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BIOCHEMICAL STUDIES ON UTILIZATION OF *CRATAGUS SINAICA* IN PREVENTION OF ATHEROSCLEROSIS IN HYPERLIPIDEMIC MALE RABBITS

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ABSTRACT

The effect of Hawthorn fruit, *Cratagus sinaica*, that found in desert on normal and hyperchlosteremic male Newzeland rabbits for various lipid profile in serum, heart and liver were studied. Four groups of eight animals were each grouped: Group I: untreated control received normal non-atherogenic diet (ND) and was designated ND; Group II: Received normal non-atherogenic diet and were treated simultaneously with the tincture of *Cratagus* (TCR) as alcoholic extract (0.5 ml/100 g body weight per day for 6 weeks). This group was designated as ND+TCR group; Group III: Received atherogenic diet (AD) and designated AD group; Group IV: Received atherogenic diet (AD) and were treated simultaneously with the TCR- extract (0.5 ml/100 g body weight per day for 6 weeks) and was designated as AD+TCR group.The results indicated that: 1) Feeding of AD caused significant increase in lipid components of serum, liver, and heart; 2) TCR administration simultaneously with AD could prevent the rise in lipid levels in serum and tissues; 3) TCR administration and simultaneously with ND did not affect the normal lipid profiles.The levels of total serum cholesterol, triglycerides (TG), VLDL, and LDL-cholesterol which are actually raised in atherogenic diet, and can be lowered significantly with TCR of *Cratagus*. Moreover, its hypolipidemic effect may have a protective mechanism against the development of atherosclerosis. These results proved that the *Cratagus sinaica* arid zoon can be used for medicinal purposes as hypolipedemic drug, and can be added to the other previously known of *Cratagus* in North America, Europe and Asia.

INTRODUCTION

Hyperlipidemia is the major risk factor in the initiation and progression of the atherosclerotic lesions. Evidence from studies both the animals and humans indicates that progression can be slowed if elevated serum concentration of the atherogenic lipoprotein and triglycerides are reduced which in turn prevents coronary heart disease (Shanthi *et al.*, 1994 and Tankanow *et al.*, 2003). It is known that tincture of *Cratagus* (TCR), an alcoholic extract of the berries of hawthorn, *Cratagus oxyacantha, C. rosaceae,* is widely used today in herbal medicine as a heart tonic (Eric, 1976). Its main constituents are amines (β-phenylethylamine, tyramine, acetylcholine), triterpene saponins (oleanolic, ursolic, crataegolic acids), flavonoids and their glycosides (Quercetein, hyperoside, rutin, vitexine), monomeric catechins and oligomeric procyanidines (Totte and Vlientinck, 1986). TCR is used in the treatment of hypertension, angina pectoris, arthritis and rheumatism (Josie Holtmom and William Hylton, 1979). It has also been recognized as an antispasmodic and sedative treatment and it is used also in the treatment of incsomnial. Another interesting and valuable property of TCR is its ability to prevent arteriosclerosis (Cynthia, 1981 and Baughaman and Bradley, 2003). It is said to have a solubilising effect on the crustaceous and calcareous deposits in arteries (Cynthia, 1981 and William, 1997). This activity of TCR could in part be due to its possible beneficial effect on hyperlipidemia which is a major risk factor for atherosclerosis. There are more than 100 species of *Cratagus* in North America, Europe and Asia. However, only a few are used for medicinal purpose. These include (*Cratagus laevigata, Cratagus oxyacanthia, Cratagus monogyna* and less after *Cratagus pentagyes* (Wichtl, 1996). Systematic study on the anti-atherosclerotic or hypolipidemic action of *Cratagus sinaica* has not been conducted so far (Shanthi et al., 1994). Hence some preliminary studies on the effect of the hyperlipdemic action of the *Cratagus sinaica* fruit, collected from desert has been done.

This necessitate the determination of total cholesterol (TC) phospholipids (PL), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) in serum, besides total cholesterol (TC), triglycerides (TG) and phospholipids in liver and heart tissues.

MATERIALS AND METHODS

Experimental animals:

Male pure New Zealand rabbits aged 6-8 months and weighing 2.1-3.2 kg were used in the present study. They were divided into 4 groups each of eight animals.

ND-Group I: 8 male rabbits were fed normal diet (ND) composed of green grass, fresh lettuce, wheat bran, breed, and fresh carrots and used as control group.

ND+TCR Group II: 8 male rabbits were fed the normal diet (ND) and simultaneously with TCR of the *Cratagus sinaica* fruit (0.5 ml per 100 gm body weight per day for 6 weeks as indicated by Bajwa *et al.* (1971) and used by Santhi *et al.* (1994).

AD-Group III: 8 male rabbits were fed on atherogenic diet (AD) composed of sucrose (61%) casein (24%), saturated fat (10%), cholic acid (0.5%), vit. D_2 (1.25 million USP units of vitamins per kg diet, cholesterol (1.5%) and salt (4%).

AD+TCR Group IV: 8 male rabbits were fed on atherogenic (AD), composed of sucrose (61%), casein (24%), saturated fat (10%), cholesterol (1.5%), cholic acid (0.5%), vitamin D_2 (1.25 million USP units per kg diet, salt mixture (4%) as reported by Bajwa *et al.* (1971) and used by Shanthi *et al.* (1994), simultaneously with TCR of the *Cratagus* sp. (as in group II) daily for 6 weeks.

Preparation of tincture of *Cratagus sinaica* (TCR) :

This was done according to Shanthi *et al.* (1994). The alcoholic extract of the berries of *Cratagus sinaica* was prepared by mixing 100 gm fresh pulp of ripe berries of the hawthorn (*Cratagus sinaica*) with 635 ml of ethyl alcohol. This is then made up to one liter with distilled water. This extract is further diluted taking 1 part of the extract with 4 parts of distilled water and 5 parts of alcohol.

At the end of 6 weeks, the rabbits were anaesthetized by ether, blood was directly collected from the heart without any anticoagulant and allowed to clot at room temperature for 10 min. Centrifuged at 1000 r.p.m. for 10 min, the serum was kept at 4 °C until used. VLDL and LDL (Friedewald *et al.*, 1992) and total cholesterol (Zlaktis *et al.*, 1953), triglycerides (Gottfried and Rosberg, 1993), phospholipids (Zilversant and Davis, 1950) and HDL cholesterol (Burstein *et al.*, 1970) were analyzed from the serum. Lipid

TABLE 1

Effect of *Cratagus sinaica tincture* (TCR) extract from *Cratagus Sinaica o*n serum lipid composition (mg/dl) of both normal and atherogenic male rabbits

Animal Groups* I - IV	Diet	TC	TG	PL	HDL	LDL	VLDL	TC/ PL Ratio	HDL (%)	Atherogenic Index
1. Effect of TCR on Normal Rabbits										
I (Control)	ND	88.9 ± 1.4	54.4 ± 1.0	110.2 ± 2.2	32.9 ± 1.6	42.6 ± 1.2	13.5 ± 0.7	0.81	58.7	1.705
II	ND + TCR	87.8 ± 1.2	53.8 ± 1.3	109.6 ± 1.2	33.0 ± 1.9	42.5 ± 1.8	13.6 ± 0.4	0.80	60.2	1.700
2. Effect of TCR on Atherogenic Rabbits										
III (Control)	AD	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	2.597
IV	AD + TCR	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*	2.230*

*Group I represented animals fed normal diet (ND); Group II fed ND and treated simultaneously with tincture of *Cratagus* fruit extract (TCR) (0.5 ml/ 100 gm b.w. per day for 6 weeks); Group III were fed AD, and Group IV were fed AD + TCR.

*$P \leq 0.01$ as compared to the corresponding control.

TC= Total cholesterol; TG= Triacylglycerides: PL= Phospholipids; HDL= High density lipoproteins; LDL= Low density lipoproteins; VLDL= Very low density lipoproteins: TC/PL; HDL % and atherogenic index (see page 5).

extraction of liver and heart muscle were performed according to the method of Folch *et al.* (1957) band total cholesterol (Zlaktis *et al.*, 1953), triglycerides (Gottfried and Rosberg, 1993) and phospholipids (Zilversant and Davis, 1950) were determined in the lipid extracts of tissues of liver and heart.

Statistical evaluation of the analytical data was done using Student's t-test and P value of < 0.05 was considered to be significant (Snedecor and Cochran, 1970 and Kulkami, 1994).

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

$$HDL\ (\%) = \frac{HDL}{Total\ Cholestrol - HDL} \times 100$$

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

$$Atherogenic\ Index = \frac{LDL + VLDL}{HDL}$$

RESULTS

The values for the control (ND- group I) and the ND + TCR (Group II) were the same (Table 1). This indicated that TCR did not affect the lipid components of serum, liver and heart of normal animals. ND- control (group I) when compared with the high lipid fed group (AD) (atherogenic) animals (group III), indicates that feeding of high lipid diet caused significant increase in lipid components of serum, liver and heart (Table 2). ND- control (group I) when compared with the high lipid fed AD + TCR (group IV) indicated that TCR administration prevent the rise in serum lipids as well as the lipid depositive in liver and heart. Drastic increase in cholesterol triglyceride and phospholipids concentrations of lipoprotein fractions especially LDL and VLDL was observed in high lipid fed rabbits, i.e. the atherogenic rate also increased due to high lipid diet. TCR could prevent the increase of the atherogenic index.

DISCUSSION

The present study shows that TCR prevents hypercholesterolemia. High fat diet in combination with cholesterol feeding raised the serum cholesterol, LDL and VLDL cholesterol. After treatment with TCR all the

TABLE 2

Effect of *Cratagus sinaica tincture* (TCR) extract on lipid composition of liver and heart tissues (mg/g) in both normal and atherogenic male rabbits

Animal Groups* I - IV	Diet	Liver				Heart			
		TC	TG	PL	TC/ PL Ratio	TC	TG	PL	TC/ PL Ratio
1. Effect of TCR on Normal Rabbits									
I (Control)	ND	5.5 ± 0.36	3.90 ± 0.24	6.40 ± 0.18	0.85	2.10 ± 0.1	3.40 ± 0.20	5.20 ± 0.32	0.40
II	ND + TCR	5.5 ± 0.37	3.90 ± 0.23	6.38 ± 0.15	0.85	2.11 ± 0.1	3.39 ± 0.21	5.10 ± 0.31	0.40
2. Effect of TCR on Atherogenic Rabbits									
III (Control)	AD	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
IV	AD + TCR	6.60 ± 0.24*	4.60 ± 0.14*	6.20 ± 0.16	1.06	7.20 ± 0.21*	4.90 ± 0.23*	5.90 ± 0.38*	1.22

*$P \leq 0.01$ as compared to the corresponding control group mean (± SEM). *Abbreviations: see details as Table 1.

above parameters were lowered. This could be possibly due to an increase in the liver-LDL receptor activity (Brown and Goldstein, 1983) and decreased hepatic triglycerides synthesis (Wong *et al.*, 1984). It was further shown that high C/P ratio decreased significantly in contrast to an increase in HDL ratio after treatment with TCR in rabbits fed with atherogenic diet. High C/P ratios are usually associated with atherosclerosis (Sharma *et al.*, 1991 and Sharma and Dixit, 1995).

TCR 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 associated also in metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose tolerance (Lees and Lees, 1976). TCR reduced triglycerides in rabbits fed with atherogenic diet and may prevent the progression of atherosclerosis (Ginsberg, 1994 and Austin and Hokanson, 1994) and complication due to hypertriglyceridemia.

Our studies confirm the hypolipidemic activity of TCR. The effective lowering of the atherogenic index shows that *Cratagus sinaica* is a good hypocholesterolemic agent in rabbits fed on HLD. This effect of TCR is probably due to the presence of triterpene-saponins and catechins which are hypocholesterolemic. Tea catechins were found to decrease total and esterified cholesterol and the atherogenic index in cholesterol fed rats (Muramatsu *et al.*, 1986). Saponins from various plant sources are reported to be hypochlosterolemic (Sauvaire *et al.*, 1991). Quercetin, a flavonoid present could be contribute to the hypocholesterolemic and hypotriglyceridemic effects (Kato *et al.*, 1983).

The effect of TCR on phospholipids of plasma as well as the tissues is probably due to the other flavonoids and procyanidines which in general may have hypolipidemic properties (Beretz and Cazenave, 1988). However, complete prevention of lipid accumulation in the tissues may not be expected in the presence of constant exogenous lipid stimuli.

The hypolipidemic action of TCR can be related to some biological effects of its various constituents. The synergistic action of these constituents is probably responsible for the observed hypolipidemic activity. It is not known whether TCR would have similar effects on hyperlipidemia associated with conditions other than high lipid diet feeding. Nevertheless, TCR appears to be a promising hypolipidemic agent in high lipid diet. It also has the

advantage of being non-toxic even on hypolipidemics which have undesirable side effects
(Malinow, 1986). However, further studies on the possible mode of action of TCR are warranted.It can be concluded from the data that the levels of total serum cholesterol, triglycerides, VLDL and LDL cholesterol which are actually raised in atherogenic diet, can be lowered significantly with concurrent feeding of TCR. Moreover, its hypolipidemic effect may have a protective mechanism against the development of atherosclerosis (Lewis *et al.*, 1974). TCR can be utilized for providing dietary management in the prevention of atherosclerosis in hyperlipidemic patients. The *Cratagus sinaica* can used for medicinal purposes as hypolipidemic drug and can be added to the list of other *Cratagus* of North America, Europe and Asia.

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التأثير الخافض لمحتوى الليبيدات للمستخلص الكحولى لثمرة الزعرور البرى فى مصل الدم ، وأنسجة الكبد والقلب فى ذكور الأرانب

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لـم تجـرى أية دراسات على الأثر الخافض لليبيدات على الحيوان أو الإنسان بالنسـبة لنبات الزعرور البرى الصحراوى بالرغم أن هناك كثير من البحوث المنشورة علـى فصـائل أخـرى معينة من هذا النبات فى كل من أمريكا ، أوربا ، وشرق آسيا. وفـيها تم إستخلاص المواد الفعالة لثمرة النبات المماثل واستخدمت ف إنتاج دواء شائع الإسـتعمال لمرضـى القلـب. ولمـا كانت بعض فصائل النبات ليس لها أثر فعال فى تخفـيض الدهـون والقلـيل منها ذو أثر فعال (حسب المراجع العلمية)فكان الهدف من الدراسـة الحالـية هو هل الفصيلة الموجودة فى صحارينا ذات تأثير خافض للدهون أم لا ؟

وقـد أسـتخدمت أربعـة مجموعـات مـن ذكـور الأرانب (ثمانية أرانب فى المجموعة) :

تجـربة أولـى : مجموعـة ضابطة غير معالجة Non-Atherogenic Diet غذيت بغذاء عادى ، ومجموعة معالجة بمستخلص نبات الزعرور وغذيت بنفس الغذاء العادى

- أن المستخلص الكحولى لثمرة نبات الزعرور يؤثر معنوياً بالخفض على مكونات مصل الدم والأنسجة من الدهون المختلفة فى الأرانب التى تتغذى على Atherogenic Diet وذلك بالمقارنة بالمجموعة Atherogenic والتى لم تعالج بالمستخلص والتى أرتفعت فيها مستوى الليبيدات.
- المستخلص الكحولى لثمرة نبات الزعرور المستجلب من الصحراء ليس له تأثير وقائى على الأصحاء من الأرانب Non-Atherogenic
- المستخلص الكحولى لثمرة نبات الزعرور فقط له تأثير خافض على الأرانب Atherogenic ، ولذك يوصى باستخدامه للمرضى فقط ، وأن تعمل شركات الأدوية على أنتاج مثل هذا الدواء من النبات النامى فى صحارينا.