The Anti-atherogenic Activity of Artemisia, Thyme & Soya bean

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Background: Atherosclerosis is a focal disease of the antima of large and medium sized arteries characterized by thickened and harden lesions with possible correlation to medicinal plants in reducing arthrogenesis.

Methods: This study was carried out in Al-Kadhmiya teaching hospital between January 20١1 to May 20١1 on 60 healthy males who were allocated to five groups; each group was given one of the following agents:
Artemisia, thyme, soya bean, Simvastatin and normal distilled water. Clinical manifestations (radial pulsation and arterial blood pressure), lipid profiles (serum cholesterol, triglycerides, HDL, LDL & VLDL) free radicals, platelets function tests are the parameters used in this study.

Results: All the tested agents have anti-arthrogenic activity. They reduce both the serum cholesterol levels & total blood free radicals. The platelet count significantly changed by thyme & soya bean at the meantime the mean arterial pressure significantly decreased by Thyme when these agents used before & after treatment for 15 days.

Conclusion: Artemisia, Thyme & Soya bean have anti-arthrogenic activity and the possibility to be used against atherosclerosis since they are accessible, safe and favorable additives.

Keywords: Medicinal plants, Atherosclerosis, free radicals & platelets function tests.

الفاعلية المضادة للتعصيد لكل من الزنجبيل والزعتر وفول الصويا الخلاصة

خلفية الدراسة: التصلب العصيدي هو اختلال التهابي متقدم لجدار الشريان ومن الممكن ان يتسبب في حصول انسداد خثري او انصمام قصوى للشريان.

الطرق: لقد اختبرت نباتات الزنجبيل، الزعتر وفول الصويا على (٦٠) متطوعا سليما من الذكور في مستشفى الكاظمية التعليمي للفترة من كانون الثاني ٢٠١١ وحتى مايس ٢٠١١ حيث توزعوا على خمسة مجاميع بضمنها مجموعتي السيطرة والسيمفستاتين، ولقد اعتمدت المؤشرات التالية للتقييم: مظاهر سريرية كفرط الضغط، الصورة الجانبية للدهون، الجذور الحرة الكلية للدم، فحوصات وظائف الصفائح الدموية.

النتائج: تمتلك كل النباتات المختبرة فعالية مضادة للتعصيد ولها تاثيرا ملحوضا في تقليل مستوى الكولستيرول والجذور الحرة في الدم مع تغيير لتعداد الصفائح الدموية بشكل ملحوظ باستعمال الزعتر وفول الصويا. كما ان ضغط الدم الشرياني انخفض بشكل ملحوظ باستعمال الزعتر قبل وبعد العلاج لمدة ١٥ يوم.

الاستنتاج: يمتلك كل من الزنجبيل والزعتر وفول الصويا فاعلية مضادة للتعصد ويمكن استعمالها للوقاية ولعلاج التصلب العصيدي لكونها امينة، زهيدة الثمن ومكملات مفضلة للغذاء.

مفتاح الكلمات: النباتات الطبية، تصلب الشرايين، الجذور الحرة، وظائف الصفائح الدموية.

Introduction

Atherosclerosis is a progressive inflammatory disorder of the arterial wall that is characterized by focal lipid rich deposits of atheroma that remain clinically silent until ulceration & disruption of the lesion result in thrombotic occlusion or distal embolisation of the vessel [(1)]. It is ubiquitous and underlies the commonest causes of death (myocardial infarction, stroke, arrhythmia & heart failure) [(2)].

Many risk factors can predispose for development and progress of atherogenesis, these include aging, family history, hypercholesterolaemia, obesity, hypertension, diabetes mellitus, cigarette smoking [(3)], alcohol, vitamins deficiency socioeconomic status and hypothyroidism [(4)]. In addition to raised C - reactive protein, homocysteine, or coagulation factors (e.g. factor VII & fibrinogen) [(5)].

The pathophysiology of the disease is of chronic inflammation in response to injury. Endothelial dysfunction (due to altered PGI_2 & NO biosynthesis) lead to loss of protective mechanisms: monocyte / macrophage and T- cell migration, uptake of LDL-c & its oxidation, uptake of oxidized LDL by macrophages, smooth muscle cell migration and proliferation and deposition of collagen. Plaque rupture lead to platelet activation and thrombosis [(5)]. It is well documented that administration of the aqueous extract of plants such as Allium sativa, Medicago sativa & Trigonella foenum graecum have been shown to reduce hypercholesterolaemia in diabetes induced rabbit model [(6)], also it has been reported that Artemisia absinthium (ginger), Thymus vulgaris (thyme) & Soya bean have significant serum cholesterol lowering effect in atherosclerosis induced rabbit model [(7)].

The current study was performed to investigate the antiatherogenic activity of artemisia, thyme & soya bean in comparison with Simvastatin in normal subjects in order to confirm their significant clinical benefit.

Subjects & Methods

The study was performed in Al-Kadhmiya teaching hospital between January 20١١ to May 20١١. Sixty healthy males participated in this study. Their age was 30-35 years with 62-67 Kg body weight. They have no history of chronic disease or any surgical problem with no family history of hyperlipidaemia.

The present study was approved by ethic committee of college of medicine. The subjects had been informed about the procedure and the purpose of this study & they were examined after an over night fasting to limit their food & drugs. The normal subjects were randomly allocated to five groups (each group contained 12 volunteers). All the drugs were given orally at 9:00 a.m.

Group 1 (normal control): They were given 100 ml of distilled water once daily for 15 days.

Group 2 (treated control): They were given Simvastatin 20 mg in 100 ml of water once daily for 15 days.

Group 3: They were given 5 grams of dried powdered of artemisia in 100 ml of water once daily for 15 days.

Group 4: They were given 5 grams of dried powdered of thyme in 100 ml of water once daily for 15 days.

Group 5: They were given 10 ml of purified soya bean oil with 90 ml of water once daily for 15 days.

Blood pressure was achieved simply by taking the sitting posture using conventional mercurial column sphygmomanometer both at 8:00 am and 10:00 am every 72 hours for 15 days. The mean arterial pressure was then documented before and after the treatment. Human radial pulsation was used as indicator that reflects valuable information regarding cardiac pulsation and vascular status.

Blood samples were taken at 8:00 am & 10:00 am every 72 hours for 15 days to measure platelet count by using Rees-Ecker diluting fluid and platelet adhesion test (in vivo) by using IVY's procedure [8] and also to measure the biochemical changes of serum cholesterol, triglyceride, HDL, LDL & VLDL by using kits of Reflotron type (Roche Diagnostic Ltd. Bell lane BN7 ILG Lewes E. Sussex GB) and Reflotron spectrophotometer. The evaluation of total free radicals (in vivo) was done by highly sensitive amperometer (1 microampere / small degree) KI solution and platinum electrode.

All plants have been identified by Iraqi national center of herbs. The dried aerial parts of artemisia & dried leaves of thyme were used. Plants were cleaned to remove abnormal materials & then crushed & grounded to be used as powder. Soya bean oil was prepared from the seeds by traditional oil extractor. All the results were coded & analyzed by using the regression coefficient test with significance at $p < 0.05$.

Results

The obtained results of the tested agents revealed a significant lowering effect of arterial blood pressure with thyme at the first day & 15th day after the treatment with insignificant reduction of arterial blood pressure of Simvastatin & soya bean (table 1).

The obtained results showed a significant decrease in the platelet count of the thyme, soya bean with no significant reduction of PAT% (table 2). All the results of serum cholesterol of artemisia, thyme, soya bean & Simvastatin were significantly reduced at the mean time, the serum triglycerides, LDL & VLDL of soya bean were significantly decreased (tables 4&5). The total blood free radicals of artemisia, thyme & soya bean were highly significant compared with Simvastatin (table 3).

Discussion

Atherosclerosis is a focal disease of the intima of large & medium sized arteries, characterized by thickened & hardened lesions [(5)]. The clinical manifestations, lipid profiles, free radicals & platelet function tests were the main parameters used to evaluate the anti-arthrogenic activity of tested agents in comparison with Simvastatin. The effect of distilled water (group 1) had no change in all the parameters used in this study. Distilled water was used instead of saline to avoid influence on arterial blood pressure & arthrogenesis since hypernatremia is an atherogenic factor [(9)]. Atherosclerosis is a causative factor in hypertension; this fact can explain that blood pressure lowering effect of an agent is a good causative criterion.

Simvastatin is a potent inhibitor of endogenous cholesterol synthesis (group 2). It acts through inhibiting HMG- reductase activity so it is widely used for treating hyperlipidaemia.

Simvastatin improves the endothelial function, decreases inflammation at site of sclerosis & inhibits platelet aggregation [(10)]. It is beneficial agent in comparing the results of tested agents with its results. Similarly, platelet count & adhesiveness revealed no significant correlation with Simvastatin treatment both in human & rabbit models [(7)].

The effect of artemisia (group 3) exhibited variable cardiovascular & metabolic effects. A marked platelet aggregation inhibition had

been observed by others [11] which was similar to our study in rabbit [7], on the other hand a polysaccharide fraction of the leaves of artemisia may cause a selective activation of heparin co-factor II [12]. Blood glucose was significantly lowered with administration of artemisia extract in Alloxan induced diabetic rat

model [13] a mechanism that is related indirectly to lipid profile lowering effect since blood sugar level correlated positively with hypercholesterolaemia.

Thyme contains resin (group 4) & it's highly potent antioxidant agent [14]. The useful antioxidant property of thymol had agreed with free radical results in our study since they have a highly significant role in pathogenesis of atherosclerosis. The current results showed a significant antihypertensive, lipid lowering, free radical effects which are compatible to the results obtained in rabbit model [7].

Soya bean is a chemical analogue of cholesterol (group 5). Its oil is rich with phytosterol [15] also it is a source of decosahexanoic acid which may have a role in hypertension, heart attack and platelet aggregation & probably decrease the severity of atherosclerosis [16].

Finally, the medicinal plants such as thyme, soya bean & artemisia showed anti-atherogenic activity & the possibility to be used as prophylactic or therapeutic agents against atherosclerosis since they are accessible, cheap, safe & favorable additives.

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Groups	Acute use		Chronic use	
	Before treatment	2h after treatment	Before treatment at first day	After treatment of first day
Normal control (distilled water)	90 ± 2.0	88 ± 5.0	90 ± 1.0	90 ± 3.0
Simvastatin	95 ± 1.0	95 ± 2.0	95 ± 1.0	90 ± 3.0
artemisia	92 ± 3.0	90 ± 3.0	92 ± 1.0	93 ± 2.0
thyme	97 ± 1.0	* 73 ± 5.0	97 ± 1.0	* 77 ± 3.0
Soya bean	95 ± 2.0	95 ± 2.0	95 ± 2.0	90 ± 2.0

* Results were significant at (P<0.05).

Table 2: The platelet count reducing effect of the tested agents & their platelet adhesion test PAT % (*in vivo*).

Groups	Platelet count 1000 per cubic mm		PAT % mean
	Before treatment at 1st day	2h after treatment at 15th day	
Normal control	198 ± 6.0	202 ± 5.0	45 ± 3.0
Simvastatin	220 ± 11.0	223 ± 6.0	20 ± 3.0
artemisia	193 ± 4.0	212 ± 9.0	18 ± 2.0
thyme	218 ± 6.0	*145 ± 3.0	15 ± 3.0
Soya bean	234 ± 11.0	*143 ± 5.0	23 ± 5.0

* Results were significant at (P<0.05).

Table 3: The mean of total blood free radicals in micro mol/L after administration of the tested agents.

Groups	Free radicals micromole/L 1st day before treatment	Free radicals micromole/L at 1st day after treatment
Normal control	**43.2 ± 10.2**	**41.6 ± 3.7**
Simvastatin	**50.8 ± 3.8**	***48.0 ± 1.9**
artemisia	**46.6 ± 8.5**	***41.2 ± 10.1**
thyme	**48.5 ± 11.0**	***31.0 ± 6.4**
Soya bean	**44.4 ± 12.6**	***30.9 ± 3.8**

* Results were significant at ($P<0.01$).

Table 4: Effect of the tested agents on serum cholesterol, triglyceride & HDL in mg/dl during 15 days of treatment.

Groups	Serum cholesterol conc. (mg/dl)						Serum triglyceride conc. (mg/dl)						Serum HDL conc. (mg/dl)					
	1st day	3rd day	6th day	9th day	12th day	15th day	1st day	3rd day	6th day	9th day	12th day	15th day	1st day	3rd day	6th day	9th day	12th day	15th day
Normal control	132 ± 4	135 ± 2	136 ± 3	130 ± 1	134 ± 5	136 ± 2	126 ± 4	125 ± 3	130 ± 2	127 ± 3	130 ± 5	136 ± 2	25	25	30	27	30	29
Simvastatin	139 ± 4	138 ± 3	134 ± 2	128 ± 4	121 ± 2	*120 ± 1	171 ± 3	169 ± 2	170 ± 1	170 ± 1	171 ± 1	175 ± 2	28	27	30	28	29	29
artemisia	151 ± 3	148 ± 3	144 ± 3	130 ± 2	135 ± 5	*133 ± 4	141 ± 3	140 ± 2	138 ± 5	135 ± 3	135 ± 4	133 ± 3	30	29	29	30	31	31
thyme	146 ± 1	146 ± 1	135 ± 1	110 ± 3	105 ± 1	*100 ± 2	166 ± 2	164 ± 4	165 ± 1	175 ± 3	175 ± 2	168 ± 3	26	24	25	27	25	26
Soya bean	156 ± 3	150 ± 3	142 ± 2	138 ± 2	132 ± 3	*120 ± 1	150 ± 3	146 ± 3	145 ± 4	138 ± 2	138 ± 2	*130 ± 6	27	27	28	29	29	30

* Results were significant at ($P<0.05$).

Table 5: Effect of the tested agents on LDL & VLDL in mg/dl during 15 days of treatment.

Groups	Serum LDL concentration (mg/dl)						Serum VLDL concentration (mg/dl)	
	1st day	3rd day	6th day	9th day	12th day	15th day	Before treatment At 1st day	After treatment At 15th day
Normal control	78	75	77	77	78	79	25.2 ± 4.0	27.2 ± 5.1
Simvastatin	76	77	75	74	73	73	34.2 ± 5.1	35 ± 3.5
artemisia	77	76	77	76	76	76	28.2 ± 4.1	26.6 ± 4.3
thyme	75	75	74	74	75	* 74	33.2 ± 1.7	33.6 ± 3.1
Soya bean	76	73	72	70	69	67	29.2 ± 3.3	* 25 ± 4.1

* Results were significant at ($P<0.05$).