

## THERAPEUTIC EFFECT OF *CRATAEGUS SINAICA* FRUIT ON VASCULAR DISEASES IN MALE RABBITS

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### ABSTRACT

*We used in this study the effect of Crataegus sinaica (hawthorn fruit) that found in St Catherin (South Sinai Desert, Egypt) on male Newzeland rabbits fed on atherogenic diet on various lipid profiles in serum, heart and liver. Four groups of six animals each were used ( untreated normal control ) received basal non-atherogenic diet, (Group II) received the basal non-atherogenic diet as in group I and were treated simultaneously with the hawthorn fruit (2.0 g of hawthorn fruit powder /100 g body weight for 6 weeks), (group III) Received atherogenic diet without treatment. (Group IV) Received atherogenic diet and were treated simultaneously with the hawthorn fruit. The results showed that the feeding of atherogenic diet caused significant increase in lipid components of serum as well as liver and heart tissues. Crataegus sinaica administration simultaneously with atherogenic could prevent the rise in lipid levels in serum and tissues. Crataegus sinaica administration simultaneously with basal non-atherogenic diet did not affect on the normal lipid profiles. We concluded that the levels of total serum cholesterol, triglycerides (TG), VLDL and LDL which are actually raised in atherogenic diet, can be lowered significantly with Crataegus sinaica fruit. Serum nitric oxide (NO) concentrations from rabbits were increased up to 5 fold in high-fat fed treated rabbits (group VI) showing significant effect of hawthorn treatment. so, its hypolipidemic effect may have a protective mechanism against the development of atherosclerosis. The present results proved that the Egyptian Crataegus sinaica fruit can be used for medicinal purposes as a hypolipidemic and anti-atherosclerotic drug.*

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## INTRODUCTION

**Tankansa et al. (2003)** reported that hyperlipidemia is the major risk factors in the initiation and progression of the atherosclerotic lesions. Evidence from studies both the animals and human indicates that progression can be slowed if elevated serum concentration of the atherogenic lipoprotein and triglycerides are reduced, which in turn prevents coronary heart diseases. **Ho, et al., 1997 and Chang, et al., 2005** stated that the fruit of *Crataegus* enhances cholesterol catabolism and suppresses cholesterol synthesis in-vitro and in-vivo. The decrease in LDL-Cholesterol has been attributed to an increase in LDL-receptor activity of hepatic membrane. **Ljubuncic, et al., 2005** stated that hawthorn fruit is also an excellent source of antioxidants. Previous studies on the antioxidant activity of hawthorn fruit identified seven antioxidants, namely, hyperoside, isoquercitrin, epicatechin, chlorogenic acid, quercetin, rutin and protocatechuic acid, all of these compounds protected human LDL from Cu-mediated oxidation. They also prevented the peroxy-free radical induced oxidation of a-tocopherol in human LDL (**Zhang, et al., 2001**). **Li and Billiar, 1999** stated that nitric oxide (NO), which is a short-lived free radical, important signaling molecule and potent vasodilator, influences physiological processes in every organ and tissue. NO is enzymatically synthesized by the oxidation of the terminal guanido-nitrogen atom of L-arginine, which is catalyzed by three different isoforms of endothelial nitric oxide synthase (eNOS). **Baughaman, and Bradley, 2003** said that the hawthorn have a solubilising effect on the crustaceous and calcareous deposits in arteries. **Rigelsky, et al., 2002; Baughaman, and Bradley, 2003; Pittler, et al., 2003** stated that hawthorn, *Crataegus Sp.* is widely used today in herbal medicine as a heart tonic. This study aimed to explore serum and tissues lipids profile after feeding with hawthorn fruits in hyperlipidemic rabbits and mechanism of reducing atherosclerosis to prove that whether the Egyptian *Crataegus sinaica* can be used for medicinal purposes as a hypolipidemic and anti-atherosclerotic drug.

## MATERIAL AND METHODS

This experiment was done at Marriott research station, Desert Research Center, at 35 k of Alexandria.

### I- Preparation of hawthorn (*Crataegus sinaica*):

This was done according to **Bajwa et al., 1971**.

### II- Experimental animals:

Male pure New Zealand white rabbits (24 animals) aged 3 months and weighing 2.3 - 3.6 kg and housed (one per cage) in an animal room at 25°C with a 12-h light:dark cycle. The rabbits consumed food and tap water ad libitum. They were divided into 4 groups of 6 animals each: **Group I: (Non Cholesterol fed group NCF):** 6 male rabbits were fed with normal basal diet composed of concentrate rations as pellets. This diet per 100 g contained "66.6 g carbohydrate, 17.0 g protein, 1.2 g fat, 6.2 g ash and 9.0 g moisture" used as control group. **Group II: (Non Cholesterol fed treated group NCFT):** 6 male rabbits were fed with the normal basal diet as group 1 and supplemented with the hawthorn fruit (2.0 g of hawthorn fruit powder /100 g body weight) per day for 6 weeks as specified by *Bajwa et al., 1971*. **Group III: High (Cholesterol fed group HCF):** 6 male rabbits were fed with an atherogenic diet composed of (sucrose 61%, casein 24%, saturated fat 10%, cholesterol 1.5%, cholic acid 0.5%, vitamin D<sub>2</sub> 1.25 million USP units/kg diet and vitamins with mineral mixture 5%). **Group IV: (High Cholesterol fed treated group HCFT):** 6 male rabbits were fed with on atherogenic diet and supplemented with the hawthorn fruit (2.0 g of hawthorn fruit powder /100 g body weight) per day for 6 weeks. At the end of 6 weeks, the rabbits were slaughtered and blood was directly collected from the heart without any anticoagulant and allowed to clot at room temperature for 30 min. centrifuged at 1000 x<sup>9</sup> for 10 min. The serum was kept at -20°C until used for determination of VLDL, LDL (*Friedewald, et al., 1992*),

total cholesterol, triacylglycerol in serum and tissues (*Zlaktis, et al., 1953*), phospholipids in serum and tissues (*Zilversant, and Davis, 1950*) and HDL cholesterol (*Cuyckens and Claeys 2002*), serum Nitric oxide (NO) (*Cadman et al., 1979*). The organs, including liver and heart were removed, washed with saline and stored at  $-80^{\circ}\text{C}$ . Lipids extraction of liver and heart muscle were performed according to the method of *Folch, et al., 1957*. Thiobarbituric acid reactive substances (TBARS) were determined in the liver tissues (*Han and Pak, 1999*). The thoracic aorta from the aortic bulb to the branching of the celiac artery was then removed and saved for measurement of cholesterol (*Chan et al., 1999*) and triacylglycerol (*Chen and Cunnane, 1992*). Statistical evaluation of the analytical data was done using Student's t-test and "P" values of  $P \leq 0.05$  were considered to be significant *Snedecor, and Cochran, 1970*.

HDL%, C/P ratio and atherogenic index were calculated as follows:

$$\text{HDL\%} = \frac{\text{HDL}}{\text{Total cholesterol} - \text{HDL}}$$

$$\text{C/P ratio} = \frac{\text{Total cholesterol}}{\text{Total phospholipids}}$$

$$\text{Atherogenic index} = \frac{\text{LDL} + \text{VLDL}}{\text{HDL} - \text{Total cholesterol}}$$

## RESULTS

The values for the NCF (group I) and the NCFT (group II) were not significantly affected. This indicates that hawthorn did not affect the lipid components of serum, liver, heart and aortic tissues of normal animals. NCF (group I) when compared with the HCF group (group III), indicates that feeding of high lipid diet caused significant increase in lipid components of serum, liver and heart tissues (Table 2). The results of HCF (group III) when compared with that of the HCFT (group IV)

indicate that the treatment prevents the rise in serum lipids as well as the lipid deposit in liver, heart and aorta. Drastic increase in cholesterol, triglyceride and phospholipids concentration of lipoprotein fractions especially LDL and VLDL was observed in HCF group, i.e. the atherogenic rabbits also increased due to high lipid diet. Hawthorn could prevent the increase of the atherogenic index. Serum (NO) concentrations from rabbits (Table 2) were increased up to 3 fold in group HCF and 5 fold in group HCFT, as compared with control, indicated significant effect of hawthorn treatment. Hepatic TBARS were increased 2 fold and 2.7 fold in the liver of HCF rabbits (Table 6), indicating that high fat diet increased the lipid peroxidation while hawthorn administration in group HCFT lowering the lipid peroxide levels significantly.

**Aortic lipids:** (Table 5) showed increased level of aortic cholesterol in HCF group. Hawthorn fruit, reduced significantly the accumulation of cholesterol in HCFT rabbits as compared with HCF and NCF groups .

## DISCUSSION

It has been claimed that the hawthorn fruit capable of reducing food stagnancy, stasis, blood lipids and blood pressure (*Walker, et al., 2006*). The present study shows that the atherogenic diet in (HCF group) raised the serum cholesterol, LDL and VLDL (Table 1). After the supplementation of hawthorn fruit lowered not only serum TC but also serum TG in group VI (Table 2). This could be possibly due to an increase in the liver-LDL receptor activity and decreased hepatic triglycerides synthesis (*Brown, and Goldslein 1983*). It was further shown that the high cholesterol/phospholipids (C/P) ratio in rabbits fed with atherogenic diet was decreased significantly in contrast to increase in HDL ratio after treatment with hawthorn. *Sharma, and Dixit (1995)* reported that high C/P ratios were usually associated with

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atherosclerosis. Hawthorn significantly increased HDL cholesterol concentration and HDL ratio; thus it would be useful in diseases like diabetes mellitus and coronary heart diseases because of their inverse relationship. Hypertriglyceridemia is also associated in metabolic consequences of hyper-coagulability, hyper-insulinemia, insulin resistance and glucose tolerance. The present study confirmed *by Ho, et al., (1997)* who showed that the hawthorn reduces triglycerides in rabbits fed with atherogenic diet and may prevent the progression of atherosclerosis and complications due to hypertriglyceridemia, decreased TC and TG levels and lowering effectively the atherogenic index. The underlying mechanisms for hypocholesterolemic activity of hawthorn fruit are poorly understood. One of these may be related to its “up-regulation” effect of LDL receptors on cell surfaces (*Ho, et al., 1997*). *Thirupurasundari, et al., 2005; Koçyildiz, et al., 2006* stated that the serum cholesterol level is maintained in a steady balance in which the rate of entry of cholesterol into the blood is equal to the rate of removal of cholesterol from the blood. Lowered serum cholesterol indicates a shift in this steady state, resulting from either a decrease in the rate of entry or an increase in the rate of removal (*Ho, et al., 1997*).

The present study revealed significant increase of the plasma NO concentration in HCF group as compared with control and in HCFT group as compared with all groups. The results were confirmed by the results of *Jun-Woo, et al., 2002; Rechciński and Kurpesa, 2005* who reported that the high fat diet results in subsequent production of systemic NO which did not damage the function of voltage dependent calcium channel and stimulation of endogenous nitric oxide formation increased nutritive muscular capillary blood flow and increased production of NO. In addition, the same data showing that activation of induced NOS (i-NOS) in cholesterol-fed rabbits had a stronger inhibitory effect on the progression of atherosclerosis. *Das et al., 1995* stated that the increased (i-NOS) activity may facilitate endothelium-dependent

smooth muscle relaxation through the intracellular second messenger cAMP, which in turn may increase vasa vasorum flow. *Augier, et al., 1996* suggested that the vasa vasorum flow contributes to the nutrition of the outer layers of the thoracic aorta, and its impairment induced structural changes of the aortic wall with deterioration of the elastic aortic properties, Thus, hawthorn intake may possibly restore impaired endothelium function by (i-NOS) activation and increased production of NO so, maintain the elastic properties of the aorta. These hawthorn-related effects might be mediated by (i-NOS) activation and subsequent restoration of impaired endothelium function. Thus, the effect of hawthorn in maintaining the elastic properties of the aorta provides insight into the mechanism or mechanisms of possible cardioprotective effect of hawthorn. (*Rechciński and Kurpesa, 2005*). Certainly, the chronic inhibition of NO production in the early stages of the disease has been shown to promote atherosclerosis (*Rechciński and Kurpesa, 2005*). *Jayalakshmi and Niranjali (2004)* stated that the *Crataegus* may increase inducible (i-NOS) staining which was observed in the intimae of atherosclerotic vessels obtained from long-term cholesterol-fed rabbits. When lesions were more advanced, i-NOS expression was more intense and diffused in the media, suggesting a link between the severity of the lesion and the (i-NOS) expression (*Jun-Woo, 2002; Rechciński and Kurpesa, 2005*).

The present study measured hepatic lipid peroxidation products (TBARS), and showed that they were increased 2 fold and 2.7 fold in the liver of HCF rabbits, indicating that high fat diet increased the lipid peroxidation while hawthorn administration lowering the lipid peroxide levels significantly. The present data confirmed by *Zhang, et al., 2001* who previously identified seven antioxidants in hawthorn mainly proanthocyanidin and flavonoids which very potent in protecting human LDL from oxidation and the protective activity of hawthorn fruit on the cardiovascular system may also be attributed to these antioxidants

because they reduce the production of free radicals, alleviate subsequent damage to the heart tissue and decrease deposition of oxidized LDL-C. *Bahorun, et al., 1996; Ljubuncic, et al., 2005* who stated that hawthorn fruit is not only hypolipidemic but also an excellent source of antioxidants so, it could scavenge hydrogen peroxide and superoxide species.

### Conclusion:

The concurrent inclusion of hawthorn fruit in diets may be an effective way of lowering serum cholesterol level and the accumulation of cholesteryl-esters in artery. Moreover, its hypolipidemic effect may have a protective mechanism against the development of atherosclerosis (*Vierling, et al., 2003*). Whether the hawthorn would have similar effects on hyperlipidemia associated with conditions other than high lipid diet feeding nevertheless, it appears to be a promising hypolipidemic agent in high lipid diet and has the advantage of being non-toxic. The Egyptian *Crataegus sinaicu* can be used for medicinal purposes as hypolipidemic and anti-atherosclerotic drug and can be added to the list of other *Crataegus* of North America, Europe and Asia (*Rietbrock, et al., 2001*).

**Table (1):** Effect of *Crataegus sinaica* administration in serum lipid components (mg/dl) of non-atherogenic rabbits (data were represented as mean  $\pm$  standard error)

Non atherogenic groups	treatment	TC	TG	PL	HDL	LDL	VLDL	TC/PL ratio	HDL %	NO ( $\mu\text{mol}$ )	Atherogenic index
NCF	Non-treated	88.9 $\pm 1.4$	54.4 $\pm 1.0$	110.2 $\pm 2.1$	32.9 $\pm 1.6$	42.6 $\pm 1.2$	13.5 $\pm 0.7$	0.81	58.7	20.3 $\pm 0.122$	1.705
NCFT	Treated	87.8 $\pm 1.2$	53.8 $\pm 1.3$	109.6 $\pm 1.2$	33.0 $\pm 1.9$	42.5 $\pm 1.8$	13.6 $\pm 0.4$	0.80	60.2	29.4 $\pm 0.14$	1.700

NS= Non-significant changes. \* $p \leq 0.01$  as compared to the corresponding control. TC (total cholesterol); TG (triacylglycerol); PL (phospholipids); HDL (high density lipoproteins); VLDL (very low density lipoproteins); NO (nitric oxide).



**Table(2):**Effect of *Crataegus sinaica* administration in serum lipid components (mg/dl) of atherogenic rabbits (data were represented as mean ± standard error).

Non atherogenic groups	treatment	TC	TG	PL	HDL	LDL	VLDL	TC/PL ratio	HDL %	NO (µmol)	Athero- genic index
HCF	Non-treated	544.9 ±14.8	218.2 ±10.7	275.4 ±8.6	179.3 ±4.2	412.2 ±6.2	53.4 ±2.2	1.97	49.0	80.3 + 0.18	2.596
HCFT	Treated	304.4* ±6.8	112.5* ±2.6	176.8* ±6.4	94.2* ±2.1	180.8* ±3.6	29.2* ±4.2	1.18*	44.8*	122.9 + 0.2*	2.230*

NS= Non-significant changes. \*p≤ 0.01 as compared to the corresponding control. TC(total cholesterol ; TG (triacylglycerol) ; PL (phospholipids); HDL (high density lipoproteins); VLDL (very low density lipoproteins) ; NO (nitric oxide).

**Table(3):**Effect of *Crataegus sinaica* administration in serum lipid composition of liver and heart tissues (mg/g) in non-atherogenic rabbits (data were represented as mean ± standard error).

Non atherogenic groups	Treatment	Liver				Heart		
		TC	TG	PL	TC/PL ratio	TC	TG	PL
NCF	Non-treated	5.50 ± 0.36	3.90 ±0.24	6.40 ±0.18	0.85	2.10 ± 0.10	3.40 ±0.20	5.20 ± 0.32
NCFT	Treated	5.50 ±0.37	3.90 ±0.23	6.38 ±0.15	0.85	2.11 ± 0.10	3.39 ±0.21	5.10 ±0.31

Non-significant changes. \* p≤ 0.01 as compared to the corresponding control. TC(total cholesterol ; TG (triacylglycerol) ; PL (phospholipids); HDL (high density lipoproteins); VLDL (very low density lipoproteins) ).

**Table(4):**Effect of *Crataegus sinaica* administration in serum lipid composition of liver and heart tissues (mg/g) wet in atherogenic rabbits (data were represented as mean ± standard error).

Non atherogenic groups	Treatment	Liver				Heart			
		TC	TG	PL	TC/PL ratio	TC	TG	PL	TC/PL ratio
HCF	Non-treated	16.20 ± 0.29	9.80 ±0.02	10.20 ±0.11	1.50	11.80 ± 0.31	7.20 ±0.48	8.40 ± 0.16	1.40
HCFT	Treated	6.60* ±0.24	4.60* ±0.14	6.20 ±0.16	1.06	7.20* ± 0.21	4.9* ±0.23	5.90 ±0.38	1.22

Non-significant changes. \* p≤ 0.01 as compared to the corresponding control. TC(total cholesterol ; TG (triacylglycerol) ; PL (phospholipids); HDL (high density lipoproteins); VLDL (very low density lipoproteins) ).

**Table (5):** Effect of *Crataegus sinaica* administration in lipid composition of aortic tissues (mg/g) in rabbits (data were represented as mean  $\pm$  standard error).

GROUPS	DIET	TREATMENT	$\mu\text{mol /g aortic tissue}$	
			TC	TG
NCF	Non-Atherogenic	Non-treated	1.5 $\pm$ 0.2	2.10 $\pm$ 0.21
NCFT	Non-Atherogenic	Treated	1.1 $\pm$ 0.3	3.30 $\pm$ 0.34
HCF	Atherogenic	Non-treated	28.3 $\pm$ 14.5	4.90 $\pm$ 0.30
HCFT	Atherogenic	Treated	13.9 $\pm$ 8.0*	2.70 $\pm$ 0.24

TC (total cholesterol) ; TG (triacylglycerol).

**Table (6):** Effect of *Crataegus sinaica* administration on hepatic TBARS in rabbits (data were represented as mean  $\pm$  standard error).

GROUPS	DIET	TREATMENT	TBARS
NCF	Non-Atherogenic	Non treated	1.16 + 0.11
NCFT	Non-Atherogenic	Treated	1.36 + 0.26
HCF	Atherogenic	Non treated	2.36 + 1.21*
HCFT	Atherogenic	Treated	1.27 + 1.62

**TBARS:** Thiobarbituric Acid Reactive Substances. \* = Significant at ( $p < 0.05$ ).

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التأثير العلاجي لثمرة نبات الزعرور البري السيناوى على  
أمراض الأوعية الدموية فى ذكور الأرانب

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منذ تم تصنيف نبات الزعرور البري السيناوى فى صحراء سيناء بواسطة عالم النبات المصري

أ.د. البتانوني بقسم تصنيف النباتات بالمركز القومي للبحوث بالدقي عام 1992 لم يجرى أية دراسات

على الأثر الخافض للبيبيدات على الحيوان أو الإنسان بالرغم من وجود كثير من البحوث المنشورة على

فصائل أخرى معينة من هذا النبات فى كل من أمريكا وأوروبا وشرق آسيا وفيها تم استخلاص المواد

الفعالة لثمرة النبات المماثل واستخدمت فى إنتاج دواء شائع الاستعمال لمرضى القلب غير موجود

بمصر ولما كانت بعض فصائل النبات ليس لها أثر فعال في تخفيض الدهون والقليل منها ذو أثر فعال ( حسب المراجع العلمية ) فكان الهدف من الدراسة الحالية هو تقدير التأثير الطبى الخافض للدهون للفصيلة الموجودة في مصر ... استخدمت 4 مجموعات من ذكور الأرناب ( 6 أرناب في المجموعة).

- 1- مجموعة ضابطة غير معالجة و تغذي بغذاء عادى خالى من الليبيدات .
- 2- مجموعة معالجة بمستخلص ثمرة نبات الزعرور بنسبة 2 جم / 100 جم علف و تغذي بنفس الغذاء العادى .
- 3- مجموعة غير معالجة ضابطة مغذاه بغذاء عادى الليبيدات.
- 4 - مجموعة معالجة بمستخلص ثمرة نبات الزعرور بنسبة 2 جم / 100 جم علف و تغذي بغذاء عادى الليبيدات. استغرقت عملية التغذية والمعالجة يوميا لمدة ستة أسابيع واستخدمت الجرعة الموصى بها في البحوث العالمية المنشورة. في نهاية مدة التجربة تم أخذ عينات سيرم الدم وأنسجة الكبد والقلب وتم عمل التحاليل الكيميائية.

#### أسفرت النتائج على ما يلى :-

- 1- أن ثمرة النبات لايؤثر معنويا مصل الدم وأنسجة الكبد والقلب من الليبيدات المختلفة في الأرناب التى تتغذى بغذاء عادى.
- 2- أن ثمرة النبات يؤثر معنويا بالخفض على مكونات مصل الدم والأنسجة من الليبيدات المختلفة (الكوليسترول و الجليسيريدات و الفسفوليبيد و VLDL و LDL و HDL cholesterol ) في الأرناب التى تتغذى على غذاء عادى الليبيدات وذلك بالمقارنة بالمجموعة التى تتغذى على غذاء عادى الليبيدات ولم تعالج بالمستخلص والتى ارتفعت فيها مستوى الليبيدات بصورة معنوية.
- 3 - زيادة معنوية لمادة اكسيد النيتروز التى تساعد فى انبساط و اتساع الأوعية الدموية فى المجموعة المعالجة (4) بالمقارنة بالمجموعة (3).
- 4 - نقص معنوى فى تركيز البيروكسيدات فى المجموعة المعالجة (4) بالمقارنة بالمجموعة (3)، مما يشير الى أن ثمرة نبات الزعرور لها تأثير مضاد للأكسدة.

الاستنتاج :-

- 1- أن ثمرة النبات المستجلب من صحراء سيناء ليس له تأثير وقائي على الأصحاء من الأرناب.
  - 2- أن ثمرة النبات فقط له تأثير معالج لأمراض الأوعية الدموية فى الأرناب و أن الفصيلة المصرية لها تأثير خافض للدهون و معالج لتصلب الشرايين.
- ولذلك يوصى باستخدامه للأرناب التى تعاني من أمراض الأوعية الدموية النزفية وأن تعمل شركات الأدوية في مصر على إنتاج مثل هذا الدواء من النبات المصري .