Journal of Research in Environmental Science and Toxicology Vol. 1(5) pp. 107-114, June 2012
Available online http://www.interesjournals.org/JREST

Full Length Research Paper

Regular physical activity and diferuloyl methane supplement reverses pro-inflammatory cytokines in heart

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Accepted 08 June, 2012

We have investigated the cardioprotective effects of regular physical activity and/or diferuloyl methane supplement(DMS) on cytokines such as; apelin, high sensitive C-reactive protein (HS-CRP), tumor necrosis factor TNF-α and E-selectin, and oxidative stress-related biomarkers such as; total antioxidant capacity (TAC), malondialdehyde (MDA), in male rats exposed to lead acetate(Pb). Fifty male Wistar rats were randomly divided into 5 groups: (1) pb (2) diferuloyl methane supplement (DMS), (3) regular physical activity (RPA), (4)physical activity +DMS (5) sham (saline) groups. The rats in the 1 to 4 groups received pb (20 mg/kg, 3 times a week for 8 weeks). Also, the 3 and 4 groups experienced the training of 15 to 22 m/min for 25 to 64 minutes, 5 times a week for 8 weeks, whereas, the 2 and 4 groups received DMS solution (30 mg/kg, 3 times a week for 8 weeks). However, rats in 5 group received ethyl oleat solvent. pb administration resulted in significant decrease in apelin and TAC levels, and significantly increased MDA, HS-CRP, TNF-α and E-selectin in heart homogenized tissue. In contrast, DMS, regular physical activity, and both interventions significantly reversed apelin, HS-CRP, TNF-α and E-selectin cytokines levels, and oxidative stress-related biomarkers (TAC and MDA) concentrations. These results suggest that the healthy lifestyle including regular physical activityand antioxidants caused reversing lead-induced cardiotoxicity.

Keywords: Cardiovascular Disease, Cytokines, Regular physical activity, Pb, Antioxidant.

INTRODUCTION

Recent studies have established a link between ambient air pollutants and health (Makri and Stilianakis, 2008). The American Heart Association (AHA) published a statement on the importance of air pollution in the development of cardiovascular disease. Lead acetate (Pb) is a non-essential toxic heavy metal widely distributed in the environment and a chronic exposure to low levels of pb induces a broad range of physiological, biochemical and behavioural dysfunctions (Alghazal et al., 2008; Ahamed, Siddiqui, 2007). Recent epidemiological studies have reported that low level pb exposure has a graded association with several disease outcomes such as cardiovascular disease and hypertension. Moreover, studies have proposed that one possible mechanism of pb toxicity is generate inflammation (Boris et al., 2008) and the disturbance of prooxidant and antioxidant balance by generation of reactive oxygen species (ROS) (Alghazal et al.,2008; Dabidi Roshan et al., 2011). Cardiovasculardiseases are currently the most frequent cause ofdeath in worldwide and their incidence continually rises, and recent studies have shown that there is an association between CVD, inflammation, and oxidative stress (Roshan et al., 2011).

Adipokines such as apelin, C-reactive protein (CRP), tumor necrosis factor TNF-α and E-selectin represent a family of proteins released by adipocytes that affect various biological processes including metabolism,

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Satiety, inflammation, and cardiovascular function. Apelin is an adipokine which was found to be the endogenous ligand for the G protein-coupled APJ receptor. Apelin has been shown to exert potent positive inotropic effects on both normal and failing myocardium(Farkasfalvi et al.,2007; Szokodi et al.,2002).On the other hand, there is growing evidence that shows the role of the inflammatory process in the onset of cardiac events (Gaeini et al.,2008). Studies have reported CRP has been identified as the most sensitive inflammatory marker and a strong independent predictor of CHD. For example, its increase is associated with 2 to 5 times increased risk of CHD(Gaeini et al.,2008). These biomarkers independently correlated whit access augmentation of cardiovascular diseases risk.

Lifestyle-related diseases, such as cancer, metabolic syndrome, cardiovascular and others, are constantly increasing (Hoyoku et al.,2011). Therefore, development of the methods to prevent these lifestyle-related diseases is very important. Many studies showed that regular physical activity prevents the development of cardiac diseases (Roshan et al.,2011). Furthermore, diferuloyl methane supplement (DMS), a group of phenolic compounds isolated from the plant rhizomes of Curcuma longa (also known asCurcumin) has along history in traditional medicine(Dabidi roshan et al.,2011;Sheril et al.,2004). It has many therapeutic properties including antioxidant, anticarcinogenic, anti-inflammatory, hypoglycemic and cardiovascular protective activities. As well, it is a known scavenger of free radicals in animals. Moreover, it has been suggested to have a wide range of therapeutic effects in numerous conditions including chronic inflammatory diseases such as CVD(Roshan et al.,2011).

Despite the knowledge that pbcan induce oxidative stress, studies have identified favorable effects of regular physical activityand/or antioxidants on certain cardiovascular biomarkers after acute exposure to air pollution(Roshan et al.,2011;Kalpana et al.,2007). However, there are few data available with respect to concomitant effects of regular physical activity and DMS, particularly the oxidant/antioxidant and inflammatory processes that underlie heart tissue injury during chronic exposure to pb. The aim of this current study evaluates the cardioprotective effects of regular physical activity with and without DMS on pb-induced cardio-toxicity, as determined by adipocytokines (apelin, HS-CRP, TNF-α and E-selectin) and oxidative stress-related biomarkers such as; total antioxidant capacity (TAC) and malondialdehyde (MDA) levels in heart tissue of the male rats exposed to pb. It is hypothesized that the results of this study provide novel insight about the cardiac ameliorative potential of DMS and regular physical activity.

MATERIALS AND METHODS

The study was approved by theDepartment of Physiology, University ofMazandaran and was performed according toguiding procedures in the Care and Use ofAnimals, prepared by the Council of theAmerican Physiological Society. Fifty male Wistar rats, 8 weeks of age (initial bodyweight of 240 + 20 g), were obtained from the Laboratoryof Animal Bearing and Multiplying at the Pasture Institute ofIran. Rats were randomly divided into five groups [1control group (sham) and 4 treatment groups (pb, diferuloyl methane supplement (DMS), physical activity, physical activity +DMS). Each rat was housed in single standard cages of polycarbonate(20 _ 15 _ 15 cm), made at the Pasture Institute of Iran, ina large air-conditioned room with controlled temperature of 22+ 2_C, light-dark cycles of 12:12 hours and humidity of 50%+ 5%. The pollutant standard index (PSI) was in the acceptablerange as determined by the Iranian Meteorological Organization.Rats were fed with a standard rat chow provided by ParsInstitute for animals and poultry with a daily regimen of 10 gr per100 g of body weight for each rat. Water was available ad libitum.

Rats were familiarized with the laboratory environment and running on the treadmill, then were randomly assigned into 5 experimental groups of 10 rats each. The groups treated as follows: group1— the sham-operate or control group (sham); these rats received water and ethyl oleate at a concentration of 30 mg/kg in the form of a water solution (for intraperitoneal [ip] injection), 3 days weekly for 8 weeks; group 2— Regular physical activity (Pb + physical activity) the rats in this group performed progressive running exercise of 15 to 22 m/min for 25 to 64 minutes, 5 times a week, and in addition they received pb at a concentration of 20 mg/kg in the form of a water solution (for intraperitoneal [ip] injection), 3 days weekly for 8 weeks, pb was solubilized in Milli-Q water.; group 3— the animals were exposed to pb at a concentration of 20 mg/kg in the form of a water solution, 3 days weekly for 8 weeks (ip); group 4—regular physical activity and DMS (Pb + physical activity + DMS); the rats in this group performed a regularphysical activity protocol similar to that in group 2, and in addition received pb as well asreceived diferuloyl methane supplementation 30 mg/kg 5 days weekly for 8 weeks (ip), DMS was solubilized in 50% ethanol.; group 5— diferuloyl methane supplement (DMS) similarly received DMS, as well as received pb, in the same manner and for the same duration of time as other groups.

In order to perform ip injections, DMS was solubilized in ethyl oleate and was injected at a dose of 30 mg/kg. DMS was protected from light throughout the experiment (Sheril et al., 2004). We are replicating a previously-reported pbdosing regimen that caused oxidative stress so that the doses of DMS and pb were 30 and 20 mg/kg, respectively (Sheril et al., 2004).

Rats in the physical activity groups were trained by

Table 1.The regular physical activity program during 8-weeks.

Training days	Number of week parameters of trainings	1	2	3	4	5	6	7	8
1	speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	25	30	35	40	45	50	55	60
2	speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	26	31	36	41	46	51	56	61
3	speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	27	32	37	42	47	52	57	62
4	speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	28	32	38	43	48	53	58	63
5	speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	29	34	39	44	49	54	59	64

running on a level motorized rodent treadmill, 5 days a week, for 8 weeks. The speed of the treadmill and duration of the training sessions was gradually increased from 15 to 22 m/min to 25 to 64 min, 5 times a week. (Table 1)

All groups were anesthetized with ketamine and Xaylozine and decapitated after 12 to 14 hours overnight fasting. Blood samples were collected from the heart of the participants in all the groups 24 hours after the last dose of treatment. These blood samples were initially centrifuged by a refrigerated centrifuge at 3000 rpm for 15 minutes within 30 minutes of collection and then stored at -80ºC for subsequent assay of MDA and TAC. The body cavities were then opened and the heart was quickly excised from the aortic root. Heart tissues were weighed and left ventricle placed into Petri dishes containing cold isolation medium (0.1 mol/L K_2HPO_4, 0.15 mol/L NaCl, PH 7.4) to remove the blood and were frozen immediately in liquid nitrogen and stored at -80ºC for subsequent analysis of apelin, CRP and MDA in heart tissue. Cytokines levels were assayed in total cell extracts prepared from heart tissues and blood samples. It was determined using commercially available kits. The analyses were performed according to the manufactures guidelines. TNF-α level assayed using Rat TNF-α prprotech kit(cat # 900-k25) (Bassiounyet al., 2011) (, Apelin-13 kit (Phoenix peptides, Burlingame, California, USA), using for the values of apelin, following the manufacturer's instructions, as previously described by Andersen (Andersen et al.,2009). The hs-CRP concentration was determined by Latex particle-enhanced Immunoturbidimetric assay on a Hitachi 912 automated analyzer using reagents from Diasorin (Stillwater, Minnesota) (Roshan et al.,2011) E-selectin levels were measured in serum samples (collected at each of the above time points) via commercially available ELISA methodologies using anti-E-selectin capture antibodies and HRP-conjugated secondary antibodies (R&D Systems, Oxon, UK) (Hannah et al., 2010).

All samples to be statistically compared were processed in the same assay to avoid interassay variations. Lipid peroxidation (MDA) levels in the left ventricle tissue was measured with the thiobarbituric-acid reaction using the method of Dabidi Roshan et al. and Asali et al. (Dabidi Roshan et al., 2011; Asali et al., 2011). The quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to a standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3 tetramethoxypropane. The values of MDA in left ventricle was expressed as nmol/g tissue.

Furthermore, Serum TAC was measured using a commercially available kit (Randox Laboratories, Crumlin, UK) as previously described by Dabidi Roshan et al. and Asali et al. (Dabidi Roshan et al., 2011; Asali et al., 2011). In accordance with the protocols of Daniel et al., Dabidi Roshan et al. and Asali et al. (Daniel et al. 2004; Dabidi Roshan et al., 2011; Asali et al., 2011), we analyzed the pb concentration using a spectrophotometer method only in the pb group.

Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Results are expressed as means $\pm$ SE. Data for heart tissue adipocytokinesand oxidative stress-related biomarkers were normally distributed after log-transformation. A one-way analysis of variance (ANOVA) was used to detect statistical differences between groups. A post-hoc test (Tukey test) was performed to determine differences in the various markers between groups. The differences were considered significant at $P < .05$.

RESULTS

The effect of chronic exposure to pb at the concentration of 20 mg/kg for 8 weeks on inflammation and oxidation stress parameters was assessed in fifty male Wistar rats, as shown in Tables 2 and 3. The values of apelin and TAC were lower (both $P < 0.01$) (38% and 27%

Table 2.The values of pro-inflammatory markers in different groups at the 8th week.

Groups and markers	Apelin (pg/dl)	HS-CRP (mg/L)	TNF-α (pg/ml)	E-selectin (ng/ml)
Sham (S)	3.5±0.36	0.054±0.036	2.87±0.5	68.6±10
Pb	2.13±0.52	0.157±0.09	5.01±0.71	80.8±10
Pb + physical activity	4.8±0.66	0.079±0.039	2.46±0.62	55.2±8.5
pb + diferuloyl methane	4.4±0.54	0.057±0.044	2.15±0.58	53.4±6.7
(pb + physical activity +diferuloyl methane	6±0.88	0.051±0.049	1.73±0.41	45.8±7.6

Data are presented as the mean±SD for 10 rats; *Abbreviation: high-sensitivity C-reactive protein (CRP), tumor necrosis factor (TNF-α),*

Table 3.The values of stress oxidative markers in different groups at the 8th week.

Group and markers	TAC (µmol/ml)	MDA (nmol/g)
Sham (S)	386±9	27±3
Pb	280±18	46±9
Pb + physical activity	412±14	18±3
pb + diferuloyl methane	412±15	36±7
(pb + physical activity +diferuloyl methane	450±20	13±3

Data are presented as the mean±SD for 10 rats. Abbreviation; *Antioxidant Capacity (TAC) and malondialdehyde (MDA).*

Respectively) while MDA, HS-CRP, TNF-α and E-selectin were higher (P<0.001, P<0.05, P<0.001, and P<0.01) (72%, 192%, 75% and 18% respectively) in the Pb group than controls. In the Pb+DMS group, levels of apelin and TAC was higher (both P< 0.01) (26% and 7% respectively) while MDA, HS-CRP, TNF-α and E-selectin was lower (P<0.001, P<0.05, P<0.01, and P< 0.01) (33%, 6%, 25% and 22% respectively) than in controls. However, MDA, HS-CRP, TNF-α and E-selectin was lower (P<0.001, P<0.05, P<0.01, and P< 0.01); apelin and TAC were higher (P<0.05) in Pb + DMS –group than Pb group (Table 2,3).

Furthermore, data in this study indicated that after 8-wk regular physical activity, the value of MDA, HS-CRP, TNF-α and E-selectin reduced in pb+physical activity group, as compared to those of sham group (31%, 46%, 14% and 19% respectively). While the value of apelin and TAC increased in pb+physical activity group in comparison with those of sham group (40% and 7% respectively). However, combined group(physical activity with DMS) has greater effects than physical activityor supplementation alone causing a decrease in CRP, E- selectin, TNF-α and MDA and a higherincrease in apelin and TAC Figure 1 and 2).

DISCUSSION

Effect of lead (pb)

Lead (pb) is a common environmental contaminant that affects all the organs and systems of the body and causes numerous acute and chronic illnesses (Mayllaet al., 2011). Experimental and epidemiological studies suggest a close relationship between pb exposure, hypertension and cardiovascular disease (Maylla et al., 2011). As Chronic exposure cases are more common than acute toxicity (Jackie et al., 2011), In the current study, chronic exposure of pb significantly increased MDA, CRP, TNF-α and E-selectin and significantly decreased TAC and aplin levels in the pb group compared with control group. These cytokines and markers were assayed as indicators of inflammation and tissue damage in heart degenerative cells and serum of rats treated with lead. Biochemical and molecular mechanisms of pb toxicity are poorly understood. Various mechanisms were suggested to explain them: inhibition of the calcium-pump, a transport protein, disturbances in mineral metabolism, inactivation of several enzymes, etc (Enas et al., 2010). Pathogenesis of pb poisoning is mainly attributed to lead- induced oxidative stress. Chronic pb exposure is known to disrupt the pro oxidant/antioxidant balance existing within the mammalian cells (Jackie et al.,2011). Although the exact

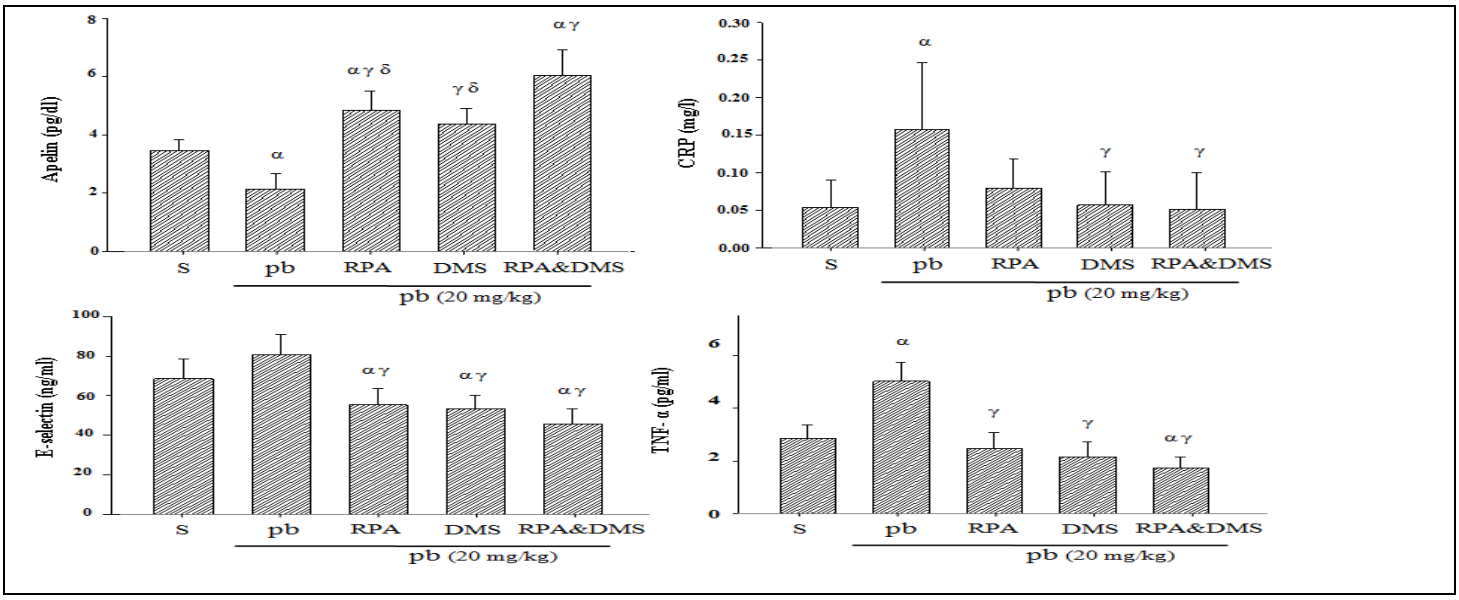

Figure 1.The levels of proinflammatory markers; apelin, high-sensitivity C-reactive protein (CRP), tumor necrosis factor(TNF-α) and E-selectin, in different groups, sham (S), lead (pb), pb + regular physical activity (RPA), pb + diferuloyl methane supplement (DMS), pb + regular physical activity + diferuloyl methane supplement (RPA & DMS). Data are presented as the mean±SD for 10 Rats; ***α*** significantly different from sham group (P < 0.001), **γ** significantly different from pb (P < 0.001), **δ** significantly different between combination (pb + regular physical activity + diferuloyl methane supplement) group with regular physical activity and or diferuloyl methane supplement groups (P < 0.05).

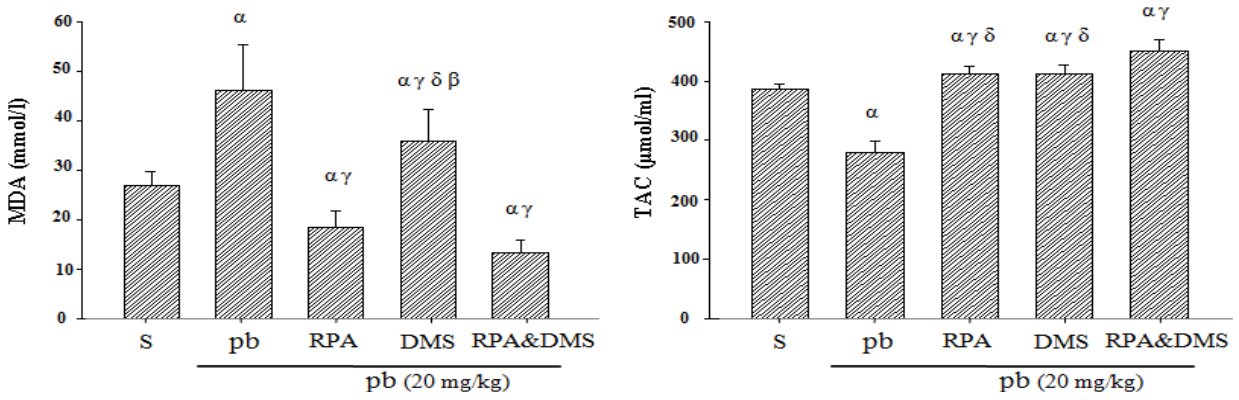

Figure 2.The levels of oxidative stress-related biomarkers; Total Antioxidant Capacity (TAC) and malondialdehyde (MDA) Levels in different groups, sham (S), lead (pb), pb + regular physical activity (RPA), pb + diferuloyl methane supplement (DMS), pb + regular physical activity + diferuloyl methane supplement (RPA & DMS). Data are presented as the mean±SD for 10 Rats; ***α*** significantly different from sham group (P < 0.001), ***β*** significantly different between regular physical activity and diferuloyl methane supplement groups (P < 0.001), **γ** significantly different from pb (P < 0.001), **δ** significantly different between combination (pb + regular physical activity + diferuloyl methane supplement) group with regular physical activity and or diferuloyl methane supplement groups (P < 0.05).

mechanisms by which pb induces oxidative stress in various tissues are not completely understood (Jackie et al., 2011), evidence indicates that Pb cause to oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides (Jackie et al., 2011). On the other hand, in cardiovascular diseases, major injury is caused by free radical generation; hence, free radical scavengers (antioxidants) form an important therapeutic (Asaliet al., 2011). This suggests that pb had damaging effects on tissues such as heart tissue during chronic exposure, which may heighten cardiovascular diseases.

Effect of regular physical activity

Whereas, Regular physical activity reduces the deleterio-

Us effects of cardiovascular and inflammatory disorders,currently, the healthy lifestyle has been associated with the regular practice of a physical activity. Evidence shows that this practice causes the individual to have more longevity, in addition to decrease the morbidity and mortality grade (Renata et al., 2012).Regular physical activity is an important treatment modality in the rehabilitation of revascularized coronary artery disease (CAD) patients (Dominique et al.,2011). Although the results of some studies (Andersson et al.,2010;Nicklas et al.,2004) suggest that physical activity is not associated with a reduction in inflammation markers, data obtained from other research support instead that regular physical activity reduces inflammation indicators (You&Nicklas., 2006;Sixt et al.,2010) by decreasing adipocytokine production and cytokine release from skeletal muscles, endothelial cells, and immune system and also improving antioxidant status (Hopps et al.,2011) and demonstrate the influence of regular physical activity in mitigating the risks of obesity and cardiovascular diseases (Hopps et al.,2011). The final analyses in Neil M. Johannsen et al. reports suggest that increased physical activity can reduce total WBC and neutrophil counts independently of changes in adiposity and inflammatory cytokines (Neil et al., 2012). On the one hand Miyazaki et al. indicated that high-intensity regular physical activity can elevate antioxidant enzyme activities in erythrocytes, and decrease neutrophil O2 production in response to exhausting physical activity (Miyazaki et al.,2001).

Furthermore, this up-regulation in antioxidant defenses was accompanied by a reduction inphysical activity –induced lipid peroxidation in erythrocyte membrane. Shahandeh et al., reported that regular physical activity seems to reduce the oxidative stress of physical activity, such that trained athletes show less evidence of lipid peroxidation for a given bout of physical activity and an enhanced defense system in relation to untrained subjects (Shahandeh et al.,2011). Also, Inal et al. indicated that both long (800 m) and short (100 m) –distance swimming increased the activities of antioxidant defense enzymes (linal et al.,2001). Regular physical activity can modulate cytokine production at the levels of gene expression, protein ligand and receptor binding, eventually having local and systemic consequences (De Salles et al.,2010). Some researchers suggested that physical activity produces an increase in release of interleukin-6 (IL-6) from active muscles, which can in turn suppress other proinflammatory markers, such as TNF- α (De Salles et al.,2010). Kasapis et al. suggested that regular physical activity reduces CRP directly by reducing cytokine production in adipose tissue, muscle and mononuclear cells, and indirectly by increasing insulin sensitivity and improving endothelial function (Kasapis& Thompson., 2005). Further Zhang et al. showed that long-term swimming training reversed the downregulation of cardiovascular expression of apelin/APJ in SHRs and effectively attenuated the hypertension and pathological cardiac hypertrophy (Jing et al.,2006). In our study, we investigated the cardioprotective effects of prolonged regular physical activity on pb-induced cytokines and oxidative stress-related biomarkers levels. The findings showed that MDA, HS-CRP, TNF-α and E-selectin in Pb group increased significantly, whereas in pb + physical activity group it was increased. Apelin and TAC levels in pb group significantly decreased, while in pb + physical activity group it was increased.

Effect of diferuloyl methane supplement

Diferuloyl methane supplement (DMS) (the common name for Curcuma Longa, known as haldi in Hindi) is an Indian spice (Aggarwal&Sung.,2009) that has been traditionally used in Ayurvedic medicine for the treatment of various ailments (Hongyu et al.,2011). Diferuloyl methane supplement, a hydrophobic polyphenol, is a principal active constituent of diferuloyl methane supplement (Hongyu et al.,2011). Numerous studies have demonstrated diferuloyl methane supplement has antioxidant, antibacterial, anti-inflammatory, and anti-atherosclerotic effects, exerting medicinal benefits against neurodegenerative diseases, cardiovascular disease and respiratory disease (Aggarwal&Harikumar., 2009). These effects of DMS are dependent on its capacity of interacting and regulation of multiple molecular targets include transcription factors, growth factors, inflammatory cytokines, apoptosis-related proteins and others (Hongyu et al.,2011). During severe infection or after severe injury, excessive synthesis and production of proinflammatory cytokines, play a major role in the development of local and systemic inflammation, causing severe pathophysiological derangement or organ failure (Hongyu et al., 2011).

Several studies have demonstrated that diferuloyl methane supplement was able to modulate the production of various inflammatory cytokines, thereby exhibiting potent anti-inflammatory activity (Chen et al., 2008;Abe et al.,1999;Srimal & Dhawan.,1973). Singh et al. firstly reported that Diferuloyl methane can down-regulate TNF-α-induced activation of NF-κB and AP-1 (Singh &Aggarwal., 1995). Fu et.al indicated that In the CCl4 rat model, diferuloyl methane supplement protected the rat liver from CCl4-induced injury and fibrogenesis by reducing inflammatory cytokines levels in the liver and in serum, including interferon-γ (IFN-γ), TNF-α, and IL-6 (Fu etal.,2008). Also, Bharat et al reported that diferuloyl methane supplement downregulated the expression of interleukin (IL)-6 protein, tumor necrosis factor (TNF), and various other chemokines, and inhibited the production of IL-8, MIP-1a, MCP-1, and IL-1a induced by inflammatory stimuli in human peripheral blood monocytes and alveolar macrophages (Roshan et al.,2011). The antioxidant activity of the curcuminoids

comes by virtue of their chemical structure. The curcuminoids consist of two methoxylated phenols connected by two a, B unsaturated carbonyl groups that exist in a stable enol form (Reason et al.,2011). DMS is a known scavenger of free radicals and can significantly inhibit lipid peroxidation.

It has been shown that Diferuloyl methane down-regulates the Inducible nitric oxide synthase (iNOS) activity in macrophages, thus reducing the amount of reactive oxygen species (ROS) generated in response to oxidative stress (Reason et al.,2011). As oxidative stress has been mainly implicated in the pb toxicity, reducing the possibility of pb interacting with cellular metabolism biomolecules and decreasing the reactive oxygen species generation by the use of antioxidant nutrients has received considerable attention in the recent past (Jackie et al.,2011). Further, in the present study, peritoneal injection of DMS while peritoneal injection of pb could reduce the harmful effects of pb on heart tissue and serum. DMSsignificantly decreased MDA,CRP, TNF-α and E-selectin and significantly increased TAC and apelin levels in the pb + DMS group compared with pb group.

In conclusion, Lifestyle changes are necessary to prevent atherosclerosis and cardiovascular events. In the research showed that combined regular physical activity with supplementation has greater anti-inflammatory and anti-oxidation effects than physical activity or supplementation alone causing a deepest decrease in CRP, E- selectin, TNF-a and MDA and a higher increase in apelin and TAC. These findings clearly show that the combination of Diferuloyl methane supplementation with regular physical activity provides numerous benefits on oxidant/antioxidant balance and inflammation over physical activity alone or supplementation alone.

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