Herb "*Asparagus curillus*" Effecting Matrix Metalloproteinases Plasma Levels in Patients with Coronary Artery Disease

Yogesh Bansal, Manveen Kaur Lall

Abstract— Asparagus species has been well known for their antioxidant, anti inflammatory and anti tumor activity. However anti atherosclerotic activity of *Asparagus curillus* extract has not been studied.The study group included 63 persons with angio-graphically verified CAD and 21 persons as healthy control. Circulating MMPs, TIMP-1, C-reactive protein and interleukins concentrations were measured by ELISA. Leucocyte subtype counts were determined in whole blood. Differences in continuous variables b/w were tested by ANOVA and correlations were analyzed by linear regression method. MMPs and TIMP-1 concentration (markers of CAD) appeared to be differentially regulated by *Asparagus curillus* extract. The plasma concentration of MMP-2 was increased in patients (Stable and Unstable CAD) having AC root extract as dietary supplement. No significant increase in plasma concentration of TIMP-1 was observed in patients (Stable and Unstable CAD) having AC root extract as dietary supplement as compare to control group ($p < 0.05$ or 0.001). Therefore AC root extract as dietary supplement showed a direct correlation with plasma concentration of MMP-2, MMP-3 and TIMP-1.MMPs and TIMP-1 concentration (markers of CAD) appeared to be differentially regulated by *Asparagus curillus* extract.

Index Terms— Asparagus, Metalloproteinase, Coronary artory disease, Anti inflammatory effect.

1 INTRODUCTION

Matrix metalloproteinase (MMPs) are proteolytic, zinc dependent enzymes involved in degradation of extracellular matrix like collagen, proteoglycan and elastin (Coker et al 1999 and Visse at al 2003). These MMPs are tightly regulated at several possible levels including transcription, zymogen activation, MMP interaction and inhibition by TIMP (Tissue inhibitor of metalloproteinase -1) (Creemers et al 2001 and Noji et al 2001). MMPs have been involved in many cellular processes, such as smooth muscle cell migration, release of growth factor, embryonic development, wound healing, angiogenesis and ECM degradation, processes that may have different effects on cardiovascular disease (Visse et al 2003 and Sang-Beom 2012). It is reported that the plasma concentration of metalloproteinase (MMP) like MMP-2, MMP-3, MMP-7 provides valuable information in patients with Coronary Artery Disease (CAD) and concentrations of MMP-2 and MMP-9 (both gelatinases) are beneficial in non-viral heart disease (Westermann et al 2011, Wilson et al 2002 and Yamazaki et al 2004).

Serum containing growth factors and inflammatory molecules, both modulates the secretion of various MMPs and TIMPs from fibroblast, smooth muscle and endothelial cells. MMPs are mostly activated by cysteine-switch (Tyagi et al 1993) and thereby promoting atherogenesis, plaque rapture and thrombosis. In coronary heart disease (CHD), serum concentration may reflect MMPs activity within the vessel wall (Bergman et al 2007 and Cheung et al 2000). A few studies have analyzed serum or plasma MMPs-2, 3 & 7 concentrations in relation to CAD. Patients with premature stable CAD, the plasma concentration of MMP-2 and MMP-3 were decreased; however MMP-2 mediates (by negative feedback mechanism) chemokine cleavage that has an important role in cardiac inflammation (George et al 2005). Concentration of MMP-2 in stable CAD and unstable CAD is conflicting, it has been shown that serum MMP-2 is increased in patient with stable angina compared with control. In other MMPs like 7 & 9 are produced in vulnerable region of atherosclerotic plaque and MMP-7 concentration is increased in patients with stable and unstable CAD (Nilsson et al 2006).

The screening of plants extracts and natural products for anti CAD activity has revealed the potential of higher plants as a source of new anti CAD agents. Herbal plant "*Asparagus curillus*" (AC) belongs to Liliaceae family. This plant is a great source of Vit-B_6 ,Ca, Mg, Zn, Vit-A, Vit- C, Vit-E , Vit-K, Thiamine, Riboflavine and Ph, Fe, K, Mn, Se and Cr as trace elements (Jagmohan et al 2010). Recently, compounds like spirotanol, furostanol glycosides, saponines, glucopyranoside and oligofurostanosides are isolated from AC (Sharma et al 1982 and 1993) . Nutritional studies have shown that six spears of Asparagus species contain 135µg of folate i.e. almost half of RDI (Recommended daily intake) and 20µg of K^+. Another report on folate showed its key role in homocysteine metabolism (Biomolecule implicated in heart disease).The root extract of AC possesses antioxidant and anti-inflammatory properties. Although, above herb has many useful claims. But the mechanisms of its medicinal effects are not understood.

So, the objectives of this study are to evaluate the plasma con-

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centrations MMPs like 2,3 & 7 together with their inhibitor TIMP-1 in patients with CAD and in patients of CAD administered with AC root extract whether as these concentrations correlates with those of CRP, cytokines and immune cells.

2 Materials and Methods

2.1 Botanical Saterial

The dried roots of *Asparagus curillus* (BSD 112754) were collected from forest region of Gupt kashi (Uttarakhand, INDIA) and identified in Botanical Survey of INDIA, Dehradun.

2.2 Extraction of Plant Material

This herbal plant material taken from for the study was under refrigerated condition till use. The sample was prepared by grinding equal amount of fresh roots in water (wt. /vol.) in pre chilled pestle and mortar. The extract thus obtained was provided to the patients for oral administration (20µl/Kg/Day) for three months.

2.3 Subjects and Clinical Course

We studied a total of 63 persons (38 males and 25 females) with angiographically verified CAD and 21 persons as healthy controls. The patients with stable CAD had effort-related angina without any worsening of symptoms in the past 6 months. Exclusion criteria were age >60 years diabetes, sever heart failure, immunologic disorder, cancer, surgery and treatment of immunosuppressive or anti-inflammatory agents. Blood was obtained by venipuncture in the morning after 12 h fast. The research protocol was approved by the local ethics committee "DAV © Dental College and Hospital".

2.4 Collection of Blood Sample and Estimations of MMPs

Blood samples were collected in Vacutainer Tubes with or without EDTA and centrifuged within 15 min to separate plasma/serum. Total serum cholesterol, high density lipoprotein (HDL) cholesterol and serum triglyceride were determined by Roche Diagnostics Kits used in Roche Modular P-900. Plasma CRP was determined by highly sensitive latex-enhanced turbidimetric immunoassay (Roche Diagnostics). The MMPs and TIMP-1 were measured with the Biotrak MMP-2, MMP-3, MMP-7 and TIMP-1 human ELISA system (Amersham Biosciences).We determined the distribution of peripheral blood mononuclear cells by Beckton Dickinson FACS cell sorter located in P.G.I.M.E.R., Chandigarh, INDIA. Data were analyzed by using CELL Quest software (Becton Dickinson).

3 Results

Mean age of study patients was 67±13 years. Thirty eight patients were males excluding healthy control i.e. (61%). All the patients were receiving beta blockers digioxin, calcium antagonists and nitrates. In addition, all the patients with UCAD were receiving angiotensin converting enzymes inhibitors or low molecular wt. heparin.

Serum TG, cholesterol, LDL and HDL levels were very high in CAD and UCAD patients as compare to healthy control (Data shown in Table 1). However, values of above parameters were significantly decreased in CAD patients with AC extract (CAD-AC) and UCAD patients with AC extract (UCAD-AC) as compare to patients without AC root extract as dietary supplement.

TABLE 1- Comparison between Lipid profile of Patients (CAD and UCAD) with and without root extract of *Asparagus curillus* as dietary supplement.

	Healthy control		Patients without *Asparagus curillus* root extract as a dietary supplement				Patients with *Asparagus curillus* root extract as a dietary supplement			
			Stable CAD (CAD)		Unstable CAD (UCAD)		Stable CAD (AC-CAD)		Unstable CAD (AC-UCAD)	
Number of patients	21		23		12		13		15	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	13	8	14	9	8	4	7	6	9	6
Mean (SD) S/TG 25-160mg/dL	90 (25)	80(15)	180(20)	170(15)	300(18)	265(12)	160 (10)	155 (15)	200 (18)	190 (12)
Mean (SD) S/Cholesterol <200 mg/dL	160(25)	154 (38)	240 (32)	220 (15)	350 (25)	320 (15)	180 (15)	170 (18)	260 (20)	210 (20)
Mean (SD) LDL < 150 mg/dL	75 (15)	80(16)	150 (12)	155 (10)	260 (25)	240 (10)	100 (10)	95 (10)	180 (20)	130 (30)
Mean (SD) HDL 30- 65 mg/dL	42 (8)	38 (8)	33 (6)	31 (8)	25 (5)	24 (6)	45 (5)	42 (8)	37 (6)	35 (7)

P<0.05compared to control. All values are expressed as mean± SD

Table 2- Basic characteristics of DLC (Differential leukocyte count) in patients with and without *Asparagus curillus* root extract as dietary supplement

	Healthy control	Patients without *Asparagus curillus* root extract as a dietary supplement		Patients with *Asparagus curillus* root extract as a dietary supplement	
		Stable CAD (CAD)	Unstable CAD (UCAD)	Stable CAD (AC-CAD)	Unstable CAD (AC-UCAD)
Mean (SD) Total Leukocyte count	3400 (800)	6800(1000)	7900 (1000)	6000 (800)	7100 (1000)
Mean (SD) Lymphocytes	1100 (500)	1800 (600)	2500(800)	1400 (500)	1500(300)
Mean (SD) Monocytes	400(100)	700(100)	1200(200)	800 (300)	1000 (200)
Mean (SD) Neutriphils	1800 (400)	4700 (1000)	5500(800)	3500 (500)	4200 (1200)

Values are expressed as mean ± SD, *p<0.05.

Patients with UCAD had higher total leukocyte counts than stable CAD and control (Table 2). This difference was accounted for by higher monocytes and neutrophilic granulocyte counts. On comparing UCAD and UCAD-AC, the level of total leukocyte was significantly reduced. On the other hand, CAD and CAD-AC, the reduction in monocytes was not very significant. So it has been inferred from the table 2 that total leukocyte count and neutrophil count were reduced significantly in patients that were administrated with AC extract.

Table 3 Plasma concentration of MMPs and other components in CAD and UCAD patients with and without *Asparagus curillus* root extract as dietary suppliment

	Healthy control		Patients without *Asparagus curillus* root extract as a dietary supplement				Patients with *Asparagus curillus* root extract as a dietary supplement			
			Stable CAD (CAD)		Unstable CAD (UCAD)		Stable CAD (AC-CAD)		Unstable CAD (AC-UCAD)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean (SD) CRP mg/dL	1.7(1.1)	1.3(0.8)	4.5(4.3)	22.8(3.1)	22.8(12.4)	20.1(16.1)	3.4(0.9)	3.1(1.1)	16.1(4.1)	15.5(3.9)
Mean (SD) IL-6 ng/dL	2.1(1.1)	1.9(1.2)	6.8(4.2)	13.5(3.3)	13.5(11.1)	12.8(8.1)	3.8(0.7)	3.5(0.9)	9.1(4.1)	8.7(3.8)
Mean (SD) IL-10ng/dL	2.9(2.0)	2.5(1.5)	3.5(1.8)	2.8(1.1)	2.8(1.8)	2.9(0.9)	2.7(1.8)	2.4(1.6)	3.0(1.6)	2.9(1.9)
Mean (SD) MMP-2 µg/dL	810(90)	780(120)	670(95)	514(130)	514(80)	488(120)	750(110)	730(140)	580(50)	565(80)
Mean (SD) MMP-3 µg/dL	25.4(9.1)	21.8(5.6)	15.7(7.1)	7.8(6.1)	7.8(2.5)	7.4(2.1)	18.1(7.6)	16.5(7.9)	10.1(3.1)	9.1(2.8)
Mean (SD) MMP-7 µg/dL	2.8(0.9)	2.3(8.1)	5.8(3.9)	4.7(3.1)	4.7(1.3)	4.6(1.7)	4.6(2.1)	4.1(2.4)	4.8(1.1)	4.7(1.7)
Mean (SD) TIMP-1 µg/dL	714(165)	650(90)	930(190)	1340(130)	1340(1.50)	1210(180)	890(160)	780(180)	910(80)	890(120)

ANOVA comparing all groups. Adjusted for age, smoking and body mass index.Compared with patients with stable CAD: $p < 0.01$, $p < 0.02$ and $p < 0.01$.

Plasma concentrations of MMPs and TIMP-1 are shown in table 3. Matrix MMP-2 plasma levels were significantly correlated with age (data not shown) whereas mean MMP-2 concentration in healthy patients were 145±20 ng /mL. Plasma concentration of MMP-2 &7 were significantly decreased in patients with UCAD compared with stable CAD and healthy control. But the patients of CAD-AC and UCAD-AC have higher values of MMP-2 & 7 (Data shown in table 3). MMP-3 level showed 2.5 times decreased in UCAD-AC (male) and 3.5 times decrease in UCAD (male) as compare to control but less significant decrease was being observed in UCAD-AC and CAD-AC female patients (shown in table 3). MMP-7 concentrations were increased by 2, 2.36 and 1.67 times in CAD (male), CAD (female) and UCAD (male and female both) patients respectively as compared to control patients. It was also being observed from table 3 that TIMP-1 concentration was very high in patients with UCAD (male and female) that was followed by stable CAD (male and female), UCAD-AC (male and female) and CAD-AC (male and female) patients respectively.

IL-6 is responsible for stimulating acute phase protein as well as neutrophils in the bone marrow. As from table 3, concentration of IL-6 and counts of neutrophils were correlated very comfortably. From table 3, it was also observed that concentration of IL-6 was high in stable CAD (male and female) patients as compare to control, UCAD, stable CAD-AC and UCAD-AC patients. CRP is a general marker of inflammation and infection. Its concentration in human serum is usually lower than 10 mg/L slightly increased with aging. Higher values are found in inflammation and viral infection and from table 3 it was very much clear that CRP values were 14 times higher in UCAD patients which was followed by 10 times increase in UCAD-AC patients as compare to control, whereas this increase of CRP concentration was not much pronounced in stable CAD and stable CAD-AC patients (about 2 to 3 times increase as compare to control).

4 DISCUSSION

MMPs are best known as proteases responsible for the degradation and remodeling of extracellular matrix protein in both physiological and pathological condition, including various cardiac pathologies (Wilson et al 2002). However, the discovery of the intracellular localization (Wang et al 2002) and function of MMP-2 to proteolyze troponin I, myosin light chain-I and α- actinin during myocardial oxidative stress injury challenged the canonical notion of extracellular only action of this enzyme (Sawieki et al 2005 and Sung et al 2007). In present study, we showed and analyzed that AC root extract have a significant effect on plasma concentration several MMPs and TIMP-1 in patients with stable and unstable CAD. It was also inferred from the above experiments that in AC administered patients (CAD and UCAD) and in without AC administered patients (CAD and UCAD) have significant difference in plasma concentration between the group of MMP-2, 3, 7 and TIMP-1. Importantly, the plasma concentration of MMP-7 were significantly increased in CAD patients, But this increase was not much pronounced in patients of CAD-AC. Previously it has been considered that MMP-7 might be a marker of atherosclerosis because it was increased equally in all patients with stable and unstable CAD (Nilsson et al 2006). Our study proves that AC root extract has intracellular effect on the MMP-7 activity. It might be possible that AC root extract have some compounds that cause different pattern of enzymatic modulation in CAD patients.

In contrast to MMP-7, Plasma MMP-2 was significantly decreased in the patients with stable CAD and was even lower in the group with CAD. Although, we analyzed that AC root extract administered patients (UCAD and CAD) has higher values of plasma MMP-2 concentration as compare to stable and unstable CAD. Reports on MMP-2 inhibition with ONO-4817 prevented ischemia/ reperfusion-induced titin degradation (Ali et al 2010) and improved the recovery of myocardial contractile function. Titin degradation (largest mammalian myofilamentous protein) was also reduced in hearts from MMP-2 knockout mice subjected to ischemia/re perfusion in vivo compared with wild-type control (Ali et al 2010 and Fukuda et al 2008).

Whereas, it has been observed that MMP-2 (in relation to plasma concentration in CAD patients) has given conflicting results (Chow et al 2007). The most significant finding by George et al, 2005 in patients with serum MMP-2 levels above 352 ng /ml are at a significant risk of death, hospitalization for heart failure, or either of these 2 end points. Low MMP-2 plasma levels are associated with intracranial location of cere-

bral atherosclerosis, suggesting that MMP-2 may play a role in the development of ICAS (Sang-Beom Jeon et al 2012). The MMP-2 concentration was also correlated with the number of NK cells (natural killer cells) in CAD patient (Gao et al 2003). The number of NK cells is lower and NK activity is impaired in patients with choronic immunologic disease and decreased NK cells function has been associated with atherosclerosis in elderly human (Flodstrom et al 2001). Indeed, we found that AC root extract causes decrease concentration of MMP-2 and also causes inhibition of plasma MMP-2 in CAD and UCAD patient and this could also be associated with low number of NK cells in patients (CAD-AC and UCAD-AC).

Lubos et al demonstrated that high serum TIMP-1 is a risk predictor for future cardiovascular death. The assay used in present study recognizes the total TIMP-1 content, i.e., free TIMP-1 and TIMP-1 complexed with MMPs (Ikonamidis et al 2005). Thus, increased plasma TIMP-1 in patients with stable and unstable CAD could reflect increased matrix-degrading activity with accumulation of MMP-TIMP-1 complex in plasma. The plasma concentration of TIMP-1 correlated strongly with makers of inflammation, such as IL-6 and CRP, in patients with manifest CAD (Sang-Beom et al 2012 and Wiernicki et al 2011)). As the concentration of IL-6 from stable CAD to UCAD patients and stable CAD-AC to UCAD-AC were increased, the counts of neutrophils were also increased up to same extant but this significant increase was not pronounced in CAD-AC and UCAD-AC patients. This interleukin is as anti-inflammatory cytokine and produced by monocytes and lymphocytes (some extant) (Schulze et al 2009). It is released by cytotoxic T-cell to inhibit the action of NK cells during the immune response to vital infection (Bruunsgaard et al 2001). In the present study, the concentration of TIMP-1 in patients (CAD and UCAD) with AC root extract was significantly decreased. These results clearly indicated the improvement in the patients suffering with CAD and UCAD.

5 Conclusion

AC root extract may contain some anti inflammatory compounds that effect on plasma concentration of MMPs and TIMPs very significantly in CAD and UCAD patients. Prospective studies are required to demonstrate whether the compounds in AC work in synergism or individually.

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