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Research Article

ADJUVANT USE OF SILYMARIN IN PATIENTS WITH HYPERTENSION AND MICROALBUMINURIA

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ABSTRACT

Microalbuminuria is an important micro-vascular complication of hypertension. It has been reported that polyphenolic compounds provide protection against the risk of cardiovascular diseases. The present study was designed to evaluate the adjuvant use of silymarin blood pressure (PB), microalbuminuria (MAU) and the lipid profile of patients with essential hypertension. A single blinded, placebo-controlled clinical trial was utilized; 80 hypertensive patients were allocated into two groups; group A, 45 patients treated with atenolol and furosemide and silymarin 420 mg/day; group B, 35 patients treated with placebo in addition to the antihypertensive drugs for 60 days. Blood pressure, lipid profile, microalbuminuria were evaluated at baseline and after 60 days. The results indicated that silymarin improves blood pressure regulation and lipid profile, in addition to decreasing microalbuminuria level compared with placebo. In Conclusion, adjuvant use of silymarin with the antihypertensive drugs (atenolol and furosemide) improves treatment of patients with essential hypertension.

Keywords: silymarin, hypertension, microalbumimuria, lipid profile, oxidative stress, adjuvant treatment

INTRODUCTION

Hypertension contributes to cardiovascular diseases[1], and about 30% of hypertensive adults display salt-sensitive hypertension, i.e. their arterial pressure increases in direct relation to dietary NaCl intake[2,3].The involvement of reactive oxygen species (ROS), not only cardiovascular diseases, but also hypertension was demonstrated in both hypertensive rat models and humans[4,5]. Humans maintain defense systems against ROS through enzymes (superoxide dismutase, glutathione peroxidase and catalase) and low-molecular-weight antioxidants. Diets with antioxidant properties include many fruits and vegetables, which are considered to be important sources of antioxidants[6]. Dietary antioxidants including vitamin E, vitamin C, carotenoids and polyphenols have received much attention in the prevention of cardiovascular disease and its risk factors[6]. Dietary polyphenols, including silymarin and others, appear to decrease these hypertensive effects and have several other health benefits in rats and humans. For example, in rats, polyphenol-containing diets are associated with decreases in blood pressure and serum total cholesterol[7,8]. Polyphenols, including catechins, silymarin and others scavenge ROS and chelate transition metal ions in a structure-dependent manner[9]. Flavonoids from natural sources scavenge nitric oxide (NO) and peroxynitrite produced from superoxide radicals and NO[10,11], effectively reducing the bioavailability of endothelium-derived NO. It was shown that any possible production of peroxynitrite could be eliminated by black tea or its characteristic constituent theaflavins by simply preventing the induction of inducible NO synthase synthesis[12]. Furthermore, epidemiologic studies suggested that tea polyphenols that can be derived from black and green tea may protect against cardiovascular diseases[13,14]. Therefore, the physiologic effects of flavonoids on cardiovascular disease risk factors such as hypertension are of interest. In the present study, the protective effects of the polyphenol silymarin on hypertension associated with microalbuminuria were examined.

PATIENTS AND METHODS

The present study was carried on 80 patients (45 males and 35 females) with essential hypertension; the patients were included in a single blind, placebo controlled study at the Out and Input patient Clinic in Baghdad Teaching Hospital during the period from September 2010 to June 2011, and the study protocol was approved by the local research ethics committee there. Each patient was asked to sign agreement consent before enrolment in the study. The patients age range was 45-75 (57.68 ± 9.85) years with disease duration of more than 5 years, and all of them were already maintained on 40 mg/day furosemide and 100mg/day atenolol, the patients were randomly allocated into 2 groups: group A, includes 45 patients treated with 420mg/day silymarin (Madus, Germany) in three divided doses, administered orally as capsule dosage form, in addition to the oral antihypertensive drugs (40 mg/day furosemide and 100mg/day atenolol) for 60 days; group B, includes 35 patients treated with placebo capsules, in addition to 40 mg/day furosemide and 100mg/day atenolol for 60 days. Systolic and diastolic blood pressure was monitored at zero-time and after 8 weeks. Blood samples (10ml) were collected from each patient before starting treatment and after 8 weeks, kept in plain tubes and left for clot formation. Serum was separated by centrifugation at 5000 rpm for 10 min and stored frozen at -18^{o}C until analyzed. The lipid profile was analyzed according to standard methods[15-17]. Urine samples were obtained for analysis of microalbuminuria[18]. The values were expressed as mean±SEM; Student's *t*-test and ANOVA were used for statistical analysis of data; values were considered significant at $P<0.05$.

RESULTS

Table 1 showed that adjuvant use of silymarin with atenolol and furosemide (group A) produced significant decrease ($P<0.05$) in MAU level (55%) compared to zero-time value after 60 days, where atenolol and furosemide did not decrease MAU levels, rather significant elevation was observed (10%, $P<0.05$) after 60 days compared to pre-treatment values. When the two approaches of treatment were compared, adjuvant use of silymarin significantly decreases MAU level compared to placebo. Table 1 indicates also that adjuvant use of silymarin significantly decreases blood pressure (22% in systolic levels and 11% in diastolic pressure) after 60 days compared to baseline values. Meanwhile, in placebo-treated

group, blood pressure values were also decreased during the treatment period, which was significantly different compared to pre-treatment values (P<0.05). When the two approaches of treatment were compared, the adjuvant use of silymarin significantly improves blood pressure levels compared to placebo. In table 2, adjuvant use of silymarin produces significant decrease in total cholesterol level (23.0%) after 60 days; while the placebo formula did not affect total cholesterol level, and significant increase rather reported (27%, P<0.05) after 60 days compared to pre-treatment values. When the two approaches of treatment were compared, adjuvant use of silymarin significantly decreases total cholesterol compared to placebo. Table 2 also showed that adjuvant use of silymarin significantly decreases serum triglycerides after 60 days. Meanwhile, the placebo formula did not shows such effect, and significant elevation was reported in this respect compared to pre-treatment values. When the two approaches were compared, adjuvant use of silymarin significantly decreases TG levels compared to placebo. Concerning the effects on serum LDL-c levels, silymarin produces 72% (P<0.05) decrease in this marker after 60 days compared to baseline values, while in group B, significant elevation in serum LDL-c levels was reported after 60 days (60%, P<0.05) compared to baseline values. Comparison between the two approaches indicates that adjuvant use of silymarin significantly decreases serum LDL-c levels compared to placebo. Finally, concerning the effect of silymarin on serum HDL-c levels, table 2 showed that silymarin significantly elevates HDL-c levels (121%, P<0.05) compared to baseline values; while in placebo-treated patients, significant decrease in serum HDL-c levels (12%, P<0.05) was reported after 60 days. Comparison between the two approaches of treatment, table 2 indicates that adjuvant use of silymarin improves HDL-c levels significantly compared to the use of atenolol and furosemide alone.

DISCUSSION

The kidney is one of the major end organ targets of hypertension. Scientific reports indicated that prevalence of renal damage secondary to hypertension continues to multiply rapidly[19]. However, the mechanisms of hypertension and the associated renal damage are not well known. One possible mechanism is the association between hypertension and renal damage with oxidative stress. Release of the superoxide anion in the kidney produces several deleterious effects, including the elevated chances for inactivation of endothelial NO. Since NO has several important renal actions such as prevention of tubular Na reabsorption and blunting of tubular glomerular feedback[20], inactivation of NO can result in excess Na reabsorption and enhanced TGF feedback and thus hypertension[21,22]. Inactivation of NO by superoxide forms peroxynitrite[23] which can nitrosylate tyrosine residues and cause renal tissue damage. Several studies indicated that a Dahl salt-sensitive rat exposed to a high Na diet will experience severe renal damage. After 2-5 weeks on high Na intake they exhibit signs of significant renal damage associated with increased oxidative stress[24]. Early signs of renal damage include increases in urinary protein excretion[25]. Numerous interventions have been tried to counteract the effects of ROS, by reinforcing the antioxidant defense systems. Dietary supplementation with vitamin E slowed the rate of progression of renal deterioration [26], where both renal structural damage and urinary protein excretion decreased when the high Na Dahl salt-sensitive rats were treated with vitamins C and E[24]. Polyphenols are a group of naturally occurring antioxidant substances found in vegetables, fruits or seeds, and are particularly abundant in *Silybum marianum* (milk thistle), where Silymarin is an active polyphenolic flavonoid extracted from the seeds of this medicinal plant[27], and proved to have well documented hepatoprotective effect; meanwhile, many reports indicated also there are some animal and nephro-protective effects[28]. In the present study, adjuvant use of silymarin with the currently followed approach significantly decreases MAU and BP levels; this result was in tune with many in vitro and animal studies, where silymarin improves kidney function[29]. Several studies have clearly shown that induction of oxidative stress initiates hypertension due to enhancement of both superoxide and hydroxyl radical production in animals[30,31], and treatment with antioxidants lowered blood pressure and increased the bioavailable nitric oxide[32]. However, the nephro-protective effects of polyphenols were attributed to large array of biological actions, such as free radical-scavenging, metal chelation and enzyme modulation abilities[33]. Other biological actions of polyphenols include the reduction in the susceptibility of LDL-c to oxidation both in vitro[34] and in vivo[35], an effect likely due to the property of these compounds to scavenge free radicals. Polyphenols also may participate in the regulation of vascular tone or in the inhibition of platelet aggregation[36]. The present study indicates also that adjuvant use of silymarin in essential hypertension significantly improves the impaired lipid profile, revealed by decreasing cholesterol, LDL-c, and triglyceride levels and increasing HDL-c levels. Previous report indicates that administration of silymarin to rats with impaired lipid profile results in significant reduction in total cholesterol and LDL-c levels associated with significant elevation in HDL-c levels[37], this profile of effects was consistent with that reported in the present study. Moreover, Kercman et al (1998) reported that silymarin inhibits development of hypercholesterolemia in rats fed cholesterol-rich diet comparable to that produced by probucol, associated with increase in HDL-c levels and decrease in the liver content of cholesterol[38]. Moreover, De Whally et al (1990) showed that certain flavonoids were potent inhibitors of the modification of LDL by mouse macrophages; flavonoids also inhibited the cell-free oxidation of LDL mediated by copper sulfate[39]. The flavonoids may also sequester metal ions like copper and iron, thereby diminishing the excessive generation of free radicals in the medium[40]. Consequently, the toxicity of oxidized LDL could be prevented by flavonoids either by inhibiting the lipid peroxidation of LDL or by blocking at the cellular level the cytotoxicity of previously oxidized LDL. In conclusion, adjuvant use of silymarin in hypertensive patients improves blood pressure regulation, microalbuminuria and the impaired lipid profile.

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Table 1: Effects of adjuvant use of 420 mg/day silymarin on blood pressure (SP and DP) and microalbuminuria (MAU) level in patients with essential hypertension

Patient group	SP (mmHg)		DP (mmHg)		MAU (mg/dl)	
	Zero-time	60 days	Zero-time	60 days	Zero-time	60 days
Group A n = 45	157.3±13.7	139.8±13.1*[a]	93.6±9.2	82.6±8.6*[a]	66.3±29.3	23.2±10.2*[a]
Group B n = 35	159.4±14.5	141.7±14.3*[b]	97.8±9.6	87.6±8.9*[b]	74.4±38.1	81.8±41.9*[b]

Values are presented as mean ± SEM; n= number of patients; * significantly different compared to Pre-treatment (P<0.05); values with non-identical superscripts (a,b) among different groups are considered significantly different ($P < 0.05$).
Group A: treatment with silymarin; group B: treatment with placebo.

Table 2: Effects of adjuvant use of 420 mg/day silymarin on the lipid profile in patients with essential hypertension

Patient group	Serum cholesterol mmol/L		Serum triglyceride mmol/L		Serum LDL-c mmol/L		Serum HDL-c mmol/L	
	zero-time	60 days	zero-time	60 days	zero-time	60 days	Zero-time	60 days
Group A n = 45	5.4±0.6	4.1±0.5*[a]	3.9±0.6	2.4±0.4*[a]	2.5±0.6	0.7±0.7*[a]	1.1±0.2	2.3±0.5*[a]
Group B n = 35	4.4±1.1	5.6±1.4*[b]	2.4±0.7	2.5±0.7*[b]	2.2± 0.8	3.5±1.2*[b]	1.2±0.1	1.0±0.1*[b]

Values are presented as mean ±SEM; n= number of patients; * significantly different compared to Pre-treatment (P<0.05); values with non-identical superscripts (a,b) among different groups are considered significantly different ($P < 0.05$). Group A: treatment with silymarin;
Group B: treatment with placebo.

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