoa could protect against weakness and oxidative stress following ovariectomy in rats. Thirty-five female albino rats, ten ٣- month old weighing (١٨٠ ± ١٠ g) were used. The first main group (n=٧) was fed on the basal diet (-ve control). The second main group (n = ٢٨) was subject to ovariectomy surgery to induced oxidative stress. Then rats were divided into ٤ subgroups (٧ rats each). Subgroup ١ was fed on basal diet (+ve control). Subgroup ٢, ٣were fed on the basal diet and supplemented with dried marjoram , cocoa at the level of ١٠٪, respectively for three month. Subgroup ٤ were fed on the basal diet and supplemented with mixed (٥ % Marjoram+٥٪ Cocoa **)** for three month. Oxidative status was evaluated by SOD, Catalase, GSH, and MDA in Liver, Heart and Kidney tissues. The marjoram-treated rats result showed significantly P<٠.٠٥ increased level of antioxidant enzymes in liver heart and kidney tissues compared to positive control. The results of antioxidant enzyme for kidney tissues showed significant increased for SOD, CAT and GSH for group treated cocoa ١٠٪ compared to positive control. Also, It could be notice that cocoa ١٠٪ group and group treated with mixed (cocoa ٥٪ + marjoram ٥٪) showed significantly P<٠.٠٥ highest level of SOD, CAT and GSH in liver and

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heart tissue. Also, the presented work showed MDA status decreased significantly in heart, liver, kidney tissues for all treated groups compared to (+ve control). Finally, consumption of coca and marjoram has a protective role against oxidative stress caused by ovariectomy surgery, it is suggested to use these components especially cocoa through postmenopausal life.

Key Words: antioxidant enzyme, Ovariectomized, postmenopausal, Super Oxide Dismutase, Catalase, Glutathione, malonyldialdehyde, rats.

ملخص البحث باللغة العربية

تأثير البردقوش و الكاكاو على الإجهاد التأكسدي الناتج عن استئصال المبايض لدى الفئران

من المعروف أن فترة انقطاع الطمث تؤثر على الحالة التأكسدية للجسم. كما أن عملية استئصال المبايض لدى الفئران لها نفس التأثير على أعضاء الجسم في السيدات خلال فترة انقطاع الطمث. في هذه الدراسة ، من المفترض أن المواد الغذائية العالية في مضادات الأكسدة مثل البردقوش والكاكاو يمكن أن تحمي من الضعف والإجهاد التأكسدي الذي يحدث بعد استئصال المبايض في الفئران. تم استخدام خمسة وثلاثين من اناث الفئران البيضاء البالغ عمرها ٣ شهور تقريبا و يتراوح وزنها (١٨٠ ± ١٠ جم). تم تقسيم الفئران الي مجموعتين رئيسيتين: تم تغذية المجموعة الرئيسية الأولى (ن = ٧) على النظام الغذائي الاساسي (المجموعة الضابطة السالبة). المجموعة الرئيسية الثانية (ن = ٢٨) خضعت لجراحة استئصال المبايض لإحداث الإجهاد التأكسدي. تم تقسيم الفئران المستئصل منها المبيض إلى ٤ مجموعات فرعية (٧ فئران لكل منهما). تم تغذية المجموعة الفرعية الاولي على النظام الغذائي الاساسي (المجموعة الضابطة الموجبة)، تم تغذية المجموعات الفرعية ٢ - ٣ على النظام الغذائي الاساسي المدعم ب (البردقوش المجفف والكاكاو عند مستوى ١٠ ٪ ، على التوالي) لمدة ٣شهور. المجموعة ٤ من المجموعة المسئصل منها المبايض تم تغذيها على النظام الغذائي الاساسي المدعم ب (٥% البردقوش المجفف + ٥% الكاكاو) على التوالي لمدة ٣شهور. تم تقييم الحالة التأكسدية عن طريق قياس SOD ، Catalase، GSH، و MDA في أنسجة الكبد والقلب والكلى. أظهرت النتائج ان الفئران التي عولجت بالبردقوش حدث بها زيادة معنوية عند مستوى P <٠.٠٥ في الإنزيمات المضادة للأكسدة في أنسجة الكبد , القلب والكلى مقارنةً بالمجموعة الضابطة الموجبة. كما أظهرت النتائج ان الانزيمات المضادة للأكسدة في أنسجة الكلى توجد زيادة معنوية في الـ SOD و CAT و GSH لمجموعة الفئران المعاملة بالكاكاو

بنسبة ١٠٪ مقارنة بالمجموعة الضابطة الموجبة. أيضا ، لوحظ أن كلا من مجموعة الفئران المعاملة بالكاكاو ١٠ ٪ ومجموعة الفئران التي تناولت نسبة مختلطة من (الكاكاو ٥ ٪ + البردقوش ٥ ٪) أظهرت . P <٠.٠٥ زيادة معنوية في كلاً من SOD ، CATو GSH في أنسجة الكبد والقلب. كما أوضحت النتائج انخفاض في نسبة الميلانوالدهيد في جميع المجاميع المعاملة مقارنه بالمجموعة الضابطة الموجبة. في النهاية تظهر الدراسة أن استهلاك الكاكاو والبردقوش له دور وقائي ضد الإجهاد التأكسدي الناجم عن جراحة استئصال المبايض ، ويوصى باستخدامهم للسيدات في فترة انقطاع الطمث وخاصة الكاكاو.
الكلمات المفتاحية: الانزيمات المضادة للأكسدة ، استئصال المبيض ، انقطاع الطمث ، سوبر أكسيد ديسماتيز. كاتاليز ، الجلوتاثيون ، ميلانوالدهيد ، الفئران

Introduction

Oxidative stress has identified as an imbalance between oxidative and antioxidative status that increased reactive oxygen species production which initiates lipid peroxidation. Antioxidant protect system prevents molecular and cellular damage by reducing free radicals **(Halliwell, ٢٠٠٧; Gunay et al., ٢٠١١; Morrone et al., ٢٠١٥).** The evaluate of antioxidant enzyme activities are useful indicator of the antioxidant status in most mammals **(Serin et al., ٢٠٠٨; Halliwell, ٢٠١٢; Kozlik et al., ٢٠١٥; Tang et al., ٢٠١٦)**. Many studies on the evaluation of oxidative/antioxidative status in women and female rodents after ovariectomy have been studied by **(Kankofer et al., ٢٠٠٧; Serin et al., ٢٠٠٨; Gunay et al., ٢٠١١ ; Szczubial et al., ٢٠١٥).**

It has known that postmenopausal life affects oxidative status and causes metabolic diseases such as osteoporosis and cardiovascular diseases **(Gurdol et al., ١٩٩٧; Kankofer et al., ٢٠٠٧; Castelao et al., ٢٠٠٨; Yang et al., ٢٠١٤; Tang et al., ٢٠١٦).** Similarly, ovariectomy in rats has long-term effects on several organs such as liver, intestines and myocardium due to deficiency of ovarian hormones, particularly estrogens following the surgery **(Morrone et al., ٢٠١٥; Tang et al., ٢٠١٦; Barp et al., ٢٠١٢; Gomez et al., ٢٠٠٢; Murphy, ٢٠١١)**. Estrogens have demonstrated to defense the liver and intestines from oxidative damage due to its antioxidative properties **(Sener et al., ٢٠٠٥). Kim et**

al. (٢٠١٢) also postulated that estrogen deficiency may develop cytokine production in peripheral blood mononuclear cells and increase interleukin-٦ (IL-٦) concentrations associated with oxidative stress after menapause in women.

Cocoa bean is loaded with the polyphenols such as quercetin (including its glucoside), clovamide, deoxyclovamide and procyanidin, Epicatechin, (+)-catechin **(Campos et al., ٢٠١٨, Hammerstone et al., ١٩٩٩)**. Research indicates that the flavonoids, a class of polyphenols, have antioxidant characteristics with potential health benefits. The specific antioxidants in chocolate (i.e., cocoa flavanols) include catechin and epicatechin, which are single flavanol molecules structurally similar to the antioxidants found in grapes and tea **(Raloff, ٢٠٠٠ ; Lodhi and Vadnere, ٢٠١٩).**Cocoa can substantially increase a person's energy level, since it contains two stimulating methylxanthines - a significant amount of theobromine and a small amount of caffeine **(Keen ٢٠٠١, Sorond et al., ٢٠٠٨).** PEA (phenylethylamine) is a chemical found in cocoa/cacao beans which increases the activity of neurotransmitters (brain chemicals) in certain areas of the brain which control the ability to focus attention and stay alert **(Lee et al., ٢٠٠٣, Crew et al., ٢٠٠٨).** Cocoa also appears to have anti-aging and anti-inflammatory properties. Cocoa is a good source of the minerals magnesium, sulphur, calcium, iron, zinc, copper, potassium, and manganese; plus some of the B Vitamins. Cocoa enhanced clot prevention afforded by cocoa flavanols **(Rein et al., ٢٠٠٠).** consumption of cocoa and dark chocolate (DC) has protective effects against cardiovascular diseases, in particular improvement of vascular endothelium function and blood pressure (BP) **(Voskoboinik et al., ٢٠١٩, Allen et al., ٢٠٠٨, Mohan and Deepa, ٢٠٠٦, and Spadafranca et al., ٢٠١٠).**

Marjoram is one of the most popular culinary herbs in the world, which was grown in Egypt over ٣,٠٠٠ years ago and Egypt produces ٩٠ % of the world's supply. It has also been prescribed in the form of a herbal tea (infusion) in folk medicine to treat different illness **(Ramadan,**

et al., ٢٠١٢). Sweet marjoram leaves contain acids (carnosic, oleanolic and ursolic acids), cis-sabinene hydrate, flavonoids (diosmetin, luteolin and apigenin), hydrocarbons (P-cymene and c-terpinene), phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin and thymonin), phenolic terpenoids (thymol and carvacrol), tannins, sitosterol and triacontane . Preliminary trials have suggested possible antioxidant properties of the sweet marjoram plant **(Ramadan, et al ., ٢٠١٢, Vagi, et al ., ٢٠٠٥ and Heo et al ., ٢٠٠٢**).

Majorana has been uses to treat wide range of infections. It could be related to extensive phytochemical, experimental and clinical investigations. Its active constituents include Monoterpene derivatives, terpenic esters, monoterpenol and sesquiterpenoids. Experimental studies have demonstrated its free radical scavenging, anti-acetyl cholinesterase, insecticidal, synergistic effects, apoptotic, anti-proliferative activity, antimutagenic, genotoxic potential, antimicrobial and anti-ulcer activity and it has calming effect on anxiety and depressant activities. As a from all the studies, that researchers done and concluded that Marjoram have be used as functional food for humans by combine with unit operations of food processing for treatment of various ailments. Since herb possesses more than one health beneficial property and there is also a possibility of synergy among them in their action, a herb diet is likely to make life not only more “spicy” but more healthy also.**(Saxena, et al., ٢٠١٦)**

The aim of this study, it can be hypothesized that dietary high in antioxidant such as marjoram and cocoa could protect against weakness and oxidative stress following ovariectomy. Evaluate the changes in oxidative status markes in liver, heart and kidney tissues in rats by measuring their antioxidant enzymes in tissues.

Material and Methods

Rats and Diet:

Female albino rats of Sprague Dawley strain weighing ١٨٠±١٠g were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt. Basal diet constituents were obtained from El- Gomhorya Company, Cairo, Egypt.

Chemicals and fed ingredient

Antioxidant status for liver, kidney and heart kits were purchased from Sigma- Aldrich Co. (St. Louis, Missouri, USA). Cocoa powder was purchased from local Market. Also, Marjoram purchased from local market , Then powdered to mixed with diet.

Methods:

Preparation of basal diet:

The basal diet (AIN-٩٣M) was prepared according to **Reeves et al., ١٩٩٣).** Diet was formulated to meet the recommended nutrients levels for rats.

Induction of ovariectomy in female rats:

Ovariectomy is could to be the procedure that gives reliable model of postmenopausal life due to deficiency of ovarian hormones **(Morrone et al., ٢٠١٥ .** The method was done according to **(Lasota and Klonowska, ٢٠٠٤)** briefly, Ten ٣- month old female rats were made operation after placing an animal on its ventral surface. Ovariectomy was preceded by a midline dorsal skin incision, ٣ cm long, approximately half way between the middle of the back and the base of the tail. Incisions of the muscles were made bilaterally. After peritoneal cavity was accessed, the ovary was found, surrounded by a variable amount of fat. Ligation of the blood vessels was necessary. The connection between the Fallopian tube and the uterine horn was cut and the ovary moved out. Because of muscle bleeding, its incision required suturing. Three single catgut stitches were placed on the skin.

Experimental animal design

Thirty-five female Ten ٣- month old albino rats were housed in well aerated cages under hygienic conditions and were fed on basal diet

for one week for adaptation. All diets were formulated to cover the nutrient requirements of rats following the recommendations of the American Institute of Nutrition (AIN-٩٣M) **(Reeves et al., ١٩٩٣).** After this week the ovariectomy operation were done for all groups rats except negative control group. Rats were divided into five groups of seven animals each as follows :

Group ١: (N = ٧) fed on Ain-٩٣M and used as a negative control (Negative control).

Group ٢: Fed on Ain-٩٣M, induction of ovariectomy were made according to the above protocol and used as positive control.

Group ٣: Induction of ovariectomy were made as above and fed on Ain- ٩٣M and mixed with + ١٠ % Marjoram daily, for ١٢ weeks.

Group ٤: Induction of ovariectomy were made as above, fed on Ain-٩٣M and mixed with + ١٠ % Cocoa daily, for ١٢ weeks.

Group ٥: Induction of ovariectomy were made as above, fed on Ain-٩٣M and mixed with + (٥ % Marjoram+٥٪ Cocoa**)** daily, for ١٢ weeks.

After ٣ month treatment, animals were anesthetized and decapitated. Liver, heart and kidney were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues were then kept in freezer at -٧٠degree until analysis.

Liver Homogenate preparation:

Liver were perfused with saline and homogenized in chilled potassium chloride (١.١٧٪) using a homogenizer. The homogenates were centrifuged at ٨٠٠ g for ٥ minutes at ٤◦C to separate the nuclear debris. The supernatant so obtained was centrifuged at ١٠,٥٠٠ g for ٢٠ minutes at ٤◦C to get the post mitochondrial supernatant which was used to assay SOD, CAT, GSH and MDA, HNE activity.

Heart Homogenate preparation:

Homogenates were prepared on ice in the ratio ٤ g tissue for ١٦ ml of phosphate p H ٧.٥, containing ١ mM/L Na٢ EDTA, ١٠ ml of ٥٠٠ mM/L BHT (butylated hydroxytoluene)in acetonitrile was added to prevent formation of new peroxides during the assay. The homogenates were centrifuged at ٢٠٠٠ minutes at ٤٠ C and frozen at -٧٠٠C until analysis.

Kidney Homogenate preparation:

Kidney homogenates were obtained by using a tissue homogenator, Ultra Taurax T-٢٥ Polytron, at ٤o C. The homogenates (١:١٠ w/v) were prepared by using a ١٠٠ mmol KCl buffer (٧.٠ p H) containing EDTA ٠.٣ mM, All homogenates were centrifuged at ٦٠٠ g for ٦٠ minutes at ٤oC and the supernatant was used for biochemical assays.

Biochemical analyses in liver, heart and kidney tissues:

Estimation of Super Oxide Dismutase levels SOD Levels in the cell free supernatant was measured by the method of **(Kono et al., ١٩٧٨)**. Estimation of Catalase activity CAT activity was assayed by the method of **(Sinha , ١٩٧٢)**. Estimation of Glutathione GSH activity was determined by the procedure of **(Carlberg and Mannervik ١٩٨٥).** Estimation of malonyldialdehyde (MDA) was determined spectrophotometrically according to the method by **(Ohkawa et al., ١٩٧٩).**

Results and discussion

Table (١): Effect of Marjoram and Cocoa powders on ovariectomy rats on oxidative status in Liver tissue.

Parameters / Groups	SOD (U/g tissue)	CAT (mmol/g tissue)	GSH (U/g tissue)
Control (-ve)	١٠١٦.٦٦±٤.٩٧ b	١٠٦.٣٣±٢.٠٢ ab	٥.٢١±٠.٠٦ a
Control (+ve)	٥٢٤.١٢±٧.٨٤ e	٣٧.٦٢±٤.٧٠ d	١.٣٦±٠.٣١ c
Marjoram ١٠٪	٩٠٦.٣٠±٥.٥٤ d	٧٤.٠٠±٦.٢٤ c	٣.٠٠±٠.٢٨ b
Cocoa ١٠٪	١٠٨٩.٣٥±٢.٩٠ a	٩٨.٠٠±٢.٠٨ b	٣.٨١±٠.٥٤ b
(Cocoa ٥ % + Marjoram ٥٪)	٩٩١.٠٠±٤.٦١ c	١١٣.٢٨±٢.٣٣ a	٤.٠٤±٠.٤٣ b

Means ± SE with different letters superscripts in the same column are significant at P < ٠.٠٥ using one way ANOVA test. n= ٨ rats/group.

In the present study, The result of antioxidant enzyme on liver tissue on ovariectomy rats showed Super Oxide Dismutase **SOD**, Catalase **CAT** and Glutathione **GSH** decreased significantly on ovariectomy group without any treated (positive control) compared to other groups. Rats treated with Marjoram showed increased significant level of liver **SOD** (٩٠٦.٣٠±٥.٥٤, at P<٠.٠٥), **CAT** (٧٤.٠٠±٦.٢٤, P<٠.٠٥), **GSH** (٣.٠٠±٠.٢٨, P<٠.٠٥) compared to positive control (table ١). Also , best result showed both groups cocoa ١٠٪ and group treated mixed with cocoa٥٪ + marjoram ٥٪ showed increased significantly at P<٠.٠٥ in SOD and CAT compared to all group.

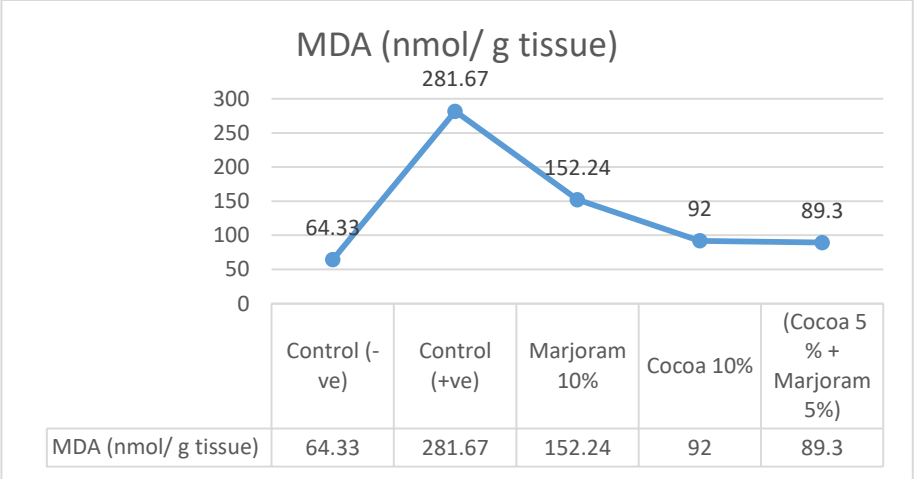

Figure (١): Effect of Marjoram and Cocoa powders on ovariectomy rats on malonyldialdehyde MDA status in Liver tissue.

The result showed that significant decrease in malonyldialdehyde MDA for liver tissue in all treated groups marjoram ١٠٪ , cocoa ١٠٪ and group mixed with (cocoa ٥٪+ marjoram ٥٪) P<٠.٠٥ (١٥٢.٢٤±٣.٥٢ ^{b}, ٩٢.٠٠±٢.٠٨ ^{c} and ٨٩.٣٠±٤.٦٣ ^{c}, P<٠.٠٥, respectively) compared to control positive ٢٨١.٦٧±٦.١٧ ^{a}. The lowest value of MDA was significantly in control negative ٦٤.٣٣±٣.١٧ ^{d} , then groups cocoa ١٠٪ and group mixed with (cocoa ٥٪+ marjoram ٥٪). No significant differences between last groups as showed in figure (١).

Table (٢): Effect of Cocoa and Marjoram powders on ovariectomy rats on oxidative status in heart tissue.

Parameters / Groups	SOD (U/g tissue)	CAT (mmol/g tissue)	GSH (U/g tissue)
Control (-ve)	١٠٠٨.٩٣±٤.١٨ a	١٠٠.٠٦±١.٧٣ b	٤.٩٦±٠.١٠ a
Control (+ve)	٤٠٥.٠٠±٥.٥٠ e	٤٠.٠٠±٢.٣٠ e	٠.٧٢±٠.٠٧ d
Marjoram ١٠٪	٧٧٤.٠٠±٣.٢١ d	٧٤.٦٠±٢.٣٠ d	١.٨٤±٠.٠٨ c
Cocoa Powder ١٠٪	٩٨٨.١٠±٤.١٠ b	١١٩.١٣±٤.٨٢ a	٢.٦٠±٠.٢١ b
(Cocoa ٥ % + Marjoram ٥٪)	٩٠١.٠٠±٢.٠٨ c	٩٠.٠٠±٣.٢١ c	٢.٨١±٠.١٢ b

Means ± SE with different letters superscripts in the same column are significant at P < ٠.٠٥ using one way ANOVA test. n= ٨ rats/group.

The parameter analyzed in heart tissue showed that all group treated with marjoram ١٠٪, cocoa ١٠٪ and mixed them showed significantly increase in antioxidant enzyme compared to positive control. The highest content significantly at P<٠.٠٥ in antioxidant enzyme showed in group ovariectomy treated with ١٠٪ cocoa powder, level of SOD (٩٨٨.١٠±٤.١٠), CAT (١١٩.١٣±٤.٨٢) GSH (٢.٦٠±٠.٢١) compared to positive control (٤٠٥.٠٠±٥.٥٠, ٤٠.٠٠±٢.٣٠ and ٠.٧٢±٠.٠٧ , respectively) as showed in table (٢).

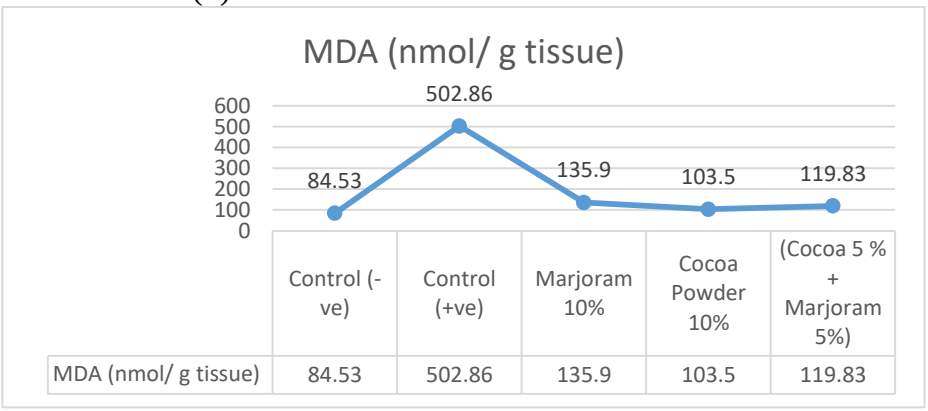

Figure (٢): Effect of Marjoram and Cocoa powders on ovariectomy rats on malonyldialdehyde MDA status in heart tissue.

In the present study MDA status in heart tissue showed, all groups treated with marjoram ١٠٪, cocoa ١٠٪ and mixed them showed significantly decrease MDA (١٣٥.٩٠±٢.٥٢ ^{b} , ١٠٣.٥٠±٣.٢٧ ^{d} and ١١٩.٨٣±٤.١٤ ^{c} ,P<٠.٠٥ , respectively) compared to positive control (٥٠٢.٨٦±٤.١٢ ^{a}). More highly decrease in MDA was in ١٠٪ cocoa powder group compared to positive control and other treated groups figure (٢).

Table (٣): Effect of Marjoram and Cocoa powders on ovariectomy rats on oxidative status in kidney tissue.

Parameters / Groups	SOD (U/g tissue)	CAT (mmol/g tissue)	GSH (U/g tissue)
Control (-ve)	١٠٣٦.٨٣±١.٦٤ ^{a}	١٠٠.١٠±١.٨١ ^{b}	٥.١١±٠.١٠ ^{a}
Control (+ve)	٤٨٣.٣٣±٧.٨٨ ^{d}	٤٤.٣٣±٢.٧٢ ^{d}	١.٣٨±٠.١٣ ^{c}
Marjoram ١٠٪	٨٦٨.٢٣±٣.٨٤ ^{c}	٨٠.٧٠±١.٤٥ ^{c}	٣.٤١±٠.٢١ ^{b}
Coca Powder ١٠٪	١٠٠٥.٦٧±٨.٢٩ ^{b}	١٣٨.٥٣±١.٨٦ ^{a}	٣.٩٨±٠.٢٥ ^{b}
(Cocoa ٥ % + Marjoram ٥٪)	٩٩٠.١٠±٣.٧٥ ^{b}	٩٨.٧٦±٢.٤٢ ^{b}	٤.٠١±٠.٣٥ ^{b}

The results of antioxidant enzyme for kidney tissues showed significant increased for **SOD**, **CAT** and **GSH** for groups treated with marjoram ١٠٪, cocoa ١٠٪ and mixed them compared to positive control. It could be notice that cocoa ١٠٪ group and group treated with mixed (cocoa ٥٪ + marjoram ٥٪) showed highest level of SOD (١٠٠٥.٦٧±٨.٢٩ , ٩٩٠.١٠±٣.٧٥ P<٠.٠٥, respectively), CAT (١٣٨.٥٣±١.٨٦, ٩٩٠.١٠±٣.٧٥, P<٠.٠٥, respectively) and **GSH** (١٠٠٥.٦٧±٨.٢٩, ٩٩٠.١٠±٣.٧٥, P<٠.٠٥, respectively). As showed in table ٣ no significant differences between CAT level for control negative (١٠٠.١٠±١.٨١, P<٠.٠٥) and mixed (cocoa ٥٪ + marjoram ٥٪) group (١٠٠.١٠±١.٨١, P<٠.٠٥) table (٣).

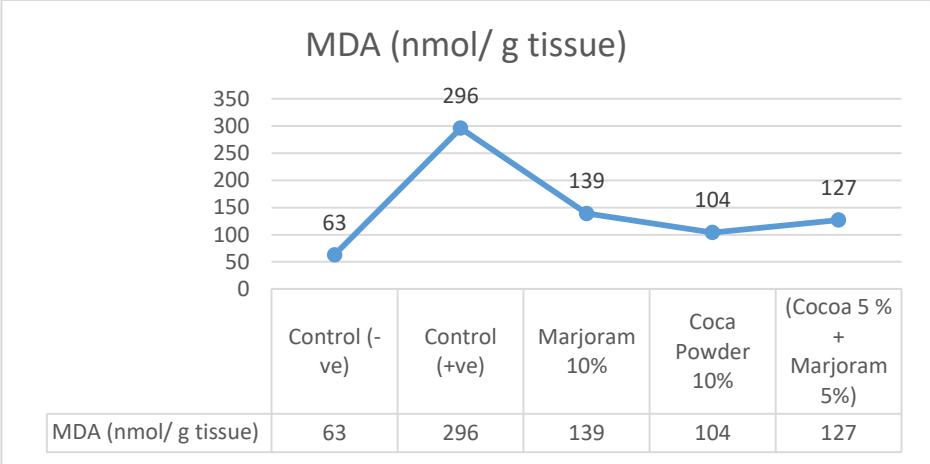

Figure (٣): Effect of Marjoram and Cocoa powders on ovariectomy rats on malonyldialdehyde MDA status in kidney tissue.

In the present study MDA status in kidney tissue showed, all groups treated with marjoram ١٠٪, cocoa ١٠٪ and mixed them showed significantly decrease MDA (١٣٩.٠٠±٤.٧٢ [b], ١٠٤.٠٠±٢.٨٨ [d] and ١٢٧.٠٠±٣.٦٠ [c], P<٠.٠٥ , respectively) compared to positive control (٢٩٦.٠٠±٤.٥٨ [a], P<٠.٠٥). More highly decrease in MDA was in ١٠٪ cocoa powder group compared to positive control and other treated groups as showed in figure (٣). Also, it is notice that control negative was the lowest significant value (٦٣.٠٠±٢.٠٨ [e] P<٠.٠٥) compared to all groups.

Discussion

It has known that postmenopausal life affects oxidative status for bodies (**Yang et al., ٢٠١٤; Tang et al., ٢٠١٦**). Ovariectomy in rats was the same effect on postmenopausal for several organs **(Morrone et al., ٢٠١٥; Tang et al., ٢٠١٦; Barp et al., ٢٠١٢**), because estrogens have demonstrated to defense the liver and intestines from oxidative damage due to its antioxidative properties **(Sener et al., ٢٠٠٥**).Several studies indicated that ovariectomy resulted in antioxidative/oxidative imbalance

in most mammals **(Muthusami et al., ٢٠٠٥; Kankofer et al., ٢٠٠٧; Serin et al., ٢٠٠٨; Günay et al., ٢٠١١; Tang et al., ٢٠١٦).**

The our result indicated that decrease in antioxidant enzyme and increase in MDA in liver, heart, kidney tissue for ovariectomy group rat without any treated (control positive), This result it could be related to serous inflammation after ovariectomy may cause oxidative stress in body organs **(Cronauer et al., ١٩٩٩)**. Also, it has been indicated that ovariectomy causes to alterations in oxidative/ antioxidative balance because of anaesthetic agents **(Serin et al., ٢٠٠٨; Gunay et al. ٢٠١١; Szczubial et al., ٢٠١٥).** The obtained result for positive control was agreement with **(Anadol, et al., ٢٠١٦)** which reported that serum MDA concentration significantly increased while SOD and GPx activities decreased on day ١ after ovariectomy surgery.

In the present study marjoram successfully improved the undesirable effects caused by ovariectomy and success in restored almost all variables antioxidant enzyme AST, CAT, GSH and decrease MDA in liver, heart, kidney tissue to near their negative control levels. Also, Combination of cocoa and Marjoram caused a significant modulation of deleterious effect of oxidative stress. The our result was agreement with **Saleh et al.(٢٠١٨)** who study the effect marjoram against oxidative stress induced by paracetamol in male albino rats , and mentioned that Marjoram or moringa+ marjoram at dose ٢٥٠mg/kg/day increase in antioxidant enzyme and decrease in MDA compared to positive control. Marjoram are rich in nutrients, minerals, vitamins and antioxidants which improve the body health in general and can improve the immunity suggesting them as valuable medicinal plants to protect against the deleterious toxic effects **(Auwal et al., ٢٠١٣ and Frank et al., ٢٠١٤).** Consistently, **Fakurazi et al. (٢٠١٢)** stated that β- Carotene in Moringa leaves is efficiently converted into vitamin A in the body that has shown significant hepatoprotective effect. Marjoram was well documented to increase globulin and have high antioxidant capacity that could protect from liver, heart and kidney damage **(Abd El-Ghany and El-Metwally, ٢٠١٠).**

Fakurazi et al., (٢٠١٢) stated that certain phenolic compounds in marjoram may induce production of glutathione-S-transferase and other antioxidant enzymes. Additionally, the ability of these phenolic compounds to bind to some minerals as copper and iron can protect against their oxidative effects **(Ferguson, ٢٠٠١). Abd El-Ghany and El-Metwally, (٢٠١٠)** used Marjoram leaves to protect against liver injury induced by carbon tetrachloride due to its high content of antioxidant compounds that are released during toxicity and can protect cells against reactive oxygen species.

In present study, we determined the oxidative status of Liver, Heart and Kidney tissues for ovariectomy rats after prolonged treated of Cocoa powder in rats. Prolonged treated of cocoa and combination with cocoa and marjoram showed decrease accumulation of MDA in heart , liver and kidney tissue, implicated oxidative stress,. It has been reported that malondialdehyde is a well-characterized mutagen **(Esterbauer et al., ١٩٩٠)** that reacts with deoxyguanosine to form a major endogenous adduct with DNA in human livers.

Increased SOD level , Catalase and GSH level in liver heart and kidney tissues for treated group with Cocoa powder was observed (Table ١ , ٢ and ٣). SOD is the major antioxidant enzyme that provides the body's first enzymatic step in the defense system against oxidative stress. **(Landmesser et al., ٢٠٠٢).** Catalase is used by cells to defend against the toxic effects of hydrogen peroxide **(Michiels et al., ١٩٩٤).** High intracellular GSH levels promote better survival under such conditions **(Kurosawa et al., ٢٠٠٥, Ruzaidi et al., ٢٠٠٥).** Increased activity of these enzymes as a result of polyphenol intake has been reported in the literature **(Young et al., ٢٠٠٠).** It could be reported that increasing enzyme antioxidant in tissues in our result for group cocoa treated was to cocoa have antioxidant properties and contains a number of different compounds such as polyphenols, caffeine, sterols, terpenes, and methylxanthines. Cocoa has been potential mechanism for beneficial effects **(Spadafranca et al., ٢٠١٠, Mursu et al., ٢٠٠٤**). **Analikumar et al., (٢٠٠١)**

suggesting an enhanced protection of the liver, heart against oxidative stress situations by these antioxidants.

The our result agreement with **Noori, et al., (٢٠٠٩)** who examined the oxidative status in terms of lipid peroxidation and antioxidant enzymes in different tissues and found that group rats treated with cocoa at level (١g/kg b.w.) showed significantly increased level of GSH in liver and heart tissue, Catalase in liver and heart, SOD in liver, and decrease in MDA in liver tissues. **Fraga et al., (٢٠٠٥)** reported a decrease in serum MDA levels after ١٥ days of consuming milk chocolate in young healthy adults, while those who ate white chocolate showed higher levels of oxidative stress. **Rein et al., (٢٠٠٠)** and **Wang et al., (٢٠٠٠)** both observed an inverse association between different amounts of flavanol-rich dark chocolate and plasma thiobarbituric acid reactive substances (TBARS) concentrations in healthy subjects ٢ hours after injection dark chocolate riches in cocoa .

Conclusion

The consumption of coca and marjoram have a protective role against oxidative stress caused by ovariectomy in rats and have ability to improvement of almost all evaluated parameters. Therefore, it is suggested to use these components especially cocoa as nutritional habits in diet to protect body organs through postmenopausal life for women.

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