Atherosclerotic Regulation of *Asparagus racemosus* Root Extract on Lipid Profile in Male Swiss Albino Mice, Subjected to Inorganic Lead Compound

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**ABSTRACT**

The present study has been made to reveal the regulatory action of *Asparagus racemosus* on inorganic lead induced toxicity in the Swiss albino mice (*Mus Musculus*). The experiment design with thirty-six male Swiss albino mice with the average weight 29 g, were divided into six experimental groups and each group with six mice. Group I – (Control; Without any treatment), Group II- (Lead nitrate;20 mg/Kg body weight, orally), Group III- (ARRE; 50mg/Kg body weight, orally), Group IV- (ARRE;150mg/Kg body weight, orally), Group V- (Lead nitrate;20 mg/Kg body weight, orally + ARRE; 50mg/Kg body weight, orally), Group VI- (Lead nitrate;20 mg/Kg body weight, orally +ARRE;150mg/Kg body weight, orally) respectively treated for 45 days. Administration of lead nitrate for 45 days increased the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol; however VLDL-cholesterol and HDL-cholesterol values are low in lead exposed subjects. Individual and simultaneous administration of ARRE along with lead for the both doses, *Asparagus racemosus* showed the positive amelioration in lipid profile at different levels to some extent with all variables. The findings of present study suggest the possible hypercholestramnic abnormalities induced by lead can be neutralized by *Asparagus racemosus* in the lead exposed population.

**Key words:** Lead nitrate, *Asparagus racemosus*, lipid profile, lipoprotein levels, Mice.

**INTRODUCTION**

Lead is a ubiquitous environmental and industrial pollutant that has been detected in almost all phases of environment and biological systems. The acute and chronic lead poisoning cause impairment of heart and vessel function and the rate of death from cerebrovascular disease are significantly increased in lead affected subjects¹. Extensive evidence of an association between serum lipid and lipoprotein levels and coronary artery disease has been well documented²-⁵. Thus, it has been suggested that one of the underlying mechanisms in the association between cardiovascular damages and lead exposure is the induction or acceleration of atherosclerosis⁶⁻⁹.

In Ayurveda, *Asparagus racemosus* Willd, (Family Asparagaceae), is a popular rasayana, commonly used for the treatment of various disorders and diseases. Increase in serum lipid levels especially cholesterol along with the generation of reactive oxygen species are the major reasons for the development of coronary artery disease and atherosclerosis. High lipid levels and high cholesterol levels are major risk factors for atherosclerosis and cardiovascular diseases¹⁰⁻¹⁴. Natural plants have also been investigated for the reduction of cholesterol levels in hypercholesteremic rats¹⁴⁻¹⁷. The root of plant has also been claimed by traditional healer to possess anti-diabetic protection; studies have reported reduced blood glucose level in rats and rabbits¹¹, ¹². The constituents of...
Asparagus racemosus root extracts have wide ranging stimulatory effects of physiological insulinotropic pathway and as a source of active components may provide new opportunities for diabetes therapy. Therefore, the present study sought to elucidate the possible effectiveness of aqueous extract of Asparagus racemosus root (ARRE) on lipid profile levels in lead exposed mice.

MATERIAL AND METHODS

Chemicals

Lead nitrate was purchased from Central Drug House (India). All other chemicals were of analytical grade and obtained from Sisco Research Laboratories (India), Qualigens (India/Germany), SD Fine Chemicals (India), HIMEDIA (India), and Central Drug House (India).

Plant material

The plant Asparagus racemosus (Family: Asparagaceae) was collected in October month from Krishi Vigyan Kendra of Banasthali University, Rajasthan, India. The plant material was taxonomically identified by a plant taxonomist of our Institute.

Preparation of plant extract

Root powder of plant material was used for the preparation of aqueous extract. A known quantity (5 g) of the powdered material was extracted using alternative treatment in water bath and over to mental heater at 40-50°C temperature, using distilled water (500 ml) as a solvent for 5 days approximately. The extract was then filtered through Muslin cloth. For complete evaporation of the solvent the extract was kept in hot air closed oven (40-50°C). The final residue left behind was weighed (3.089 g) in an electrical balance and stored at 4°C. It was dissolved in distilled water whenever needed to treat the animals.

Experimental animals

Male Swiss albino mice weighing 15-30 g (2-2.5 months) were obtained from Haryana Agricultural University, Hisar, India. The Animal Ethics Committee of Banasthali University, Banasthali, India approved the study. All experiments were conducted on adult male albino mice when they weighed 25-35 g (3-4 months old).

Thirty six adult male Swiss albino mice were left for 2 weeks before experimentation to adapt to laboratory conditions. They were housed in polypropylene cages in an air-conditioned room at 25±3°C, relative humidity of 50±5% and 12-h alternating light and dark cycles. The mice were provided with chow diet (Hindustan Lever Limited, India) and drinking water ad libitum.

Experimental Design

Adult male Swiss albino mice (36) weighing 25-30 g (3-4 month old) were used for the current investigation. For serum lipid profile, 36 mice were divided into six groups of 6 mice each. The groups for each parameter were as follows:

Group I - Control (Normal diet and water)
Group II - Lead nitrate (20mg/kg bwt, orally)
Group III- AR root extract (50 mg/ kg body weight, orally by gavage)
Group IV- AR root extract (150 mg/ kg body weight, orally by gavage)
Group V- AR root extract (ARRE-I + Lead nitrate)
Group VI- AR root extract (ARRE-II + Lead nitrate)

All the above groups were treated once daily for the period of 45 days. All these groups of mice i.e. group II to VI served as treated groups against group I as a control. The dose for lead nitrate was decided on the basis of experiments conducted in the laboratory and the concentration of lead nitrate was decided according to experiment of Plastunov and Zub, 2008. The plant doses were selected on the basis of experiments of the earlier published reports of Velavan et al., 2006, and on this base modified method was conducted in our own laboratory.

After the administration of the last dose, animals were given rest overnight and then weighed on the next day and blood was collected from eye-orbit in small blood collecting vial rinsed without anticoagulant. After withdrawal of blood, the obtained blood was allowed to coagulate at room temperature. A thin glass rod was moved gently along the side of the tube to retract the clot. After 1 h free serum was collected by a Pasteur pipette in a centrifuge tube. The tube with remaining clot was left overnight in cold for a final collection of serum. Blood cells were removed by centrifugation at 3000 rpm (REMI 9001:2000 CM-12) at 4°C for 15 min.
and the top most layer of clear serum was collected by means of Pasteur pipette. After collection of serum; it was used to assess lipid profile in various treated and untreated groups of animals.

**Serological lipid profile assays**

Serum lipid profile parameters such as total cholesterol (TC) \(^{21}\), triglyceride (TG) \(^{22}\), high density lipoprotein-cholesterol (HDL-C) \(^{23}\) were estimated by using respective diagnostic kits (Erba Mannheim cholesterol kit, transasia Biochemicals Ltd; Daman) and semiautomatic analyzer (Erba Mannheim, CHEM-5 Plus VZ). The protocols for the estimation of TC, TG, and HDL-C were followed as per instruction given in the kit literature.

LDL and VLDL-cholesterol were calculated as per Friedevald's equation \(^{24-26}\), according to which-

\[
\text{LDL-Cholesterol} = \text{Serum total cholesterol (TC)} - (\text{VLDL-C}) - (\text{HDL-C})
\]

\[
\text{VLDL-Cholesterol} = \frac{\text{Serum triglyceride}}{5}
\]

Results are expressed in mg/dl.

**Statistical analysis**

Results are expressed as the mean ±SEM. The data were analyzed by analysis of variance (ANOVA) followed by Tukey test using the Statistical Package for the Social Sciences (S.P.S.S. 16). The level of significance was set at p<0.05.

**RESULTS**

**Total Cholesterol (TC)**

After 45 days treatment, animals showed approximately \(\frac{1}{2}\) fold increase of total cholesterol concentration in lead treated animals in comparison to control (untreated) group I. The significant difference level was \(p<0.001\) in group I & II. Both dose of ARRE (low; 50mg/kg body wt. & high; 150 mg /kg body weight) on treatment significantly decreased (\(p<0.001\)) the level of TC in respective treated animals of groups III & IV as compared to lead exposed animals. However, no significant difference in the level of TC was observed in animals treated with two different doses of *Asparagus racemosus* when compared with control animals. That means ARRE stimulated TC content near to that of control values. Lower dose of plant extract tried to restore TC content to normal level to some extent in group III. Lead and ARRE administration amplified the TC content in both respective groups V & VI. The significant altered TC level for group V was \(p<0.001\) and for group VI was \(p<0.02\) when compared with lead exposed animals.

**Triglyceride (TG)**

Under the influence of lead nitrate treatment, lead augmented the TG content in treated animals. The increment level was significant (\(p<0.05\)) when compared with control animal group I. TG levels were found to be insignificantly less when treated with ARRE both dose individually in group III & IV as compared with control group I but on the other hand TG levels were found to be significantly lower (group III- \(p<0.05\); group IV- \(p<0.02\)) as compared with lead exposed mice group II. However complex dose of lead and ARRE have altered TG level in group V & VI when compared with control group I and lead treated animals. TG level in group V was significantly (\(p<0.001\)) reduced in comparison to lead treated animals but insignificant with control treated animals. This alteration was insignificant in group VI when compared to group I & II.

**High Density Lipoprotein-cholesterol (HDL-C)**

The HDL- cholesterol level in lead treated animals was insignificantly diminished (group II) when compared to control animal group I. Administration of individual doses of ARRE showed significant higher level (\(p<0.001\)) of HDL-C in both group III & IV in comparison to lead exposed animals. The low & high dose of *Asparagus racemosus* root extract individually but in combination with lead showed removal of lead toxicity to some extent but insignificant by improving the HDL-C level in group V & VI.

**Low Density Lipoprotein-cholesterol (LDL-C)**

The level of LDL- C in serum was significantly (\(p<0.001\)) higher in lead treated mice group II than the control mice group I. Both dose (low & high) of aqueous ARRE insignificantly reduced the LDL- C level in groups III & IV when compared with control but showed significant (\(p<0.001\)) depletion in LDL-C when compared to lead nitrate group II. The co-administration of low & high dose of aqueous ARRE extract along with lead showed significant (\(p<0.001\)) depletion in LDL-C.
Table 1: Effect of Lead nitrate and aqueous root extract of *Asparagus racemosus* (ARRE) either individually or in combination on TG, TC and HDL-C

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Treated Doses (mg/kg/oral)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I- (Control)</td>
<td>Nil</td>
<td>99.82 ± 4.82</td>
<td>75.22 ± 5.69</td>
<td>26.04 ± 1.61</td>
</tr>
<tr>
<td>Group II- (Lead Nitrate); (LN)</td>
<td>20 mg/kg body weight</td>
<td>147.38 ± 6.48^*c</td>
<td>96.05 ± 6.14^*c</td>
<td>19.15 ± 2.02^ ns</td>
</tr>
<tr>
<td>Group III- (ARRE-1)</td>
<td>50 mg/kg body weight</td>
<td>94.02 ± 9.00^*d</td>
<td>73.8 ± 6.13^*c</td>
<td>29.08 ± 2.11</td>
</tr>
<tr>
<td>Group IV- (ARRE-2)</td>
<td>150 mg/kg body weight</td>
<td>103.64 ± 8.78^*d</td>
<td>71.49 ± 2.42^*b</td>
<td>35.12 ± 1.33</td>
</tr>
<tr>
<td>Group V- (LN+ ARRE-1)</td>
<td>20 mg/kg body weight + 50mg/kg body weight</td>
<td>113.24 ± 6.23^*d</td>
<td>67.18 ± 3.42^*e^*f</td>
<td>22.43 ± 1.71^ns</td>
</tr>
<tr>
<td>Group VI- (LN+ARRE-2)</td>
<td>20 mg/kg body weight + 150 mg/kg body weight</td>
<td>126.45 ± 4.79^*b</td>
<td>78.47 ± 6.03^*b</td>
<td>24.06 ± 1.91^ns</td>
</tr>
</tbody>
</table>

Abbreviations- ARRE: Asparagus racemosus root extract, LN: Lead nitrate, TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol. Very low density lipoprotein cholesterol. All parameters are expressed in mg/dl. Values are expressed as the mean ± SEM for n=6 mice per group. *change with respect to control group I, †change with respect to toxicant treated group II, ^ns= not significant change with respect to control group I, †ns= not significant change with respect to toxicant treated group II, ns= not significant change with respect to both groups control group I and toxicant treated group II; a, b, c, d; different letters are used to show different levels of statistically significant, a= p<0.01, b=p<0.02, c= p< 0.05, d= p< 0.001; (Tukey’s multiple comparison test).

Table 2: Effect of Lead nitrate and aqueous root extract of *Asparagus racemosus* (ARRE) either individually or in combination on LDL-C and VLDL-C.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Treated Doses (mg/kg/oral)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I- (Control)</td>
<td>Nil</td>
<td>58.68 ± 2.64</td>
<td>14.94 ± 1.18</td>
</tr>
<tr>
<td>Group II- (Lead Nitrate); (LN)</td>
<td>20 mg/kg body weight</td>
<td>113.98 ± 4.15^*bd</td>
<td>14.29 ± 0.48^ ns</td>
</tr>
<tr>
<td>Group III- (ARRE-1)</td>
<td>50 mg/kg body weight</td>
<td>48.27 ± 6.32^*d</td>
<td>16.66 ± 1.18^ns</td>
</tr>
<tr>
<td>Group IV- (ARRE-2)</td>
<td>150 mg/kg body weight</td>
<td>49.3 ± 6.44^*d</td>
<td>19.20 ± 1.22^*b^c</td>
</tr>
<tr>
<td>Group V- (LN+ ARRE-1)</td>
<td>20 mg/kg body weight + 50mg/kg body weight</td>
<td>79.04 ± 3.48^*d</td>
<td>13.43 ± 0.68^ns</td>
</tr>
<tr>
<td>Group VI- (LN+ARRE-2)</td>
<td>20 mg/kg body weight + 150 mg/kg body weight</td>
<td>86.61 ± 1.76^*d</td>
<td>15.69 ± 1.20^ns</td>
</tr>
</tbody>
</table>

Abbreviations- LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol. All parameters are expressed in mg/dl. Values are expressed as the mean ± SEM for n=6 mice per group. *change with respect to control group I, †change with respect to toxicant treated group II, ^ns= not significant change with respect to control group I, †ns= not significant change with respect to toxicant treated group II, ns= not significant change with respect to both groups control group I and toxicant treated group II; a, b, c, d; different letters are used to show different levels of statistically significant, a= p<0.01, b=p<0.02, c= p< 0.05, d= p< 0.001; (Tukey’s multiple comparison test).
level in both groups V & VI when compared with lead nitrate exposed animals of group II.

**Very Low Density Lipoprotein -cholesterol (VLDL-C)**

Lead declined the VLDL-C content in lead treated animals. The declined level was insignificant when compared with control animal group I. VLDL-C levels were found to be elevated when animals were treated with ARRE both doses individually in group III & IV as compared with control group I. Only the higher dose of extract produced significant effect when compared with lead exposed animals (p<0.02) and control animals (p<0.05). However complex dose of lead and ARRE treated mice have modified VLDL-C levels in group V & VI when compared with control group I and lead treated group II. No significant differences were observed with both control and lead treated animals.

**DISCUSSION**

The main finding of this study is that serum cholesterol and lipoprotein levels were higher in subjects who were exposed to lead toxicant than in those who were not exposed (Table 1and 2). Lead nitrate increased level of TC, TG and LDL as well as lowered level of HDL has been identified as contributors in the development of lead toxicity. The decrease of VLDL content in serum of male mice was altered to some extent but not significant. The elevation of lipid components is a risk factor for coronary heart disease27-29. Microscopic analysis of lead-intoxicated ani-mals has indicated fatty degeneration of the myocardium and sclerotic changes in the aorta and walls of the small arteries, especially the renal, cerebral and coronary arteries [7-9, 30], and atrophy of elastic fibers in the aorta31. Thus, it has been suggested that one of the underlying mechanisms in the association between cardiovascular damage and lead exposure is the induction or acceleration of atherosclerosis32. The present results were found similar with few studies of the effects of lead exposure with different dose on serum lipids and lipoprotein levels in either animals5,31 or humans1,5. Revis et al., 1980; Tarugi et al., 1982; Stofen, 1974; Xiao et al., 1989; Yagminas et al., 1990 reported increase in serum cholesterol levels. The assessment of a possible rela-tionship between serum lead level and lipids is an important step in elucidating the mecha-nisms underlying the excess cardiovascular morbidity among lead-exposed subjects2. An increase in HDL cholesterol was reported by Cocco *et al.*, (1995). The association between lead exposure and high serum lipid levels is biologically plausible and could be due to either increased synthesis or decreased removal of lipoproteins. Decreased removal may occur as a result of the alteration of cell surface receptors for lipoproteins33 or as a result of the inhibition of hepatic lipoprotein lipase activity37. Further-more, lead has been shown to depress the activity of cytochrome P-450 38, 39. This can limit the biosynthesis of bile acids, which is the only significant route for e1imi-nation of cholesterol from the body. Increased synthesis may be due to a lead induced increase in hepatic enzymes at important control points for de novo cholesterol synthesis, as has been found in Wistar rats6, or it may be due to impaired feedback inhibition4.

Statistically very high significant decrease of TC, TG and LDL were found in the serum of ARRE treated mice group III and IV. On the contrary, the changes in HDL and VLDL were also noticed after ARRE alone treatment with two different doses. Effect of the extract tested on serum lipid profile showed that the extract had significant effect on cholesterol. It however reduced triglyceride significantly. Like other plant constituents ARRE reduced TG level and it could be suggested that ARRE increased lipase activity which hydrolyzed the triglyceride level. In human nutrition, triglycerides are the most prevalent glycerol esters encountered. They constitute 95% of tissue storage fat and are the predominant form of glycerol esters found in plasma. Following absorption, triglycerides are resynthesized in the epithelial cells and combine with proteins to form chylomicrons which travel through the lymphatic system to the thoracic duct and eventually to the jugular vein41. The significant reduction of triglyceride by the extract is indicative of the lipid-lowering potential of the extract in mixed hyperlipidaemic states16. ARRE may act as inhibitor for enzyme such as hydroxyl-methyl-glutaryl-CoA reductase, which is the key enzyme in de novo cholesterol biosynthesis as has been suggested for some plants earlier. Hanna *et al.* (2007) found that ARRE responsible to enhance the insulin
secretion and able to inhibit the \( K_{ATP} \) channel opener, diazoxide, \(^{42}\) reduced the insulin-releasing action. This reduction could be beneficial in improving lipid metabolism and complications in diabetes \(^{14}\). The total cholesterol and triglycerides in serum, and LDL-cholesterol were significantly reduced with both doses of ARRE and showed alteration with lead nitrate supplementation. However, HDL-cholesterol level increased in both treated groups significantly. This observation indicates that *Asparagus racemosus* root powder, as an aqueous extract form is effective in reducing serum LDL-C and alteration in VLDL-cholesterol levels. It is well known that increased HDL-cholesterol levels have a protective role in CAD \(^{43}\). The decreased lipids including cholesterol and triglycerides in treated animals along with increased bile acid, cholesterol and neutral sterols content in fecal matter indicate that *Asparagus racemosus* may reduce the absorption of dietary cholesterol and enhance its excretion. A similar result was reported when soy protein was used as feed supplement \(^{44,45}\). Authors found increased HMG CoA reductase activity in experimental groups. This could be due to an increased cholesterol excretion and decreased cholesterol absorption through the gastrointestinal tract. Thus the decreasing cholesterol levels in the body under the influence of *Asparagus racemosus* could have enhanced the enzymatic activity by a positive feedback mechanism. Further, increased bile acid production also indicates the turnover of endogenous cholesterol into bile acid that could be under the influence of supplementary feeding with *Asparagus racemosus*. 'Abana', a herbo-mineral formulation containing 10 mg *A. racemosus* extract per tablet, was found to have significant hypocholesterolaemic effect in rats and therefore demonstrated a potential for use as a cardioprotective agent \(^{10}\). They found that the total cholesterol, phospholipids and triglyceride levels were significantly lower (37–45%) as against the control. Since 'Abana' is a polyherbal formulation, further research needs to be conducted on the exact role that the *A. racemosus* component plays in the hypolipidaemic action.

**CONCLUSION**

The present work shows that the *Asparagus racemosus* treated groups have higher levels of antioxidative parameters (catalase and superoxide dismutase) and decreased level of lipid peroxidation indicating its efficacy to reduce the LDL-cholesterol oxidation. The present study concluded that ARRE could modify lead toxicity in mice to some extent. The results of this study indicate that the potent therapeutic phyto-components present in ARRE, could be responsible for increased HDL, elimination of excess cholesterol and elevation of reverse conditions of lipid profile. Thus the present study might be fruitful to a major extent in reducing risk of toxicity in population exposed to lead nitrate.

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