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**THE IMPACT OF VARYING CONCENTRATIONS OF HAWTHORN
(*CRATAEGUS OXYACANTHA*) ON CARDIOVASCULAR HEALTH IN
ADRIAMYCIN-INDUCED CARDIOVASCULAR DISEASES RATS**

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ABSTRACT: Since the 1st century, Hawthorn, a *Crataegus* plant, has been used to treat heart disease. Both leaves and blossoms are used in modern medicine. Hawthorn may help manage mild to moderate heart failure, so the current study aims to Promote cardiovascular health using different levels of Hawthorn (*Crataegus oxyacantha*) in heart disorder rats. Thirty (30) Male albino rats of the Sprague-Dawley strain weighing 150 ± 10 g, ten weeks old were separated into four groups, one of them as negative control (normal), and other groups were treated with adriamycin (ADM) for two days a week by intraperitoneal injection (five mg/kg) two time per week throughout two weeks, to induce CVD. One of the adriamycin-induced CVD groups was assigned as a positive control group and fed the standard diet, and the remaining three groups were fed 7%, 10%, and 15% Hawthorn for twenty-eight days. Upon completion of the investigation, the blood samples were collected for biochemical analysis. The results indicate that exposure rats to ADM that fed on basal diet +15 % Hawthorn led to a significant increase in the levels of TC, TG, LDL, and VLDL in the blood serum, as opposed to HDL. Also Rats were fed a diet supplemented with a distinct concentration of Hawthorn resulting in a significant reduction (P below 0.05) the level of creatinine & urea serum.

Key words: *Crataegus species* - Heart disorder – Bio Cemical Analysis- Hawthorn.

INTRODUCTION

Hawthorn (*Crataegus species*) is a widespread thorny rose family shrub that can attain a maximum height of 5 cm and is found in sunny woodlands and slopes worldwide. They develop into pink, red, or white clusters. Hawks, which are diminutive berries, emerge subsequent to the blooms. When mature, they are typically red, but can also be black. The glossy foliage of hawthorn trees exhibit a diverse array of shapes and sizes. (Cloud *et al.*, 2019). Since the 1st century, people have been using hawthorn, which is a plant of the *Crataegus* genus, to cure heart problems. By the early 1800s, it was being used as a treatment for circulatory diseases and respiratory ailments by medical professionals in the United States. Historically, the berries were utilized to treat a wide range of cardiovascular conditions, Such as but not restricted to irregular heartbeat, high blood pressure, chest discomfort, hardening of the arteries, and heart failure.

Presently, both the flowers & foliage are utilized for medicinal purposes. Research findings even indicate that hawthorn may exhibit potential efficacy in the management of modest to moderate heart failure. (Corte *et al.* , 2016). According to laboratory and animal studies, hawthorn is rich in antioxidants, such as quercetin and oligomeric procyanidins (OPCs), which are also present in grapes. Hawthorn's antioxidants may prevent a portion of the damage caused by free radicals, particularly in relation to cardiovascular disease. (Daiber *et al.*, 2017). Many of the substances found in hawthorn may be beneficial to the heart. OPCs & other antioxidant flavonoids have the potential to aid in vasodilation, enhance blood circulation, and prevent damages.for medicinal purposes, the fruit, foliage, and flowers of the hawthorn plant have been utilized. The foliage and flowers are utilized in the majority of contemporary preparations because they are thought to contain a greater quantity of flavonoids compared to the

fruit (Dehghani *et al.*, 2019). Hawthorn is utilized to aid in protection against heart disease, as well as the management of hypertension and lipids. Research on both animals and humans indicates that hawthorn enhances circulation, reduces blood pressure, and increases coronary artery blood flow. Additionally, it has been applied topically to address abscesses as well as skin ulcers. (Dong *et al.*, 2017) and (Du *et al.*, 2019) exhibited that further research is required to determine its potential efficacy. Several studies have reached the conclusion that hawthorn enhanced heart function markedly. Additionally, research indicates that the herb may improve an individual's capacity to engage in physical activity subsequent to experiencing cardiac failure. According to the accounts of study participants, hawthorn significantly alleviated disease symptoms (including fatigue and shortness of breath). discovered that modest doses of captopril (a prescription heart medication) were just as efficacious as 900 mg per day of hawthorn extract for two months in alleviating symptoms of heart failure. (Ferrucci, *et al.*, 2018).

Aim of study

The purpose of this research was to determine the impact of varying Hawthorn (*Crataegus* species) concentrations on rats' biochemical markers of cardiovascular disease.

MATERIALS & METHODS

1- Materials

1.1- preparation of Hawthorn (*Crataegus species*): *Crataegus* species of hawthorn were purchased from the local market in Jeddah, Saudi Arabia. After being washed and dried in an oven designed explicitly for drying at fifty degrees Celsius for three days, the hawthorn was pulverized and milled into a fine powder.

1.2- Experimental animals: In the experiment, we employed thirty male albino Sprague Dawley rats that weighed 150 plus or minus 10 grams.

1.3- Adriamycin injectable solution: A bottle of Adriamycin injectable solution with a concentration of twenty-five mg per ml was obtained from Mina pharm Co. in Cairo, Egypt. El-Gomhoreya Company in Cairo, Egypt provided all of the additional chemicals, kits, and reagents that were used in this study.

2- Methods

2.1- Biological experiment

Basal diet composition of rats

The basal diet comprised the following components: five percent cellulose, ten percent maize oil, 0.25 percent choline chloride, one percent vitamin mixture, 0.35 percent methionine, & four percent salt mixture (Morsi, 1992).

CaCO₃ (600 mg), MgSO₄.2H₂O (204 mg), K₂HPO₄ (645 mg), CaHPO₄.2H₂O (150 mg), Fe(C₆H₅O₇)₂.6H₂O (55 mg), ZnCl₂ (0.5 mg), MnSO₄.4H₂O (10 mg), NaCl (334 mg), CuSO₄.5H₂O (0.06 mg), as well as KI (1.6 mg) Comprised the composition of salt mixture utilized in the experiment, (Hegsted, Mills, Elvehjem, & Hart, 1941).

The vitamin mixture of the standard diet composed of; , Vitamin E (10 Iu), Calcium panthothenic acid (0.40 mg), Thiamin (0.50 mg), Vitamin A (200 Iu), Vitamin K (0.50 Iu), Pyridoxine (1.00mg), Niacin (4.00 mg), Para-amino – benzoic acid (0.02 mg), Vitamin D (100 Iu), Folic acid (0.02 mg), Choline chloride (200 mg), Inositol (24 mg), Vitamin B12 (2.00 g) (Campbell, 1963).

2.2- Induction of Cardiovascular disease

According to Young *et al.* (2002), cardiovascular disease groups were given adriamycin (ADM) via intraperitoneal injection (five mg/kg) twice a week for a total of two weeks to induce CVD.

Table (1): The components of the fundamental & experimental diets.

Component (g)	Basal diet	7% Hawthorn	10% Hawthorn	15% Hawthorn
Hawthorn	---	7	10	15
Casein	20	20	20	20
Corn oil	4.7	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1
Cellulose	5	5	5	5
Choline chloride	2	2	72	2
Sucrose	10	10	10	10
Corn starch	Up to 100	Up to 100	Up to 100	Up to 100

2.3- Design of Experiments & Animal Groups

This experiment was conducted on a cohort of 30 fully matured male Sprague-Dawley albino rats aged between fourteen and sixteen weeks. These rats had an average weight of 150±10 g. The animals were housed in strain-free metallic coverings secured to plastic Cages, and stringent hygiene protocols were adhered to. The rats were given the basal diet for seven days prior to the start of the experiment to allow for adaptation. Specialized non-scattering feeding containers were utilized to administer diets to Rats in order to prevent food loss and contamination. Ad libitum water was supplied through a narrow mouth container equipped with a metallic tube securely fastened to its mouth using a rubber tube. As previously stated, Rats were acclimated to the experimental protocol by being exposed to a twelve hour light & twelve hour dark cycle for a duration of seven days prior to its commencement. Rats were Classified into five Equal groups (6 in each group) as follow:

- Group one : provided with a basal diet (negative control).
- Group two: (positive control)

- Group three: CVD group fed the basal diet comprising 7% Hawthorn.
- Group four: CVD group fed the basal diet onstisting of 10% Hawthorn.
- Group five: CVD group fed the basal diet consisting of 15% Hawthorn.

2.4- Biological evaluation

Daily feed consumption was documented, while body weight was assessed on a weekly basis for the duration of the 28-day experiment. In accordance with (Chapman *et al*, 1959),

Blood sampling

After fasting for 12 hours, blood samples in initial times were obtained from retro orbital vein, while it obtained from hepatic portal vein at the end of each experiment. Blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 28minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean Eppendorf tube and stored frozen at -20°C till analysis according to the method described by T by Curry and Wallington (1967).

2.5) Biochemical analysis

2.5.1) *Quantification of Lipids in Serum:*

2.5.1.1) Triglycerides: Triglycerides were determined by enzymatic calorimetry in accordance with *Fassati and Prencipe's (1982)* methodology.

2.5.1.2) Total Cholesterol: primary application of TC measurement, as described by *Allain (1974)*.

2.5.1.3) HDL-cholesterol: According to *Lopez (1977)*, phosphotungstic acid & magnesium ions selectively precipitating all lipoproteins other than the HDL fraction-cholesterol contained in the supernatant may be measured via similar procedure utilized for TC.

2.5.1.4) V-LDL & LDL- cholesterol: The technique developed by *Lee & Nieman (1996)* was utilized for the measurement of both very low density lipoproteins (VLDL) & low density lipoproteins (LDL).

2.5.1.5) Total Lipids : According to *Schmit 1964*, the colorimetric approach was used to determine the amount of total lipids.

2.5.2) Estimation of liver functions

2.5.2.1) Estimation of Alanine transferase (ALT): The approach proposed by *Tietz (1976)* was utilized for the analysis necessary to establish the ALT. Pyruvate & L-Glutamate are produced as a byproduct of the reaction that ALT catalyzes, which involves the amino group transfer from L-alanine to α -ketoglutarate.

2.5.2.2) Estimation of AST: The approach developed by *Henry (1974) & Yound (1975)* was utilized throughout the process of determining the (AST).

2.5.3) Estimation of Kidney functions

2.5.3.1) Estimation of Creatinine: The kinetic method developed by *Henry (1974)* was utilized to ascertain creatinine.

2.5.3.2) Estimation of urea: Urea was quantified utilizing enzymatic technique described by *Patton and Crouch in 1977*.

2.5.3.3) Statistical Analysis: All data were presented as mean and standard deviation. One-way classification was employed to compute statistical analyses. In accordance with *Snedcor & Cochran (1967)*, analysis of variance (ANOVA) as well as least significant variance (LSD) are utilized.

RESULTS & DISCUSSION

The objective of this research was promoting cardiovascular health using different levels of Hawthorn (*Crataegus species*) in CVD rats

1) Biological effects

1.1) The impact of various levels of Hawthorn (*Crataegus species*) on Lipids Profile and Atherogenic Index in Negative Control and CVD

The effect of (*Crataegus species*) on lipids profile of control negative & adriamycin groups are illustrated in the Table (2). Injection of rats with ADM resulted in a considerable rise in the levels of TC, total fat, LDL & VLDL in the serum, in contrary to HDL, that demonstrated a significant decrease compared with negative control (P less than 0.05). The identical outcomes were achieved by (*Corte et al ., 2016*). According to what they claimed, hawthorn (a species of *Crataegus*) has been utilized to treat cardiovascular conditions as long back as the 1st century. By the early 1800s, it was being used as a treatment for circulatory diseases and respiratory ailments by medical professionals in the United States. Historically, the berries were utilized to treat a wide range of cardiovascular conditions, containing, but not restricted to, irregular heartbeat, high blood pressure, chest discomfort, hardening of the arteries, & heart failure. In modern times, both the leaves and the flowers have been employed in medical preparations. There is also data that suggests

hawthorn could be useful when taken in the management of mild to severe heart failure.

The effect of (*Crataegus species*) on of atherogenic index of control negative & adriamycine groups are illustrated in Table (3). Injection rats by ADM caused a significant rise in concentrations of CRR, AC & AI compared with negative control (P less than 0.05). In accordance with Gao *et al.*, (2019) AI can be utilized on a daily basis in clinical Procedures as a routine monitoring index for CVD. Furthermore, de Quadros *et al.* (2017) demonstrated, hawthorn is rich in antioxidants, such as quercetin and oligomeric procyanidins (OPCs), The impact of (*Crataegus species*) on the atherogenic index of the control negative and adriamycin groups is demonstrated in Table (3). The injection of rats with ADM resulted in a notable increase in CRR, AC, and AI concentrations compared to the negative control (P < 0.05). Quadros *et al.* (2017) proved that

hawthorn contains many antioxidants, specifically quercetin and oligomeric procyanidins (OPCs). Thus, the atherogenic index of rats treated with hawthorn may be enhanced due to their content of antioxidants.

1.2) Impact of distinct levels of Hawthorn (*Crataegus species*) on Liver Functions in CVD rats.

Table 4 presents the impact of Hawthorn (*Crataegus species*) on the liver functions of the negative control as well as adriamycine groups. ADM injection resulted in a statistically significant elevation of AST & ALT concentrations in the serum of rats, as Compared with negative control group (P less than 0.05). Alp *et al.* (2015) reported similar findings, stipulating that hawthorn (*Crataegus oxyacantha*) is a botanical remedy renowned for its diverse array of polyphenolic compounds that exhibit antioxidant as well as hypolipidemic properties.

Table (2): impact of distinct levels of Hawthorn (*Crataegus species*) on lipids profile in CVD rats

Variables	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Negative control	57.50 \pm 5.69	67.94 \pm 7.19	27.00 \pm 0.82	16.91 \pm 4.08	13.59 \pm 1.44
Positive Control	239.00 \pm 6.48	187.50 \pm 1.90	23.75 \pm 1.71	77.75 \pm 8.54	37.50 \pm 2.80
7% Hawthorn	102.00 \pm 1.63	61.75 \pm 3.69	25.25 \pm 1.50	64.40 \pm 2.83	12.35 \pm 0.74
10% Hawthorn	105.75 \pm 3.20	62.25 \pm 1.71	25.50 \pm 2.08	67.80 \pm 2.28	12.45 \pm 0.34
15% Hawthorn	108.25 \pm 9.25	62.00 \pm 2.94	25.25 \pm 1.50	70.60 \pm 8.87	12.40 \pm 0.59

All data are presented as mean and standard deviation. Means in the same column subscribed with different levels indicate significant differences between these values at P less than 0.05 as calculated by ANOVA and LSD follow-up test.

Table (3): Impact of distinct levels of Hawthorn (*Crataegus species*) on atherogenic coefficient (AC), cardiac risk ratio (CRR) and atherogenic (AI) in CVD rats.

Variables	AC (mg/dl)	CRR (mg/dl)	AI (mg/dl)
Negative control	2.0 \pm 0.18	1.09 \pm 0.18	0.40 \pm 0.05
Positive Control	8.99 \pm 0.75	9.99 \pm 0.75	0.90 \pm 0.05
7% Hawthorn	3.05 \pm 0.25	4.05 \pm 0.25	0.39 \pm 0.02
10% Hawthorn	3.16 \pm 0.26	4.16 \pm 0.26	0.39 \pm 0.03
15% Hawthorn	3.29 \pm 0.26	4.29 \pm 0.26	0.39 \pm 0.04

All data are presented as mean and standard deviation. Means in the same column subscribed with different levels indicate significant differences between these values at P less than 0.05 as calculated by ANOVA and LSD follow-up test.

Table (4): The impact of distinct levels of Hawthorn (*Crataegus species*) on Liver Functions in Compared rats.

Variables	ALT(U/L)	AST(U/L)
Negative control	34.99 ^c ±2.16	39.90 ^e ±3.99
Positive Control	59.99 ^a ±6.85	55.89 ^{ab} ±4.89
7% Hawthorn	54.50 ^{ab} ±1.29	58.00 ^{ab} ±4.69
10% Hawthorn	53.50 ^{ab} ±4.04	45.50 ^d ±6.03
15% Hawthorn	48.00 ^{bc} ±4.24	63.50 ^a ±3.87

All data are presented as mean and standard deviation. Means in the same column subscribed with different levels indicate significant differences between these values at P less than 0.05 as calculated by ANOVA and LSD follow-up test.

1.3) The impact of distinct levels of Hawthorn (*Crataegus species*) on kidney functions in heart disorder rats.

Table (5) illustrates the influence of Hawthorn (*Crataegus species*) on renal functions in the negative control and adriamycin groups. The administration of ADM to rats significantly elevated creatinine and urea levels in the bloodstream ($P < 0.05$). The rats were provided with a diet containing a certain concentration of supplements, which led to a noteworthy decrease ($P < 0.05$) in the creatinine and urea levels in their blood. These results correspond to the findings of Kurkin *et al.* (2022), who found that the shoot-thick extracts of *Crataegus sanguinea* Pall and *Crataegus submollis* Sarg affect the excretion of creatinine through increased diuresis. In contrast, they found that the concentrated extract of *Crataegus monoline* Jacq did not impact the kidney's excretory function.

Furthermore, *Crataegus sanguinea* Pall. and *Crataegus submollis* Sarg. bloom during the flowering season. Shoots can be harvested as a raw material for drugs that enhance renal excretory function. *Crataegus submollis* Sarg., akin to many hawthorn species, exhibits therapeutic characteristics.

1.4) Effect of different levels of Hawthorn (*Crataegus species*) on Antioxidant Activity in heart disorder rats.

The effect of Hawthorn on antioxidant activity of negative control & adriamycin groups are illustrated in Table (6). When ADM was administered into rats, malonaldehyde (MDA), and glutathione peroxidase (GPx) levels significantly higher in contrast to negative control (P below 0.05). The identical outcomes were achieved by Dehghani *et al.*, (2019). They showed that ADM produce substantial quantities of reactive oxygen species (ROS) and causes oxidative stress in the body. Hawthorn is also found to contain numerous substances that may have cardiovascular benefits. OPCs & other antioxidant flavonoids have the potential to aid in vasodilation, enhance blood circulation, and prevent vasodamage. The results reported by Dehghani *et al.* (2019) were found to be identical. They demonstrated that ADM generates significant amounts of reactive oxygen species (ROS) and induces oxidative stress in the body. Hawthorn has also been discovered to possess various compounds that could offer advantages for cardiovascular health. OPCs and other antioxidant flavonoids can facilitate vasodilation, augment blood circulation, and avert vasodamage.

Table (5): The impact of distinct levels of Hawthorn (*Crataegus species*) on kidney functions in CVD rats.

Variables Groups	Creatinine(mg/dl)	Urea(mg/dl)
Negative control	0.87 ^c ±0.04	6.94 ^h ±0.83
Positive Control	1.78 ^a ±0.09	27.00 ^a ±1.41
7% Hawthorn	0.88 ^c ±0.09	17.50 ^{cd} ±1.29
10% Hawthorn	1.16 ^b ±0.11	20.00 ^b ±1.41
15% Hawthorn	0.84 ^{cd} ±0.09	15.88 ^{def} ±1.65

All data are presented as mean and standard deviation. Means in the same column subscribed with different levels indicate significant differences between these values at P less than 0.05 as calculated by ANOVA and LSD follow-up test.

Table (6): The impact of various levels of Hawthorn (*Crataegus species*) on Antioxidant Activity in heart disorder rats.

Variables Groups	MDA (nrol/mL)	GPx (mu/ml)
Negative control	15.62 ^d =fc 1.55	5.22 ^f ±1.55
Positive Control	38.90 ^a ±4.23	15.94 ^{de} ±1.28
7% Hawthorn	35.35 ^{ab} ±3.01	14.54 ^e ±1.44
10% Hawthorn	31.58 ^{bc} ±3.24	28.32 ^a ±3.11
15% Hawthorn	34.73 ^{abc} ±2.37	16.41 ^{de} ±.98

All data are presented as mean and standard deviation. Means in the same column subscribed with different levels indicate significant differences between these values at P less than 0.05 as calculated by ANOVA and LSD follow-up test.

Conclusion

It is recommended to utilize hawthorn (*Crataegus species*) for heart cases. Also various concentrations of hawthorn (*Crataegus species*) could potentially be recommended for the reduction of LDL & atherogenic index levels.

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تأثير التركيزات المختلفة من نبات الزعرور (*Crataegus oxyacantha*) على صحة القلب والأوعية الدموية لدى الفئران المصابة باضطرابات القلب نتيجة حقنها بادراميسن.

مقبولة سالم هلال الزهراني

قسم الأحياء، كلية العلوم، جامعة الباحة، المملكة العربية السعودية

الملخص العربي

استخدم الانسان الزعرور منذ القدم ، وهو نبات من جنس *Crataegus*، لعلاج مشاكل القلب. وفي بداية القرن التاسع عشر كان الزعرور يستخدم بالفعل كعلاج لمجموعة متنوعة من أمراض الدورة الدموية والجهاز التنفسي حيث كان يتم استخدام كل من الأوراق والأزهار في الممارسات الطبية الحديثة. كما تشير نتائج الأبحاث إلى أن الزعرور قد يُظهر فعالية محتملة في علاج قصور القلب البسيط إلى المتوسط، لذا تهدف الدراسة الحالية إلى تعزيز صحة القلب باستخدام مستويات مختلفة من الزعرور (*Crataegus oxyacantha*) في الفئران التي تعاني من اضطرابات القلب. تم استخدام ثلاثين (30) فأر ألبينو ابيض ذكور من سلالة سبراج داولي بوزن 150 ± 10 جم، بعمر عشرة أسابيع تم تقسيم الفئران الى مجموعتين اساسيتين المجموعة الاساسية الاولهى مجموعة فئران طبيعية (المجموعة الضابطة السالبة) و المجموعة الاساسية الثانية و التي تم تقسيمها الى اربع مجاميع فرعية تم حقنها بالأدرياميسين (ADM) مرتين أسبوعياً لمدة أسبوعين عن طريق الحقن تحت الجلد (خمسة ملغم/كغم) لتحفيز الإصابة بأمراض القلب والأوعية الدموية وتم تقسيم هذه المجموعة الى ثلاث مجاميع تجريبية و التي تتغذى على الوجبة الاساسية بجانب ثلاث مستويات مختلفة من نبات الزعرور (7% و 10% و 15%) لمدة ثمانية وعشرين يوماً و ايضا مجموعة ضابطة موجبة و هي التي تتغذى على الوجبة الاساسية فقط . وبعد الانتهاء من التجربة تم ذبح الفئران و اخذ عينات الدم لاجراء التحليل البيوكيميائي. وقد اوضحت النتائج إلى أن مجموعة الفئران المصابة و التي تغذت على الوجبة الاساسية بجانب 15% نبات الزعرور إلى زيادة معنوية في مستويات TC و TG و LDL و VLDL في مصل الدم، مقابل HDL، مما أظهر انخفاضاً ملحوظاً مقارنة مع مجموعة الكنترول السلبية ($P < 0.05$).

الكلمات المفتاحية: - نبات الزعرور - التركيزات المختلفة - اضطرابات القلب