

A Study Of antioxidant capacity of different doses of alcoholic extract of *Marticaria Chamomilla* flower in comparison with vitamin E in oxidative stressed male rats

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دراسة قابلية أصداد الأكسدة لجرع مختلفة من المستخلص الكحولي لأزهار البابونج بالمقارنة مع فيتامين هـ في ذكور الجرذان المعرضة للأجهاد التأكسدي

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المستخلص

تمت هذه الدراسة على ذكور الجرذان للكشف عن تأثير المستخلص الكحولي لأزهار البابونج على الحالة التأكسدية بالمقارنة مع فيتامين هـ. تم استخدام 30 من ذكور الجرذان البالغة. قسمت الحيوانات الى ست مجاميع (5 جرذان لكل مجموعة). المجموعة الاولى اعطيت الماء فقط، المجموعة الثانية عرضت الى 0.75% بيروكسيد الهيدروجين مع الماء، المجموعة الثالثة جرعت فمويا 50 ملغم/كغم من فيتامين E بالإضافة الى بيروكسيد الهيدروجين، اما المجاميع الثلاثة الاخرى (T3, T4, T5) جرعت فمويا بالمستخلص الكحولي لأزهار البابونج (40,80,120 ملغم/كغم) على التوالي. عينات الدم اخذت خلال فترة زمن الصفر وبعد 30 يوم من المعالجة، تم فحص المصل لكل من فعالية انزيم الكاتلاز، تركيز بيروكسيد النايتروجين، الكوليسترول الكلي، الكليريدات الثلاثية، كوليسترول البروتينات الدهنية عالية الكثافة، كوليسترول البروتينات الدهنية واطئة الكثافة جدا وكوليسترول البروتينات الدهنية واطئة الكثافة.

اظهرت النتائج وجود ارتفاع معنوي في مستوى بيروكسيد النايتروجين، الكوليسترول الكلي، الكليريدات الثلاثية، كوليسترول البروتينات الدهنية واطئة الكثافة جدا وكوليسترول البروتينات الدهنية واطئة الكثافة في المجموعة المعالجة ببيروكسيد الهيدروجين فقط مقارنة مع مجموعة السيطرة وعزيت هذه النتيجة الى الهدم التأكسدي المتزامن مع التعرض الطويل لبيروكسيد الهيدروجين. بينما المجاميع الاخرى (المجموعة المعالجة بفيتامين هـ والمجاميع المعالجة بالمستخلص الكحولي لأزهار البابونج) اظهرت اختزال معنوي بمستوى بيروكسيد النايتروجين، الكوليسترول الكلي، الكليريدات الثلاثية، كوليسترول البروتينات الدهنية واطئة الكثافة جدا وكوليسترول البروتينات الدهنية واطئة الكثافة مع زيادة معنوية في مستوى كوليسترول البروتينات الدهنية عالية الكثافة بالمقارنة مع مجموعة المعالجة ببيروكسيد الهيدروجين وعزيت هذه النتيجة الى التأثير المضاد للأكسدة لكل من فيتامين هـ والمستخلص الكحولي لأزهار البابونج. مع ذلك فإن المجموعة المعالجة بـ 120 ملغم/كغم من المستخلص الكحولي لأزهار البابونج سجلت اعلى تأثير موجب لمواجهة الاجهاد التأكسدي وابدى هذا التأثير معادلة مع تأثير فيتامين هـ، بالإضافة الى ذلك فإن كلا المجموعتين (المجموعة المعالجة بفيتامين هـ والمجموعة المعالجة بـ 120 ملغم/كغم من مستخلص أزهار البابونج) اظهرت عدم وجود اختلاف معنوي بالمقارنة مع مجموعة السيطرة خلال جميع معايير التجربة. هذه النتيجة اشارة الى التأثير المضاد للأكسدة للمستخلص الكحولي لأزهار البابونج بطريقة معتمدة على الجرعة.

Abstract

This study was conducted to investigate the effect of alcoholic extract of *Marticaria chamomilla* flowers on the antioxidant status in comparison with vitamin E in male rats. Thirty mature male Albino Wister rats were used in this study, the animals were divided into six groups (5 each), the first group was administered with water only, the second one was subjected to H₂O₂ 0.75% in drinking water. The third group was orally intubated with 50mg/kg vitamin E concurrently with H₂O₂. The other three groups (T3, T4 and T5) were orally administered with M.Ch.E (40, 80 and 120 mg/kg BW) respectively. Blood samples were taken at zero time and then after 30 days, serum sample were examined for CAT, peroxynitrite radical concentration, TC, TAG, HDL-C, VLDL-C and LDL-C.

The results showed a significant elevation in ONOO concentration, TC, TAG, VLDL-C and LDL-C associated with a reduction in CAT and HDL-C in H₂O₂ treated group in compared with control group, this result may be contributed to oxidative damage associated with long term H₂O₂ exposure. While other groups (vitamin E group and M.Ch.E treated groups) showed a significant reduction in ONOO concentration, TC, TAG, VLDL-C and LDL-C with an elevation in HDL-C if compared to H₂O₂ treaded group. This may be contributed to the antioxidant effect of vitamin E and M.Ch.E. However, 120mg/kg BW M.Ch.E treated group recorded the highest positive effect against oxidative stress and its effect seems to be normalizing with vitamin E effect, as well as both groups (vit. E and 120mg/kg BW M.Ch.E) clarified a non-significant different with control group in all experiment parameters. This result pointed to antioxidant effect of M.Ch.E in a dose dependent manner.

Introduction

For a long time plants have played a very important role for human life. Nowadays, the use of plants as a way of treatment is still very important for human beings. The use of medicinal plants is as old as the evolution of man himself. Medicine in Iraq can be traced back to the Sumerian period (3000-1970 B.C.) and then to the Babylonian and Assyrian periods (1970-539 B.C.) [1].

Chamomile, *Marticaria chamomilla*, is widely used throughout the world. The use of chamomile comes from two Greek words meaning "ground apple" for its apple like smell [2]. *Matricaria chamomilla* flowers contain active compounds such as volatile oil consists of alphabisabolol, bisabolol oxide – A, bisabolol oxide – B (2), trans – farnesene and spathaulenol [3]. Also the flowers of chamomile contain phenolic compounds as flovonoides such as flavon glycoside, a glycogen apigenin and lutoline. The flowers contain glycosides such as anthamic acid, anthamedine and matricarin [4]. In Iraqi folk medicine chamomile has been used as analgesic, anti-microbial, diuretic and in treatment of peptic ulceration [5]. A review of the medical literature reported a number of beneficial effects for chamomile *in vitro* and animal tests. Research with animals suggests antispasmodic anxiolytic, anti-inflammatory and some anti-mutagenic and cholesterol-lowering effects for chamomile. It also showed some benefit in an

animal model of diabetes [6]. Alcoholic and aqueous extracts of flowers powder are used for therapeutics of skin infections caused by some pathogenic bacteria, therapy drug of injuries in mouth, therapeutics of respiratory system infection and treatment of digestive disorders [7]. Also *Matricaria chamomilla* extracts are used as anti – contractive development of immunity system and increasing the white blood cells [8]. *In vitro* chamomile has demonstrated moderate antioxidant properties [9]. The latter use was based on observational impression rather than on clinical or experimental studies. Therefore, the aim of the present study was to induce oxidative stress in rats and to investigate the antioxidative effect of different doses the alcoholic extract of chamomile in comparative to the well-known antioxidant vitamin E.

Materials and methods

1- Chamomile extract preparation:

Chamomile flowers were purchased from the local Iraqi market, and their classification was received from (Ministry of Agriculture/ State Board for Seeds Testing and Certification S.B.S.T.C. in Abu Graib/ Baghdad. After grinding the dried flowers, the plant material (powder) was extracted with 70% ethanol according to Harbornes' method [10]. The extract (MCE) was further diluted with distilled water to prepare different concentration 40, 80 and 120 mg/kg.

2- Vitamin E dosage:

Vitamin E group was dosage with 50 mg/kg of vitamin E (The Arab Company for Manufacturing Veterinary and Agricultural Products, Jordan). The dose was prepared by dissolving 0.5 ml of stock solution (100 mg/ml) with 1 ml of distilled water according to [11]. The used form of vitamin E is "water soluble" in which certain compounds during a manufacturing process were added that made it more efficiently dissolve in water.

3- Animals and methods:

Thirty mature male Albino Wister rats with average weight of 280-300g and age of 10-12 weeks were used in this study; all animals were obtained from the central animal house of The College of Veterinary Medicine/University of Baghdad, animals in all stages of the experiments were housed in plastic cages in a conditioned room (22-25 °C) also had free access to standard pellet diet throughout the experimental period. After acclimatization for two weeks, the animals dividing into the following groups consisting of 5 rats each: The first group (C), received normal drinking water only; the second group (T1), received H₂O₂ 0.75% in drinking water only according to [12]; the third group (T2), received H₂O₂ 0.75% in drinking water and vitamin E (50 mg/kg) administered by stomach tube daily; the fourth group (T3), received H₂O₂ 0.75% in drinking water and *Matricaria chamomilla* extract (40mg/kg B.W) administered by stomach tube daily. The fifth group (T4), received H₂O₂ 0.75% in drinking water and *Matricaria chamomilla* extract (80mg/kg B.W) administered by stomach tube daily. The sixth group (T5), received H₂O₂ 0.75% in drinking water and *Matricaria chamomilla* extract (120mg/kg B.W) administered by

stomach tube daily. The animals maintained in their respective groups for 30 days. Blood sample were collected by cardiac puncture technique, serum were separated and then deep frozen until analyzed for some physiological and biochemical parameters.

4- Analytic procedure:

- A- Spectrophotometric assay for serum Catalase activity was determined according to the method of [13].
- B- Peroxynitrite ONOO^- determination based on formation of nitrophenol which was absorbed at 412nm. The amount of nitrophenol that form in the serum which reflecting the level of peroxy nitrite in the serum [14].
- C- Enzymatic estimation of serum TAG concentration was carried out using Triacylglycerol kit according to [15].
- D- Serum total cholesterol (TC) concentration was enzymatically measured using enzymatic assay kit according to [16].
- E- Serum (HDL-C) concentration was measured enzymatically using enzymatic assay kit according to [17, 18].
- F- Serum very low density lipoprotein-cholesterol concentration was calculated by dividing serum TAG by five according to [19].

$$\text{VLDL-C (mg/dl)} = \text{TAG} / 5$$

- G- Serum (LDL-C) and (VLDL-C) concentrations were calculated by the following two formulas respectively according to [19].

$$\text{LDL-C (mg/dl)} = \text{TC} - (\text{HDL-C} + \text{VLDL-C})$$

Statistical analysis

Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P < 0.05$). Specific group differences were determined using least significant differences (LSD) as described by [20].

Results

Statistical analyze revealed no significant ($P > 0.05$) differences between groups during the zero time period, however, continuous administration of 0.75% H_2O_2 for thirty days in drinking water alone (group T1) or in a combination with M.Ch.E 40mg/kg B.W (group T3) pointed to a significant decrease ($P < 0.05$) in serum catalase activity compared to control and other treated groups, while administration of H_2O_2 in concurrently with vitamin E (group T2), 80 mg/kg BW (group T4) and 120 mg/kg BW (group T5) clarified no significant ($P > 0.05$) difference in serum catalase activity comparing to control group and such groups at zero time period (table 1). On the other hand, results of serum peroxynitrite radical showed no significant ($P > 0.05$) difference between groups at zero time periods. Within the time, an a significant ($p < 0.05$) increase in ONOO concentration appears just in (T1 and T3), moreover, no significant ($P > 0.05$) different in ONOO appear in (T2), (T4) and (T5) groups in comparative to group (T1) and such groups at zero time period (table 2).

Tables 3, 4, 5, 6, 7, illustrated the mean value of TC, TAG, HDL-C, LDL-C and VLDL-C concentration in serum of control and the other treated groups along the experimental period.

Table (3) showed a non-significant ($P>0.05$) different in TC values between groups at zero time period. While at the end of experiment H_2O_2 group (T1) recorded the highest significant ($p<0.05$) elevation in this parameter with mean value (109.29 ± 1.86) in comparative to other treated groups which seem to trend to the normalization and their values showed no significant ($P>0.05$) difference specially at T2 and T5 groups with mean value (93.02 ± 1.57 and 94.66 ± 1.32) respectively in comparative to control group value (90.95 ± 0.82) at same period.

Also the result of serum TAG concentration (table 4) showed that exposure of animals to 0.75% H_2O_2 alone in drinking water significantly ($p<0.05$) increased the mean value of TAG (85.37 ± 1.36) after 30 days of the experiment comparing to the mean values of control (59.80 ± 1.04) and T2, T3, T4 and T5 (62.91 ± 0.64 , 69.04 ± 1.33 , 64.04 ± 1.02 , 60.04 ± 1.38) respectively. It appears that M.Ch.E at the all different doses and vitamin E reduced the elevated TAG concentration caused by H_2O_2 exposure.

Besides oral intubations of vitamin E or M.Ch.E concurrently with H_2O_2 in drinking water significantly suppressed ($p<0.05$) the elevated VLDL-C concentration after 30 days of experiment and this suppression appeared clearly in (T2 and T5) groups with mean values (12.58 ± 0.32 and 12.01 ± 0.41) which seems to normalize the value of the control group (11.96 ± 0.22) and been non significantly ($P>0.05$) different (table 5).

Also the results showed a significant ($p<0.05$) reduction in the mean value (24.24 ± 0.65) of (T1) group after 30 days of H_2O_2 intubation in compared to control group value (32.24 ± 0.61) at same period, while a significant ($p<0.05$) increase ($p<0.05$) in mean value of serum HDL-C concentration Were detected after 30 days of experiment in vitamin E treated group value (31.74 ± 0.63) and all M.Ch.E treated (T3, T4 and T5) groups with a mean value of (28.15 ± 0.40 , 29.15 ± 0.51 and 31.03 ± 0.47) respectively comparing to H_2O_2 treated group. Moreover, increasing the dose of intubation of M.Ch.E had positive effect on HDL-C concentration (table 6).

It seems that vitamin E intubations concurrently with H_2O_2 normalized LDL-C value (48.7 ± 0.98) with that of control group (46.75 ± 0.55) after 30 days, also the M.Ch.E treated groups value seems to reach very close from that of control at the end of experiment compared to the H_2O_2 treated group specially in M.Ch.E 120 mg/kg BW treated group value (51.62 ± 2.0) (table 7).

Table (1): The effect of different doses of *Matricaria Chamomilla* alcoholic extract and vitamin E on serum catalase activity (KU/L) in H₂O₂ treated rats

GROUP \ Period	C CON.	T1 H ₂ O ₂	T2 Vitamin E+ H ₂ O ₂	T3 M.Ch.E 40mg/kg B.W + H ₂ O ₂	T4 M.Ch.E+ 80mg/kg B.W + H ₂ O ₂	T5 M.Ch.E+ 120mg/kg B.W + H ₂ O ₂
zero time	119.33±2.63 Aa	118.94±3.11 Aa	118.82±2.68 Aa	119.99±4.73 Aa	119.22±3.01 Aa	118.86±2.02 Aa
After 30 days	119.65±3.05 Aa	93.65± 4.11 Cb	118.05±4.04 Aa	108.85±5.02 Bb	113.57±2.67 ABa	117.22±3.54 Aa

- LSD=6.5, Values are expressed as Means ± SE, n = 5 / group
- Capital letters denote between groups differences, p<0.05 vs. control.
- Small letters denote within group differences, p<0.05 vs. zero time period.

Table (2): Effect of different doses of *Matricaria Chamomilla* alcoholic extract and vitamin E on serum peroxynitrite radical concentration (M/L) in H₂O₂ treated rats

GROUP \ Period	C CON.	T1 H ₂ O ₂	T2 Vitamin E+ H ₂ O ₂	T3 M.Ch.E 40mg/kg B.W + H ₂ O ₂	T4 M.Ch.E+ 80mg/kg B.W + H ₂ O ₂	T5 M.Ch.E+ 120mg/kg B.W + H ₂ O ₂
zero time	32.08±0.8 Aa	31.32±0.5 Ab	32.58±0.3 Aa	31.98±0.9 Ab	31.33± 0.8 Aa	30.66± 0.7 Aa
After 30 days	32.74± 0.98 Ca	65.71±1.45 Aa	35.46±0.61 Ca	42.87±1.32 Ba	34.03± 0.80 Ca	33.42±1.11 Ca

- LSD=2.9, Values are expressed as Means ± SE, n = 5 / group.
- Capital letters denote between groups differences, p<0.05 vs. control.
- Small letters denote within group differences, p<0.05 vs. zero time period.

Table (3): The effect of different doses of *Matricaria Chamomilla* alcoholic extract and vitamin E on serum total cholesterol (TC) concentration (mg/dl) in H₂O₂ treated rats.

GROUP \ Period	C CON.	T1 H ₂ O ₂	T2 Vitamin E+ H ₂ O ₂	T3 M.Ch.E 40mg/kg B.W + H ₂ O ₂	T4 M.Ch.E+ 80mg/kg B.W + H ₂ O ₂	T5 M.Ch.E+ 120mg/kg B.W + H ₂ O ₂
zero time	90.87±0.98 Aa	90.43±0.91 Ab	91.79±0.93 Aa	90.44±0.89 Ab	91.39±0.94 Ab	91.02±0.88 Ab
After 30 days	90.95± 0.82 Da	109.29±1.86 Aa	93.02 ± 1.57 Da	100.9 ±1.84 Ba	97.67±1.84 BCa	94.66±1.32 CDa

- LSD=3.4, Values are expressed as Means ± SE, n = 5 / group.
- Capital letters denote between groups differences, p<0.05 vs. control.
- Small letters denote within group differences, p<0.05 vs. zero time period.

Table (4): The effect of different doses of *Matricaria Chamomilla* alcoholic extract and vitamin E on serum triglycerides (TAG), with concentration of (mg/dl) in H₂O₂ treated rats.

GROUP Period	C CON.	T1 H ₂ O ₂	T2 Vitamin E+ H ₂ O ₂	T3 M.Ch.E 40mg/kg B.W + H ₂ O ₂	T4 M.Ch.E+ 80mg/kg B.W + H ₂ O ₂	T5 M.Ch.E+ 120mg/kg B.W + H ₂ O ₂
zero time	61.63±0.82 Aa	60.77±1.21 Ab	60.65±0.87 Aa	59.88±0.99 Ab	60.21±1.02 Ab	61.30±0.93 Aa
After 30 days	59.80±1.04 Da	85.37±1.36 Aa	62.91±0.64 CDa	69.04±1.33 Ba	64.04±1.02 Ca	60.04±1.38 Da

- LSD=3.2, Values are expressed as Means ± SE, n = 5 / group.
- Capital letters denote between groups differences, p<0.05 vs. control.
- Small letters denote within group differences, p<0.05 vs. zero time period.

Table (5): The effect of different doses of *Matricaria Chamomilla* alcoholic extract and vitamin E on serum very low density lipoprotein-cholesterol (VLDL-C), with concentration of (mg/dl) in H₂O₂ treated rats.

GROUP Period	C CON.	T1 H ₂ O ₂	T2 Vitamin E+ H ₂ O ₂	T3 M.Ch.E 40mg/kg B.W + H ₂ O ₂	T4 M.Ch.E+ 80mg/kg B.W + H ₂ O ₂	T5 M.Ch.E+ 120mg/kg B.W + H ₂ O ₂
zero time	12.33±0.20 A	12.15±0.23 A	12.13±0.21 A	11.98±0.27 A	12.04±0.24 A	12.26±0.28 A
After 30 days	11.96±0.22 D	17.07±0.51 A	12.58±0.32 CD	13.81±0.29 B	12.80±0.37 C	12.01±0.41 CD

- LSD=0.8, Values are expressed as Means ± SE, n = 5 / group.
- Capital letters denote between groups differences, p<0.05 vs. control.
- Small letters denote within group differences, p<0.05 vs. zero time period.

Table (6): The effect of different doses of *Matricaria Chamomilla* alcoholic extract and vitamin E on serum high density lipoprotein-cholesterol (HDL-C), with concentration of (mg/dl) in H₂O₂ treated rats.

GROUP Period	C CON.	T1 H ₂ O ₂	T2 Vitamin E+ H ₂ O ₂	T3 M.Ch.E 40mg/kg B.W + H ₂ O ₂	T4 M.Ch.E+ 80mg/kg B.W + H ₂ O ₂	T5 M.Ch.E+ 120mg/kg B.W + H ₂ O ₂
zero time	31.97±0.56 A	32.04±0.51 A	32.56±0.42 A	31.87±0.55 A	32.22±0.61 A	31.90±0.49 A
After 30 days	32.24±0.61 A	24.24±0.65 D	31.74±0.63 A	28.15±0.40 C	29.15±0.51 BC	31.03±0.47 AB

- LSD=1.6, Values are expressed as Means ± SE, n = 5 / group.
- Capital letters denote between groups differences, p<0.05 vs. control.
- Small letters denote within group differences, p<0.05 vs. zero time period.

Table (7): The effect of different doses of *Matricaria Chamomilla* alcoholic extract and vitamin E on serum low density lipoprotein-cholesterol (LDL-C), with concentration of (mg/dl) in H₂O₂ treated rats.

GROUP Period	C CON.	T1 H ₂ O ₂	T2 Vitamin E+ H ₂ O ₂	T3 M.Ch.E 40mg/kg B.W + H ₂ O ₂	T4 M.Ch.E+ 80mg/kg B.W + H ₂ O ₂	T5 M.Ch.E+ 120mg/kg B.W + H ₂ O ₂
zero time	46.57±0.41 A	46.24±0.62 A	47.1±0.6 A	46.59±0.7 A	47.13±0.62 A	46.86±0.71 A
After 30 days	46.75±0.55 D	67.98±1.6 A	48.7±0.98 CD	58.94±1.3 B	55.72±1.6 B	51.62±2.0 C

- LSD= 3.3, Values are expressed as Means ± SE, n = 5 / group.
- Capital letters denote between groups differences, p<0.05 vs. control.
- Small letters denote within group differences, p<0.05 vs. zero time period.

Discussion

The present study showed a significant increase in serum Peroxynitrite radical concentration in the hydrogen peroxide treated group (T1), this indicating a case of oxidative stress. Oxidative stress has been suggested to be a common mechanism underlying impairment of NO production [21]. NO can react with O₂⁻ or H₂O₂ to form peroxynitrite (ONOO⁻), whose oxidant potential is greater than that of O₂⁻ or H₂O₂ alone [22]. Besides, NO inhibits catalase activity and increases intracellular ROS levels in macrophages [23] concomitant with elevation in peroxynitrite radical concentration [24]. Peroxynitrite (ONOO⁻) which is formed by the diffusion-controlled reaction of O₂^{•-} and NO has been shown to be strong reactive oxidant which oxidizes proteins, sulfhydryl, lipids and DNA leading to cellular injury [25]. It also consumed biological antioxidant like catalase leading to its depression [26]. However, ONOO⁻ elevation in this experiment concurrently with depletion of serum catalase documents this statement.

Whereas, the reduction in CAT activity explained according to the fact that oxidative impacts cause a significant increase in hepatic lipid peroxidation and a significant decrease in hepatic antioxidants including SOD and CAT activities [27]. As well as oxidative stress generates ROS with the accumulation of superoxide radicals [28], which, in turn, caused LPO and membrane damage [29] coincident with lowering antioxidant enzyme activity [30] like catalase.

However, the results of this experiment revealed that incidence of lipid profile significantly increased represented by TC, TAG, LDL-c, VLDL-C, in parallel to a significant decrease in the level of HDL-c in group treated with H₂O₂. These results documented the well known fact that H₂O₂ is one of the reactive oxygen species which has a direct effect on the level of plasma TC, TAG and atherogenic lipoproteins [31].

Such changes in serum lipid may reflect the suppression of lipid metabolism due to H₂O₂ induced oxidative stress and there might be a dynamic alteration in the process of absorption and exertion of steroid or there was reduction in bowel bile salts [32].

Oxidative stress cause a partial deficiency of lipoprotein lipase (the key enzyme determining the removal rate of TG from plasma) associated with increased output of lipoprotein from the liver may contribute to the elevation of serum TG level in H₂O₂ treated group [33]. On the other hand, oxidative stress cause a suppression in insulin excretion due to β -cell damage [34] which in turn to a decrease in lipoprotein lipase activity which in turn to TAG elevation [35]. Besides, increment of TAG level in animals received H₂O₂ in the present study may be due to an increase in serum VLDL- level which acts as a carrier for the TAG in the plasma [36]. Furthermore, serum HDL-C level was reported to be inversely correlated with serum VLDL-C, TG level, both in normolipidemic and hyperlipidemic subject [37]. Another explanation of the a negative effect H₂O₂ (T1 group) on lipid profile status, this may be due to the oxidation of apo-B-100 of the LDL-C which is accumulate due to total cholesterol elevation [38] (Table 3).

In all parameters of current study vitamin E pointed out to a protective effect against the oxidative stress indicator which represented by serum CAT and ONOO concentration, so the protective effects of vitamin E may be attribute to its antioxidant properties. As well as its ability to extinguish single oxygen species and to terminate free radical chain reactions [39]. Vitamin E may play a role as it stabilizes membranes, scavenges and quenches various reactive oxygen species (ROS) and lipid soluble by products of oxidative stress [40], thus acting as a powerful terminator of lipid peroxidation and subsequently elevation in catalase activity [41]. Also as an antioxidant, vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical which will then be oxidized by a hydrogen donor and thus return to its reduced state [42]. As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage.

Moreover, the present study showed that oral intubation of vitamin E plus H₂O₂ in drinking water caused positive changes in serum lipid profile through significant decline in the mean value of serum TC, TAG, LDL-C and VLDL-C concentration & elevation in serum HDL-C concentration in comparison to H₂O₂ treated group (tables 3, 4, 5, 6, 7). This result insures the hypolipidemic effect of vitamin E. Furthermore, previous study reported that vitamin E is a one of powerful antioxidant which achieve its antioxidant activity via suppress the formation of oxidized LDL-C by reduce its concentration and increase HDL-C level which is considers as protective agent against the elevated LDL-C [43]. Therefore, the current study findings are in agreement with [44] were they suggested there was an increase in the HDL-C component and HDL/LDL ratio and a decrease in the LDL-C component and triglycerides in the rats group treated with vitamin E. As well as, Dose response studies in humans have reported that 400 IU/day vitamin E increased its levels in plasma two-fold and prolonged the lag time before LDL oxidation. This might be beneficial in decreasing the individual risk of coronary heart diseases [45].

Beside, results of different doses of M.Ch.E intubation showed a significant positive effect against oxidative stress in all present study parameters, this effect may be due to the major components from *M. chamomilla* which are (-)-R-bisabolol and R-farnesene, and the yield of the essential oil from the flowers are about 0.4%. This plant also has high levels of polyphenolic compounds such as coumarins and flavonoids [46]. It has been recommended that presence of phenolic compounds donates electron to H_2O_2 and thus neutralizing it to water [47] and decreases its free radical activity [48]. It can be concluded that depression of peroxynitrite and elevation of catalase by M.Ch.E could be done by this way.

Results showed significant decrease in (TC, TAG, LDL-C, and VLDL-C) concentration associated with an elevation in (HDL-C) concentration in all M.Ch.E treated groups, compared with H_2O_2 treated group (T1). This effect may be due to antioxidants properties of *Marticaria chamomilla* extract. In a study researchers found that the ethanolic extract of *Marticaria chamomilla* inhibit the formation of free radicals and may scavenge the reactive oxygen metabolites through various antioxidants compounds in them [49].

While the major flavonoids in M.Ch.E components are apigenin, luteolin, patuletin, quercetin and their glucosides, which comprise 16.8, 1.9, and 9.9%, respectively, of total flavonoids. Thus, chamomile is one of the richest sources of dietary antioxidants. There is substantial evidence that these compounds have suppressive activity on oxidative damage to skins, membranes, proteins, and DNA by inhibiting free radical scavenging activity and contribute to protection against chronic health disorders such as atherosclerosis and hypertension [50]. In another study *in vivo*, they found that chamazulen, the active ingredient in *Marticaria chamomilla*, effect free radical processes and inhibit lipid peroxidation [51]. Beside, the dose M.Ch.E 120mg/kg B.W. showed the highest positive effect against oxidative effect and it seem to trend the value of such parameter to normalization this result is in agreement with [51, 52] were they reported that Chamazulene affects free radical processes and inhibits lipid peroxidation in a concentration- and time-dependent manner [52]. In addition, in recent years, scientists have focused on the preventive effects of phenols against degenerative diseases mediated by the ROS. It has been reported that the phenolic compounds are able to interact with the biological systems and act as bioactive molecules. They are particularly important inhibitors of lipid peroxidation [53], and are believed to be effective through their free radical scavenging [54] and metal chelating properties [55]. Furthermore, phenolic compound was proved to have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation [56]. It can be concluded that the supplementation of Phenolic compound and vitamins might cause increment of antioxidant defense system like catalase and the decline of lipid peroxidation [57]. However, depending on the above fact our finding suggesting that the effect of M.Ch.E is attributed to its rich content of essential compounds.

On conclusion, it appears that different antioxidant & active ingredients of marticaria chamomile contribute to the antioxidant effect. And it seems that the protective effect is a dose dependent.

Vitamin E and M.Ch.E are successes to counteract the damaging effect of H₂O₂. This may be attributed to short duration of the experiment and the short action of H₂O₂.

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