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Manufacture and Evaluation of Novel Chocolate for Girls' Dysmenorrhea

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ABSTRACT

This study aimed to produce a novel chocolate supplemented with cinnamon, ginger, and mint powder and their essential oils and assess its effect on dysmenorrhea in a sample of 48 student volunteers from Mansoura University suffering from dysmenorrhea. Girls aged between 18:25 years were divided into eight groups (6 patients in each) as follows: Group 1: Normal group (without chocolate sample). Group 2: Control fed on chocolate without herbs powder and their essential oils Group 3: fed on chocolate with cinnamon powder. Group 4: fed on chocolate with cinnamon oil. Group (5): provided chocolate with ginger powder. Group 6: fed on chocolate with ginger oil. Group 7: provided chocolate with mint powder. Group 8: fed on chocolate with mint oil. Results showed that feeding girls chocolate with cinnamon, ginger, and mint powder and their essential oils increased significantly ($p<0.05$) hemoglobin scores as compared with control chocolate. At the same time, the consumption of chocolate for three months did not cause an increase in body mass index. So, thus the previous results indicated that consumption of chocolates with cinnamon, ginger, and mint powder and their essential oils decreased significantly ($p<0.05$) serum total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein scores as compared with control chocolate. on the other hand, this study showed that eating chocolate containing (ajwa, dried fruits, cinnamon, ginger, and mint) and their essential oils effectively reduce menstrual pain and improve iron levels in the blood, which may be lost during the menstrual cycle.

Keywords: Menstrual, Dysmenorrhea, Menorrhagia, Chocolate, and Iron deficiency anemia.

INTRODUCTION

Menstruation that is so painful as to make daily activities impossible is referred to as dysmenorrhoea. At some point in their life, 80% of women experience them; often, they do not indicate a significant underlying issue. In this condition, painful menstruation occurs along with backache, nausea, vomiting, stiffness in the thighs, and cramping in the calf muscles. The discomfort typically begins in the first few days of the period and lasts for two to three days. Around 15% of women experience period symptoms that are severe enough to limit their everyday activities (Zaidi *et al*., 2012). According to Armour *et al*., (2019) young women under the age of 25 frequently have dysmenorrhea (period discomfort) and its accompanying symptoms. This period coincides with a pivotal period in young women's academic careers at both the secondary and postsecondary levels. Disrupted concentration and poor performance in class can be brought on by dysmenorrhea. Iron deficiency anemia is frequently brought on by heavy monthly bleeding, which can have an impact on a woman's quality of life. 30% of women think their menstrual cycles are too frequent. In more than half of menorrhagia women, no clear reason for the bleeding is discovered. (Lethaby and Farquhar, 2003).

Numerous phytochemicals, including polyphenols, which have been linked to anti-inflammatory, antioxidant, and pain-relieving effects, may be found in cocoa. Through a variety of ways, cocoa's other ingredients may be able to reduce or eliminate pain. (De Feo *et al*., 2020). As described by Meier *et al*., (2017), chocolate appears to increase positive mood, particularly when it is eaten mindfully. Additionally, It was found that iron in chocolate protects against iron deficiency anemia and magnesium (Haritha *et al*., 2014). Ali and Alam, (2020) revealed that giving ajwa date fruit (*Phoenix dactylifera L.*) leads to increasing hemoglobin (Hb) levels in teenage girls through study used 35 samples that were chosen on purpose. A rich source of vitamins, minerals, and antioxidants, dry fruits are an excellent source of energy. It boosts the body's many metabolic processes and offers a number of health advantages. It contributes significantly to lowering harmful effects on human health, enhancing the functionality of many physiological processes, and delivering essential nutrients like vitamins, dietary fibre, carbs, and minerals. Along with acting as a strong antioxidant and anti-inflammatory (Abobatta, 2021).

Jaafarpour *et al*., (2015) recommended that cinnamon can be viewed as a safe and effective treatment for dysmenorrhea in young women due to its strong impact on the reduction of pain, menstrual bleeding, nausea, and vomiting with primary dysmenorrhea without causing adverse effects. The popular spice ginger, also known as Zingiber officinale R. rhizome, has long been used to treat inflammatory conditions. Five days of ginger therapy for primary dysmenorrhea in students reduced pain severity and duration in a statistically meaningful way (Rahnama *et al*., 2012). Negara *et al*., (2021) resulted from that use of ginger oil several days before menstruation can reduce the menstrual pain scale in students with primary dysmenorrhea; therefore, its use can be recommended to prevent the pain. Masoumi *et al* (2016)'s study showed that all clinical indications and symptoms, pain, and severity all decreased

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after ingesting peppermint extract. Peppermint is suggested to treat the symptoms of dysmenorrhea since the side effects of herbal medicine are less severe than those of other prescription medications.

MATERIALS AND METHODS

Materials:

Cinnamon (*Cinnamomum zeylanicum*), ginger (*Zingiber officinale*), mint (*Mentha Piperita*), and their essential oils were obtained from the National Research Center, Giza, Egypt. Ajwa dates from "Elqaseem Company", dark chocolate "Dreem", Mashreq Company, and dried fruits (figs, apricots, plums, and raisins) were purchased from local markets in different areas in Mansoura city, Egypt.

Patients: Experiments were conducted on 48 volunteers girl from Mansoura University students suffering from dysmenorrhea, aged between 18 and 25 years. All the biological experimental procedures were applied according to Internationally Ethical Guidelines. And permission for the experiment was obtained from the Research Ethics Committee at the Faculty of Specific Education, Mansoura University.

Methods:

- **Preparation of herbs:** Each of the cinnamon, ginger, and mint was ground well until become fine powder and then saved in polyethylene bags until they were used.
- **Preparation of Ajwa dates: A**jwa dates were ground (Blender 2000 rpm) for 3 minutes to become soft.
- **Preparation of dried fruits (figs, apricots, plums, and raisins):** Dried fruits were cut into small pieces and then saved in polyethylene bags until they were used.
- **Preparation of chocolate:** The formulations used for making chocolate are given in Table (A). The dried fruits were cut into small pieces, then added to the ajwa dates and mixed well together. This mixture was shaped into cubes using molds. The raw dark chocolate was placed in a small bowl, then in a larger bowl containing water on the stove. The chocolate was completely melt in the bowl. It was removed from heat and stirred until it reaches a smooth consistency. The cubes of the mixture were dipped in chocolate. Chocolate cubes were packed in wrappers and stored at refrigerator temperature (± 3°C).

Table A. Formulas used for making chocolates.

Ingredients (%)	Chocolate samples						
	Control chocolate	CP chocolate	CO chocolate	GP chocolate	GO chocolate	MP chocolate	MO chocolate
Dark chocolate	25,00	25,00	25,00	25,00	25,00	25,00	25,00
Ajwa dates	35,00	32,00	34,75	32,00	34,75	32,00	34,90
Dried figs	10,00	10,00	10,00	10,00	10,00	10,00	10,00
Dried Apricots	10,00	10,00	10,00	10,00	10,00	10,00	10,00
Dried plums	10,00	10,00	10,00	10,00	10,00	10,00	10,00
Raisins	10,00	10,00	10,00	10,00	10,00	10,00	10,00
CP	–	3,00	–	–	–	–	–
CO	–	–	00,25	–	–	–	–
GP	–	–	–	3,00	–	–	–
GO	–	–	–	–	00,25	–	–
MP	–	–	–	–	–	3,00	–
MO	–	–	–	–	–	–	00,10

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil.

Sensory evaluation:

Sensory evaluation of beverages was conducted to identify their sensory characteristic according to Moor (1970) Who proposed a score of (10) excellent, (9) very good, (8) good, (7) medium, fair, (5) poor, very poor, (3) extremely poor.

Chemical composition:

Gross chemical composition content (Moisture%, Ash%, Protein%, Fat% and Fiber%) were determined according to (AOAC,2000

Total carbohydrate: Percentage carbohydrate was given by: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein) (Gul and Safdar, 2009).

- **Minerals:** Total Mg and K were estimated according to Peterburgski (1968). Fe, Zn and Cu were estimated according to the methods of Chapman and Pratt (1961).
- **Vitamins:** Vitamin C was determined according to the method described by Mazumdar and Majumder, (2003). Vitamin A IU estimated according to Aremu and Nweze (2017). Vitamin E was estimated by reversed phase high performance liquid chromatography according to Gimeno *et al*., (2000). Vitamin B6, B9 were determined according to Marsili *et al.* (1981) and Saad *et al.,* (2015).
- **Total phenols:** Total phenolic content was determined as described by Slinkard and Singleton, (1977).
- **Flavonoid:** The total flavonoid content determined by a colorimetric method of Zhishen and Jianming**,** (1999).

Designing a questionnaire:

1- Personal data: (Name of the patient- Age - Social status).

2- Anthropometric measurements form: (Body weight - Height - Body Mass Index).

- Body Weight: Weight was measured using beam balance. The dysmenorrhea patients were weighed to the nearest kilogram without shoes and in light clothes, and the scales were calibrated before use (Cai *et al* ., 2004)**.**

-Height (HT): The height of each subject was measured using a meter while the dysmenorrhea patient was in the standing position with no shoes and was recorded to the nearest centimeter (Gill *et al* ., 2017).

-Body Mass Index (BMI): Was calculated

BMI = Wt (kg) / (Height meter)²

- **The grades of obesity utilizing the BMI:**
- Underweight < 20kg / m²
- Oesirable range of BMI (normal) 20- < 25kg / m²
- Over weight = > 25 = 30kg / m²
- Obesity = > 30 kg / m² (Diehl , 1991) .

3- Data related to symptoms associated with dysmenorrhea:

- History from Patients includes symptoms associated with the discovery of the disease.
- Family member suffering from dysmenorrhea.

- Suffering from other diseases.
- Taking painkillers during menstruation.
- Heavy menstruation.
- Level of daily activities during menstruation.
- Hemoglobin percentage.

Design of biological experiment:

The dysmenorrhea patients were divided into eight groups (6 patients in each) as follows:

- **Group 1 :** (Normal group) without chocolate.
- **Group 2:** fed on control chocolate
- **Group 3:** fed on chocolate with 3% cinnamon powder.
- **Group 4:** fed on chocolate with 0.25% cinnamon oil.
- **Group 5:** fed on chocolate with 3% ginger powder.
- **Group 6** fed on chocolate with 0.25% ginger oil.
- **Group 7:** fed on chocolate with 3% mint powder.
- **Group 8:** fed on chocolate with 0.1% mint oil.

Determination of blood constituents:

- **sample collection:** Fasting morning venous blood samples (5ml) were withdrawn after 12-14 hours of overnight fasting from all subjects included in the study while they were in the supine position.
- **Measurement of blood hemoglobin:** Blood hemoglobin (Hb %) was performed at the central laboratory at El-Borg laboratory in Mansoura city by using an automated blood cell counter (Sysmex, Sysmex, Kobe and Japan) to all patients as recommended by **(**Beaton and Bengoa, 1976 **).**

Biochemical assays:

Determination of serum lipid profile: For examination of lipid profile, serum total cholesterol (TC) and triglycerides (TG) were chemically determined using specific diagnostic kits according to the methods described by Richmond, (1973) and Jacobs and Van Denmark (1960), respectively. High density lipoprotein (HDL-c) cholesterol was chemically estimated according to the method described by Richmond, (1973). All measurements were performed by specific kits using spectrophotometer (Model T80, UV / visible, double beam, UK). Low density lipoprotein (LDL-c) cholesterol was calculated according to the formula of Friedewald *et al.*, (1972) as follows

LDL-c = {TC- (HDL-c + VLDL-c)} VLDL-c = TG/5

Statistical analysis:

Recent researches have reported that all analyses were done at least in triplicate. Then these values were presented as average values in parallel with their traditional derivations. By using the minitab software data were discussed. Furthermore it was held some statistical comparisons with one-way analysis of variance, and p values < 0.05 were regarded as great (Stalikas , 2007).

RESULTS AND DISCUSSION

Sensory evaluation of prepared chocolates:

Results revealed that the percentage addition (3 % cinnamon, 3 % ginger, and 3 % mint) powder and (0.25 % cinnamon, 0.25 % Ginger, and 0.1 % mint) essential oils was the best addition percent according to the sensory evaluation. While, the level of acceptability decreases with increasing concentrations of used herbs and their essential oils. The effect of flavoring chocolate with (3 % cinnamon, 3 % ginger, and 3 % mint) powder and (0.25 % cinnamon, 0.25 % Ginger, and 0.1 % mint) essential oils on the organoleptic parameters was studied and the obtained data were presented in Table 1. Data show non-significant differences ($p < 0.05$) between control chocolate and all samples in color. Concerning mean score contents, data show that control, cinnamon oil, and ginger oil chocolate recorded the highest mean score contents (9.32±0.28, 9.54±0.09 and 9.33±0.13), while mint oil chocolate recorded the lowest mean score contents (8.76±0.16). Results obtained were in accordance with results of Rada *et al***.,** (2020) who reported that chocolates with the addition of herbs and their essential oils are distinguished by a more pleasant taste, slightly spicy, and a special aroma. Also, the addition of mint (dried leaves, aqueous extract of dried leaves, and fresh leaves) stabilized the color and sensory properties of sausages (Latoch and Stasiak, 2015).

Table 1. Organoleptic evaluation of prepared chocolates.

	Parameters					
Samples	**Color (10)**	**Taste (10)**	**Odor (10)**	**Texture (10)**	**overall acceptability (10)**	**mean score (10)**
Control chocolate	9.58a±0.39	9.30a±0.67	8.98ab±0.66	9.44a±0.44	9.28ab±0.64	9.32ab±0.28
CP chocolate	9.18a±0.53	8.86ab±0.55	8.96ab±0.65	9.08a±0.40	9.42a±0.51	9.10b±0.32
CO chocolate	9.54a±0.38	9.36a±0.61	9.62a±0.43	9.52a±0.37	9.66a±0.42	9.54a±0.09
GP chocolate	9.24a±0.48	9.10ab±0.74	8.96ab±0.53	9.34a±0.47	9.20ab±0.79	9.17b±0.15
GO chocolate	9.34a±0.43	8.94ab±0.56	9.40a±0.51	9.52a±0.37	9.46a±0.55	9.33ab±0.13
MP chocolate	9.10a±0.59	9.02ab±0.29	9.26ab±0.55	9.52a±0.37	9.36ab±0.61	9.25b±0.12
MO chocolate	9.18a±0.43	8.34b±0.65	8.52b±0.81	9.14a±0.62	8.62b±0.50	8.76c±0.16
LSD at 0.5	n.s	0.77	0.78	n.s	0.77	0.25
LSD at 0.01	n.s	n.s	1.06	n.s	1.02	0.34
LSD at 0.001	n.s	n.s	n.s	n.s	n.s	0.45

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil; A,b,c,d= Different superscripts Within the same column represent significant differences between the results (p<0.05); LSD= Least significant differences n.s= non-significant and ± = Means standard deviation.

Proximate chemical composition of prepared chocolates:

Data concerning the chemical composition (moisture, ash, protein, fat, fiber, and carbohydrates) of cinnamon, ginger, mint, and their essential oils chocolates were recorded in Table 2. Data show significant differences ($p<0.05$) between control chocolate and all samples in moisture. Mint oil and cinnamon oil chocolate recorded the highest moisture content followed by ginger oil, control, mint powder, and cinnamon powder chocolate while chocolate with ginger powder recorded the lowest moisture content. Regarding ash, data show that chocolate with mint oil recorded the highest ash content (4.46±0.06) as compared to other chocolate samples. It could be observed that ash content increased significantly ($p < 0.05$) by the addition of herbs and their essential oils to the chocolate compared to the control chocolate except for cinnamon oil chocolate which recorded the lowest ash content (4.09±0.04). As for protein, fat, and fiber contents, data show significant differences ($p< 0.05$)

between the control and all chocolate samples. The addition of herbs and their essential oils significantly increased ($p< 0.05$) the protein, fat, and fiber contents in all chocolate samples as compared to the control chocolate which recorded the lowest contents. Data show that mint oil chocolate recorded the highest protein and fat values (4.54±0.05 and 0.85±0.04). While ginger oil chocolate recorded the highest fiber content (4.83±0.05) followed by mint oil chocolate which recorded (4.63±0.04). Results were in line with Rada *et al* **.** (2020) results who mentioned that the fat content of chocolate samples with added spices presents relatively close values that are between 20.97 - 23.17 % in cinnamon chocolate, 21.07- 23.27 % in ginger chocolate, and 21.48 - 23.67 % in chili pepper chocolate.

Concerning carbohydrates contents, data show that control, cinnamon powder, and ginger powder chocolate recorded the highest carbohydrates contents (53.87±0.07, 53.93±0.07, and 53.92±0.05), respectively. While mint oil recorded the lowest carbohydrate contents (52.04±0.06). Results were in harmony with Rada *et al***.** (2020) results who stated that the concentration of carbohydrates varies depending on the type of chocolate with the addition of spices and has values between 51.38 - 53.78 % in cinnamon chocolate, 50.81 53.54 % in ginger chocolate, and 72.54 76.03 % in chili pepper chocolate. Smaller differences in carbohydrate content between ginger chocolate, cinnamon chocolate, and control chocolate.

It could be observed from the previous results that the addition of cinnamon, ginger, and mint powder and their essential oils increased significantly ($p<0.05$) the moisture, ash, protein, fat, fiber, and carbohydrates contents as compared with the control chocolate. This result is different from that obtained by (Albak and Tekin, 2015) who found that the nutritional composition of fortified dark chocolate recorded 57.80%, 8.73%, 30.70%, 2.15%, and 0.54% for carbohydrate, Protein, Fat, Ash, and Moisture. This difference is due to the addition of ajwa dates, dried fruits, herbs, and their essential oils to chocolate. The types of chocolate with the addition of cinnamon, ginger and hot peppers contain significantly higher amounts of nutrients than control chocolate (Rada *et al***.,** 2020)**.**

Table 2. Chemical composition of prepared chocolates.

	Parameters					
Samples	**Moisture%**	**Ash%**	**Protein%**	**Fat%**	**Fiber%**	**Carbohydrates%**
Control chocolate	37.54b±0.08	4.17de±0.05	3.82e±0.07	0.61e±0.03	4.34e±0.08	53.87a±0.07
CP chocolate	37.26c±0.07	4.21cd±0.08	3.96d±0.10	0.64de±0.03	4.38de±0.06	53.93a±0.07
CO chocolate	38.11a±0.07	4.09e±0.04	4.12c±007	0.71bcd±0.04	4.55bc±0.08	52.97c±0.06
GP chocolate	36.97d±0.10	4.28bc±0.06	4.16c±0.04	0.67cde±0.05	4.51c±0.04	53.92a±0.05
GO chocolate	37.61b±0.08	4.36b±0.04	4.41b±0.05	0.77b±0.06	4.83a±0.05	52.85c±0.06
MP chocolate	37.37c±0.06	4.32b±0.06	4.23c±0.09	0.72bc±0.05	4.47cd±0.05	53.36b±0.16
MO chocolate	38.11a±0.09	4.46a±0.06	4.54a±0.05	0.85a±0.04	4.63b±0.04	52.04d±0.06
LSD at 0.5	0.13	0.10	0.12	0.07	0.10	0.14
LSD at 0.01	0.18	0.13	0.16	0.10	0.14	0.20
LSD at 0.001	0.26	0.19	0.23	0.14	0.20	0.27

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil; A,b,c,d= Different superscripts Within the same column represent significant differences between the results (p<0.05); LSD= Least significant differences n.s= non-significant and ± = Means standard deviation.

Total flavonoid and Total phenols of herbs essential oils:

Data concerning the total flavonoid and total phenols of essential oils were recorded in Table 3. Concerning total flavonoid contents, data show that total flavonoid values were 56.09±0.15, 3.01±0.23, and 37.36±0.13 for cinnamon oil, ginger oil, and mint oil, respectively. It could be noticed that cinnamon oil and mint oil recorded the highest total flavonoid contents. While ginger oil recorded the lowest total flavonoid content. It is noticeable that the total phenol content was 148.17±0.47, 7.94±0.27, and 118.29±0.31 for cinnamon oil, ginger oil, and mint oil, respectively. Data show significant differences between cinnamon oil, ginger oil, and mint oil in total phenol at ($p < 0.05$). Cinnamon oil and mint oil had the highest total phenol content while ginger oil recorded the lowest total phenol content.

Table 3. Total flavonoid and total phenols of herbs essential oils.

Samples	**Total flavonoid mg/ml**	**Total phenols mg/ml**
Cinnamon Oil	56.09a±0.15	148.17a±0.47
Ginger Oil	3.01c±0.23	7.94c±0.27
Mint Oil	37.36b±0.13	118.29b±0.31
LSD at 0.5	0.35	0.72
LSD at 0.01	0.53	1.09
LSD at 0.001	0.85	1.75

A,b,c,d= Different superscripts Within the same column represent significant differences between the results (p<0.05); LSD= Least significant differences n.s= non-significant and ± = Means standard deviation.

It could be observed from these results that essential oil is rich in phenols and flavonoids. Results in cinnamon essential oil with those obtained by previous researches of Ismail *et al*., (2017) Anal *et al***.,** (2014) and Prasad *et al*., (2009).

Mineral content of prepared chocolate:

Data concerning the mineral including (Fe, Zn, Mg, K, and Cu) of cinnamon, ginger, mint, and their essential oils chocolate samples were recorded in Table 4. It is noticeable that Fe content was 1.98±0.04, 2.41±0.02, 2.64±0.03, 2.11±0.02, 2.37±0.04, 2.23±0.03 and 2.48±0.03 mg/100g for control, cinnamon powder, cinnamon oil, ginger powder, ginger oil, mint powder, and mint oil chocolate, respectively. Data show significant differences ($p < 0.05$) between control chocolate and all samples in Fe. Cinnamon oil and mint oil chocolate recorded the highest Fe contents (2.64±0.03 and 2.48±0.03) mg/100g, respectively. While control chocolate records the lowest Fe content (1.98±0.04 mg/100g). Concerning Zn, data show significant differences ($p<0.05$) between control chocolate and all samples in Zn. Cinnamon oil chocolate had the highest Zn content (0.78±0.03 mg/100g). While control and ginger powder chocolate recorded the lowest Zn contents (0.45±0.03 and 0.51±0.03) mg/100g, respectively.

Regarding magnesium, data show that chocolate with cinnamon oil recorded the highest Mg content (53.19± 0.07 mg/100g) as compared to other chocolate samples. It could be observed that Mg content increased significantly

($p<0.05$) with the addition of herbs and their essential oils to the chocolate compared to the control chocolate which recorded the lowest Mg content (51.17±0.05 mg/100g). as for potassium, data show that mint oil recorded the highest K content (811.50±1.04 mg/100g) and the lowest K value was for control chocolate (768.70±0.81 mg/100g). Concerning Cu contents, data show that cinnamon oil chocolate recorded the highest Cu content (0.51±0.02). while control and mint powder chocolate recorded the lowest Cu contents (0.31±0.03 and 0.32±0.03) mg/100g, respectively.

In general, chocolates with cinnamon, ginger, and mint powder and their essential oils are nutritionally richer in mineral content (Fe, Zn, Mg, K, and Cu) than chocolate (control). Results were in accordance with results of Cinquanta *et al*., (2016) who described that dark chocolate has been confirmed as an excellent source of magnesium, iron, zinc and selenium. Also, Gul and Safdar, (2009) showed that cinnamon contained the highest amount of potassium and the lowest amount of sodium.

Table 4. Mineral content of prepared chocolate.

	Mineral content (mg/100g)				
Samples	**Fe**	**Zn**	**Mg**	**K**	**Cu**
Control chocolate	1.98f±0.04	0.45d±0.03	51.17g±0.05	768.70g±0.81	0.31e±0.03
CP chocolate	2.41c±0.02	0.65b±0.02	52.43b±0.04	786.45d±1.02	0.43bc±0.03
CO chocolate	2.64a±0.03	0.78a±0.03	53.19a±0.07	791.40c±1.10	0.51a±0.02
GP chocolate	2.11e±0.02	0.51d±0.03	51.67e±0.07	771.53f±1.09	0.36d±0.03
GO chocolate	2.37c±0.04	0.59bc±0.04	52.12c±0.05	784.43e±0.86	0.41c±0.04
MP chocolate	2.23d±0.03	0.56c±0.05	51.53f±0.06	793.54b±1.05	0.32de±0.03
MO chocolate	2.48b±0.03	0.64b±0.03	51.93d±0.07	811.50a±1.04	0.46b±0.02
LSD at 0.5	0.05	0.06	0.10	1.75	0.04
LSD at 0.01	0.07	0.08	0.14	2.43	0.06
LSD at 0.001	0.10	0.11	0.19	3.38	0.08

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil; A,b,c,d= Different superscripts Within the same column represent significant differences between the results ($p<0.05$); LSD= Least significant differences n.s= non-significant and ± = Means standard deviation.

Vitamins content of prepared chocolate:

Data concerning the vitamins including (A, E, C, K, B9, and B6) of cinnamon, ginger, mint, and their essential oils chocolates were recorded in Table 5. Data show significant differences ($p<0.05$) between control chocolate and all samples in vitamin A. Mint oil chocolate recorded the highest vitamin A contents followed by mint powder, ginger oil, ginger powder, cinnamon oil, and cinnamon powder chocolate while, control chocolate records the lowest vitamin A content (13.84±0.06 mg/100g). Regarding vitamin E, data show that chocolate with ginger oil recorded the highest vitamin E content as compared to other chocolate samples. It could be observed that vitamin E content increased significantly ($p < 0.05$) with the addition of herbs and their essential oils to the chocolate compared to the control chocolate which recorded the lowest vitamin E content. Concerning vitamin C contents, data show that vitamin C values were 3.41±0.04, 3.56±0.05, 3.98±0.04, 3.77±0.06, 4.18±0.06, 3.91±0.05 and 4.67±0.05 for control chocolate, cinnamon powder, cinnamon oil, ginger powder, ginger oil, mint powder, and mint oil chocolate, respectively. It could be noticed that mint oil chocolate recorded the highest vitamin C content (4.67±0.05 mg/100g), while control chocolate recorded the lowest vitamin C content (3.41±0.04 mg/100g). As for vitamin K, vitamin B9, and vitamin B6 contents, data in the same table show significant differences ($p<0.05$) between the control and all chocolate samples. The addition of herbs and their essential oils significantly increased ($p<0.05$) the vitamin K, vitamin B9, and vitamin B6 contents in all chocolate samples as compared to the control chocolate which recorded the lowest contents. Data show that ginger oil chocolate recorded the highest vitamin K, vitamin B9, and vitamin B6 values (21.98±0.08, 15.11±0.07 and 0.48±0.05) mg/100g in addition to cinnamon oil chocolate recorded the highest vitamin B6 content (0.54±0.03 mg/100g).

Finally, we could observe from the previous results that the addition of cinnamon, ginger, and mint powder and their oils to chocolate increased significantly ($p<0.05$) the vitamins content (A, E, C, K, B9, and B6) contents as compared with the control chocolate.

Table 5. Vitamins content of prepared chocolate.

	Vitamins (mg/100g)					
Samples	**Vitamin(A)**	**Vitamin(E)**	**Vitamin(C)**	**Vitamin(K)**	**Vitamin(B9)**	**Vitamin(B6)**
Control chocolate	13.84g±0.06	0.64f±0.04	3.41f±0.04	21.14f±0.04	14.18e±0.04	0.26d±0.02
CP chocolate	14.11f±0.08	0.71de±0.03	3.56e±0.05	21.38d±0.06	14.56c±0.05	0.39b±0.05
CO chocolate	14.26e±0.06	0.84b±0.03	3.98c±0.04	21.60b±0.04	14.87b±0.06	0.54a±0.03
GP chocolate	14.43d±0.08	0.77c±0.04	3.77d±0.06	21.53bc±0.0.06	14.63c±0.06	0.32bcd±0.04
GO chocolate	14.71c±0.07	0.93a±0.03	4.18b±0.06	21.98a±0.08	15.11a±0.07	0.48a±0.05
MP chocolate	15.11b±0.05	0.66ef±0.02	3.91c±0.05	21.26e±0.06	14.26e±0.07	0.29cd±0.04
MO chocolate	15.84a±0.05	0.73cd±0.04	4.67a±0.05	21.43cd±0.05	14.43d±0.05	0.36bc±0.05
LSD at 0.5	0.11	0.06	0.09	0.10	0.10	0.07
LSD at 0.01	0.16	0.08	0.12	0.14	0.14	0.10
LSD at 0.001	0.22	0.11	0.17	0.19	0.20	0.14

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil; A,b,c,d= Different superscripts Within the same column represent significant differences between the results ($p<0.05$); LSD= Least significant differences n.s= non-significant and ± = Means standard deviation.

Effect of feeding on prepared chocolate on menstrual pain and using analgesics in girls :

Questionnaire results illustrated by Fig. 1 shows the percent distribution of studied subjects according to "Reducing menstrual pain and using analgesics ". Data revealed that all girls' groups suffered from menstrual pain and using analgesics rate reached 100 % before the experiment. Meanwhile, remarkable improvement was

observed for all girls' groups after feeding on prepared chocolate samples among the experiment period (3 months).

These results were in line with those obtained by (Arfailasufandi and Andiarna 2018) who stated that dark chocolate is influential in decreasing menstrual pain in primary dysmenorrhea. Cocoa contains a wide range of phytochemicals, including polyphenols, which have been shown to exert anti-inflammatory and antioxidant actions, and also to have a positive effect on pain. Other components of cocoa might be able to positively influence pain perception through various mechanisms (De Feo *et al*., 2020). The results showed that eating dried figs during menstruation reduces pain intensity and duration, PMS symptoms, and perceived fatigue, and increases the quality of life (Amanak 2020). Also Jahangirifar *et al* (2018) stated that cinnamon can be a complementary therapy to cope with menstrual pain. Meanwhile, Jenabi (2013) demonstrated that ginger is effective in minimizing pain severity in primary dysmenorrhoea.

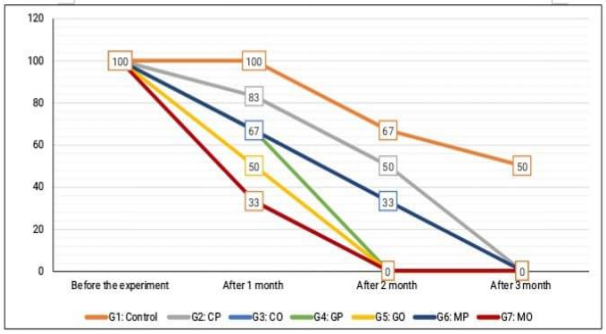

Fig. 1. Percent's of girl's menstrual pain and using analgesics.

Effect of feeding on prepared chocolate on menorrhagia in girls:

Questionnaire results illustrated by Fig. 2 show the percent distribution of studied subjects according to "Menorrhagia", Data revealed that all girls' groups suffered from menorrhagia rate reached 100 % before the experiment. Meanwhile, remarkable improvement was observed for all girls' groups after feeding on prepared chocolate samples among the experiment period (3 months). Results revealed that all groups of girls who ate chocolate had a decrease in menorrhagia because chocolate contains Ajwa dates which are rich in iron. These results were in harmony with those obtained by Wang *et al*., (2013) who stated that heavy menstrual bleeding (HMB) is associated with both iron deficiency and fatigue. Also, Ali and Alam (2020) noted that there is an increase in Hb in teenage after the intervention of ajwa date fruit. Also, our results stated that the addition of cinnamon, ginger, mint, and their essential oils to chocolate improved the absorption of iron because they contain vitamin C. Rathore and Shekhawat (2008) stated that ginger and cinnamon supply the body with calcium, iron, vitamin B, vitamin C, and carotene. Meanwhile, Zaida *et al* (2006) demonstrated that tea and vervain infusions inhibited iron availability. In contrast, mint improved it, vitamin C helped in preventing these inhibiting properties.

Effect of feeding on prepared chocolate on level of daily activities during menstruation period in girls

Questionnaire results illustrated by Fig. 3 show the percent distribution of studied subjects according to "Level of daily activities during menstruation ". It is clear that the menstruation period affected girl's performance and restricted their daily activities by 100 % before the experiment. Meanwhile, remarkable improvement in girls' activities during menstruation period was observed for all groups after feeding on prepared chocolate samples among the experiment period (3 months).

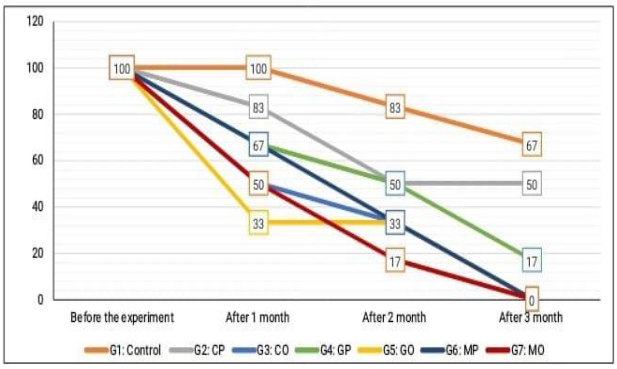

Fig. 2. percent's of menorrhagia in girls.

We noticed that all groups of girls who ate chocolate had an improvement in their activity level because chocolate reduced pain, inflammation, and heavy menstruation. Our results are in accordance with results of Abdullah *et al*., (2019) who stated that ajwa dates supplementation has the potential to alleviate stress-induced changes in mood and cognitive functions in healthy young adults without adverse effects on renal functions. Also, Negara *et al*., (2021) who stated that ginger oil use can be recommended to prevent the pain before menstruation Also Indumathi (2012) indicated that peppermint extract has been effective in students with symptoms of dysmenorrhea.

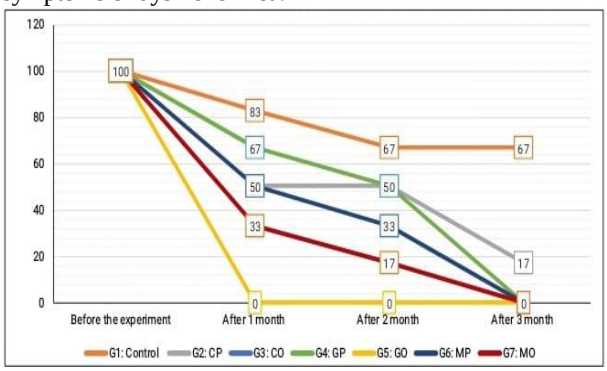

Fig. 3. percent's of girl's daily activities during menstruation.

Body mass index of dysmenorrhea girls fed on chocolate for three months:

Data concerning the body mass index (BMI) of dysmenorrhea girls fed on chocolate for three months were recorded in Table 6. Before the experiment period (zero time), it is noticeable that BMI of girls recorded 25.50±0.95, 23.47±1.00, 22.62±0.91, 24.20±0.80, 23.37±1.05, 23.43± 1.03 and 24.43±1.06 for control, cinnamon powder, cinnamon oil, ginger powder, ginger oil, mint powder, and mint oil chocolate groups, respectively. On the other hand at the end of the experiment (3 months) BMI recorded 25.70±0.90, 23.63±1.01, 22.80±0.95, 24.32±0.76, 23.50± 1.10, 23.57±0.97 and 24.63±1.03 for the same chocolate samples, respectively. After feeding girls on chocolate with cinnamon, ginger, mint, and their oils for three months, data showed that non-significant differences ($p < 0.05$) were observed in body mass index for all groups during the experiment period (3 months). It is noticeable that feeding on

chocolate with (cinnamon, ginger, and mint) powder and their essential oils for three months had no noticeable change on girls' weight as compared to their weight before the experiment at ($p<0.05$).

It can be noted that eating chocolate for three months did not cause an increase in body mass index. This is due to the (cinnamon, ginger, and mint) powder and their essential oils added to the chocolate. Results are in accordance with El Sayed and Moustafa, (2016) who showed that a combination of ginger and cinnamon could decrease levels of lipid profile in plasma ($p< 0.05$).

Table 6. Body mass index of dysmenorrhea girls fed on chocolate for three months .

Girls groups	Body mass index			
	Zero time	1 month	2 month	3 month
G1: control chocolate	25.50a±0.95	25.57a±0.90	25.60a±0.95	25.70a±0.90
G2: CP chocolate	23.47bc±1.00	23.50bc±0.95	23.57bc±1.00	23.63bc±1.01
G3: CO chocolate	22.62c±0.91	22.70c±0.85	22.77c±0.91	22.80c±0.95
G4: GP chocolate	24.20abc±0.80	24.23abc±0.87	24.27abc±1.03	24.32abc±0.76
G5: GO chocolate	23.37bc±1.05	23.40bc±1.00	23.43bc±1.10	23.50bc±1.10
G6: MP chocolate	23.43bc±1.03	23.50bc±0.98	23.53bc±0.93	23.57bc±0.97
G7: MO chocolate	24.43ab±1.06	24.53ab±1.03	24.60ab±1.01	24.63ab±1.03
LSD at 0.5	1.71	1.65	1.73	1.68
LSD at 0.01	2.37	2.29	2.41	2.34
LSD at 0.001	n.s	n.s	n.s	n.s

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil; A,b,c,d= Different superscripts Within the same column represent significant differences between the results (p<0.05); LSD= Least significant differences n.s= non-significant and ±= Means standard deviation.

Hemoglobin concentration of dysmenorrhea girls fed on chocolate for three months.

Data concerning the hemoglobin concentration was recorded in Table 7. Before the experiment period (zero time), the hemoglobin concentration of dysmenorrhea girls recorded (12.33±0.08, 11.34±0.09, 10.37±0.09, 11.51±0.03, 11.58±0.07, 11.17±0.09 and 11.44±0.08) g/dl for control, cinnamon powder, cinnamon oil, ginger powder, ginger oil, mint powder, and mint oil chocolate groups, respectively. On the other hand, at the end of the experiment (3 months) hemoglobin recorded (12.73±0.05, 13.95±0.06, 13.17±0.08, 12.92±0.07, 13.78±0.07, 12.98±0.09 and 13.44±0.07) g/dl for the same groups, respectively. Data showed that significant differences were noticed between all groups in hemoglobin scores after feeding girls on chocolate samples with herbs and oils at ($P< 0.05$). Chocolate with cinnamon powder group recorded the highest hemoglobin scores after three months (13.95±0.06 g/dl) followed by ginger oil, mint oil, cinnamon oil, mint powder, and ginger powder chocolate while, control chocolate group recorded the lowest hemoglobin content (12.73±0.05 g/dl). From the obvious data, all dysmenorrhea girls groups that ate chocolate increased in hemoglobin scores especially groups that fed on chocolate with used herbs and their essential oils and this is in agreement with (Ali and Alam, 2020) who showed that giving ajwa date fruit (*Phoenix dactylifera L.*) leads to increasing hemoglobin (Hb) levels in teenage girls.

However, we noticed from the previous results that feeding girls chocolate with cinnamon, ginger, and mint powder and their essential oils increased significantly ($p<0.05$) hemoglobin scores as compared with control chocolate. Also Hassan (2017) showed that cinnamon has an effect on increasing the level of hemoglobin in women when given during the menstrual cycle. Results also are in the same line with the studies of Ooi *et al***.** (2022) and Kulkarni *et al*., (2012) who stated that ginger supplementation was shown to enhance iron absorption and thus increase oral iron therapy's efficacy. Also Hamdia *et al***.,** (2020**)** suggested that mint powder a very effective food supplement to prevent and treat anemia.

Table 7. Hemoglobin concentration of dysmenorrhea girls fed on chocolate for three months.

Girls groups	Hemoglobin *(g/dl)*			
	Zero time	1 month	2 month	3 month
G1: control chocolate	12.33a±0.08	12.46a±0.08	12.57c±0.07	12.73f±0.05
G2: CP chocolate	11.34d±0.09	12.22bc±0.06	13.04a±0.08	13.95a±0.06
G3: CO chocolate	10.37f±0.09	11.41f±0.08	12.25e±0.07	13.17d±0.08
G4: GP chocolate	11.51bc±0.03	12.00d±0.08	12.53cd±0.05	12.92e±0.07
G5: GO chocolate	11.58b±0.07	12.33ab±0.07	13.04a±0.06	13.78b±0.07
G6: MP chocolate	11.17e±0.09	11.82e±0.07	12.43d±0.08	12.98e±0.09
G7: MO chocolate	11.44cd±0.08	12.15c±0.09	12.78b±0.07	13.44c±0.07
LSD at 0.5	0.13	0.13	0.12	0.12
LSD at 0.01	0.19	0.19	0.16	0.17
LSD at 0.001	0.26	0.26	0.23	0.24

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil; A,b,c,d= Different superscripts Within the same column represent significant differences between the results (p<0.05); LSD= Least significant differences n.s= non-significant and ±= Means standard deviation.

Lipid profile of dysmenorrhea girls fed on chocolate for three months:

Data concerning the serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-c), and low-density lipoprotein (LDL-c) of dysmenorrhea girls groups fed on cinnamon, ginger, mint, and their essential oils were recorded in Table 8. The normal group recorded 160.33±4.19, 80.00±3.00, 45.33±3.06 and 99.00±5.76 mg/dL, while the chocolate control group recorded 161.67±4.51, 91.33±2.52, 44.67±1.53 and 98.73±3.23 mg/dL for TC, TG, HDL, and LDL, respectively. After feeding girls on chocolate with cinnamon, ginger, mint, and their oils for three months data

showed that TC recorded 161.67±4.51, 154.33±2.52, 152.33± 3.51, 158.33±3.06, 155.00±3.61, 162.67±3.21 and 156.67± 3.06 mg/dL for control chocolate, cinnamon powder, cinnamon oil, ginger powder, ginger oil, mint powder, and mint oil chocolate, respectively. Data show significant differences ($p<0.05$) between control chocolate and all samples in TC. Feeding girls on control chocolate, ginger powder, mint powder, and mint oil chocolate groups recorded the highest TC contents while feeding girls on chocolate with cinnamon oil recorded the lowest TC content (152.33±3.51 mg/dL).

Data show that TG values of dysmenorrhea girls groups were 91.33±2.52, 86.00±3.00, 84.67±4.04, 87.00± 3.00, 86.67±2.52, 81.33±2.52 and 88.00±3.00 for control, cinnamon powder, cinnamon oil, ginger powder, ginger oil, mint powder, and mint oil chocolate, respectively. Control, ginger powder, ginger oil, and mint oil chocolate groups recorded the highest TG content (91.33±2.52, 87.00±3.00, 86.67±2.52 and 88.00±3.00 mg/dL) and the lowest TG value was for mint powder chocolate group (81.33±2.52 mg/dL). As for HDL and LDL contents, data in the same table show Non-significant differences were observed in serum total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein except for cinnamon oil girls group (89.40±4.70 mg/dL).

Finally, we could observe from the previous results that feeding girls chocolate with cinnamon, ginger, and mint powder and their essential oils decreased significantly ($p<0.05$) serum total cholesterol, triglycerides, and low-density lipoprotein scores as compared with control chocolate. Our results are in the same trend with Baba *et al*., (2007) who found that cocoa powder is rich in polyphenols that has been shown in various models to inhibit LDL oxidation and atherogenesis. It is possible that increases in HDL-cholesterol concentrations may contribute to the suppression of LDL oxidation and that polyphenolic substances derived from cocoa powder may contribute to an elevation in HDL cholesterol. Also, Alqarni *et al*., (2019) revealed that phenolic extracts of Ajwa dates have positive impacts on health as they reduced TC, LDL-C, and lipids VLDL-C and improved HDL-C and the antioxidant defense system in rats. Also, researches of Anderson *et al*., (2016), Ranasinghe *et al.*, (2012) and Ping *et al*. (2010) recommended that supplementation of cinnamon reduced fasting insulin, glucose, total cholesterol, and LDL cholesterol and enhanced insulin sensitivity as well as increasing HDL of subjects with elevated blood glucose. In the same trend, researches by ElRokh *et al*.**,** (2010), Al-Tahtawy *et al*., (2011) and (Bolanle, 2011) revealed that dietary consumption of ginger extract significantly reduced all lipid profile parameters in hyperlipidemic mice. However Badal *et al*. (2011) indicated that the aqueous extract of *menthe Piperita* produced a significant decrease ($p<0.05$) in elevated levels of glucose, cholesterol, triglycerides, and very low-density lipoprotein.

Table 8. Lipid profile of dysmenorrhea girls fed on chocolate for three months.

Girls groups	Serum lipids			
	TC (*mg/dL*)	**TG (*mg/dL*)**	**HDL (*mg/dL*)**	**LDL (*mg/dL*)**
G1: Normal	160.33ab±4.19	80.00d±3.00	45.33a±3.06	99.00a±5.76
G2: Control chocolate	161.67a±4.51	91.33a±2.52	44.67a±1.53	98.73a±3.23
G3: CPchocolate	154.33bc±2.52	86.00bc±3.00	45.33a±3.06	91.80ab±5.23
G4: CO chocolate	152.33c±3.51	84.67bcd±4.04	46.00a±2.00	89.40b±4.70
G5: GP chocolate	158.33abc±3.06	87.00ab±3.00	44.67a±2.52	96.27ab±3.81
G6: GO chocolate	155.00bc±3.61	86.67ab±2.52	45.67a±2.08	92.00ab±4.91
G7: MP chocolate	162.67a±3.21	81.33cd±2.52	48.33a±2.52	98.07a±5.14
G8: MO chocolate	156.67abc±3.06	88.00ab±3.00	47.33a±3.06	91.73ab±2.47
LSD at 0.5	6.08	5.16	n.s	7.84
LSD at 0.01	8.36	7.12	n.s	n.s
LSD at 0.001	n.s	9.78	n.s	n.s

CP: Cinnamon Powder; CO: Cinnamon Oil; GP: Ginger Powder; GO: Ginger Oil; MP: Mint Powder; MO: Mint Oil; A,b,c,d= Different superscripts Within the same column represent significant differences between the results ($p<0.05$); LSD= Least significant differences n.s= non-significant and ±= Means standard deviation.

CONCLUSION

Consuming the novel prepared chocolate which contains (ajwa, dried fruits, cinnamon, ginger, and mint) has proven its effectiveness in reducing menstrual pain and inflammation, and increasing the percentage of iron in the blood, which makes it compensate for the iron that may be lost during the menstrual cycle, and it also reduces the percentage of total cholesterol, triglycerides, and low-density lipoprotein.

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تصنيع وتقييم شيكولاتة مبتكرة لعسر الطمث عند الفتيات

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الملخص

هدفت هذه الدراسة إلى إنتاج شيكولاتة جديدة مدعمة بالقرفة والزنجبيل ومسحوق النعناع وزيوتها الأساسية وتقييم تأثيرها على عسر الطمث لدى عينة من 48 طالبة متطوعة من جامعة المنصورة يعانون من عسر الطمث. وقد تراوح أعمارهن بين 18:25 سنة مقسمين إلى ثماني مجموعات (6 مرضى في كل مجموعة) على النحو التالي: المجموعة 1: المجموعة التي لا تتناول شيكولاتة. المجموعة 2: الضابطة التي تتغذى على الشيكولاتة بدون مسحوق الاعشاب وزيوتها العطرية. المجموعة 3: تتغذى على الشيكولاتة مع مسحوق القرفة. المجموعة 4: تتغذى على الشيكولاتة بزيت القرفة. المجموعة 5: تتغذى على الشيكولاتة مع مسحوق الزنجبيل. المجموعة 6: تتغذى على الشيكولاتة بزيت الزنجبيل. المجموعة 7: تتغذى على الشيكولاتة مع مسحوق النعناع. المجموعة 8: تتغذى على الشيكولاتة بزيت النعناع. أظهرت النتائج أن تناول الفتيات شيكولاتة القرفة والزنجبيل ومسحوق النعناع وزيوتها الأساسية أدى إلى زيادة معنوية ($P < 0.05$) في درجات الهيموجلوبين مقارنة بالشيكولاتة الضابطة. بينما لم يؤدي تناول الشيكولاتة لمدة ثلاثة أشهر إلى زيادة مؤشر كتلة الجسم. لذلك أشارت النتائج السابقة إلى أن استهلاك شيكولاتة القرفة والزنجبيل ومسحوق النعناع وزيوتها الأساسية ادي الي انخفاضا معنوياً ($P < 0.05$) في مستوي الكوليسترول الكلي في الدم، والدهون الثلاثية، والبروتين الدهني عالي الكثافة ، والبروتين الدهني منخفض الكثافة بالمقارنة مع الشوكولاتة الضابطة. من ناحية أخرى ، أظهرت هذه الدراسة أن تناول الشيكولاتة المحتوية على (العجوة، والفواكه المجففة، والقرفة، والزنجبيل، والنعناع) وزيوتها الأساسية تقلل بشكل فعال من آلام الدورة الشهرية وتحسن مستويات الحديد في الدم ، الذى قد يفقد أثناء الدورة الشهرية.