Asparagus Root Regulates Cholesterol Metabolism and Improves Antioxidant Status in Hypercholesteremic Rats

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Hyperlipidemia/hypercholesteremia are major risk factors for atherosclerosis and cardiovascular diseases. Root of Asparagus racemosus (AR) is widely used in Ayurvedic system of medicine in India and is known for its steroidal saponin content. This study was designed to investigate the hypocholesteremic and antioxidant potential of AR root in both normo- and hypercholesteremic animals. Normal and hypercholesteremic male albino rats were administered with root powder of AR (5 and 10 g% dose levels) along with normal and hypercholesteremic diets, respectively, for a duration of 4 weeks. Plasma and hepatic lipid profiles, fecal sterol, bile acid excretion and hepatic antioxidant activity were assessed. Inclusion of AR root powder in diet, resulted in a dose-dependant reduction in plasma and hepatic lipid profiles, increased fecal excretion of cholesterol, neutral sterol and bile acid along with increases in hepatic HMG-CoA reductase activity and bile acid content in hypercholesteremic rats. Further, AR root also improved the hepatic antioxidant status (catalase, SOD and ascorbic acid levels). No significant changes in lipid and antioxidant profiles occurred in the normocholesteremic rats administered with AR root powder. AR root appeared to be useful as a dietary supplement that offers a protection against hyperlipidemia/hypercholesteremia in hypercholesteremic animals. The results of the present study indicate that the potent therapeutic phyto-components present in AR root i.e. phytosterols, saponins, polyphenols, flavonoids and ascorbic acid, could be responsible for increased bile acid production, elimination of excess cholesterol and elevation of hepatic antioxidant status in hypercholesteremic conditions.

Keywords: antioxidant – Asparagus racemosus – cholesterol metabolism – hypercholesteremia

Introduction

Atherosclerotic cardiovascular disease is rapidly becoming a major cause of death in many societies throughout the world due to changed dietary habits and occupational stress. The hallmark of atherosclerosis is the accumulation of cells containing excessive lipids (i.e. foam cells) within the arterial wall. The major risk factors for the development of atherosclerosis are hypercholesterolemia and elevated low-density lipoprotein cholesterol (LDL-C) concentration (1). Persistent hypercholesteremia results from prolonged circulation of lipid-rich lipoproteins that increase oxidative stress leading to oxidative modification of LDL to oxy-LDL (2,3). A number of plants with potent therapeutic components such as fibers, sterols, saponins, polyphenols, flavonoids and ascorbic acid, have been investigated for their antihyperlipidemic, antioxidant and antiatherosclerotic properties. These compounds are reported to be beneficial with great variation in magnitude and mechanism of action and hence have a potential therapeutic value in combating multifactorial atherosclerotic disorders (4–9). Research in such plant-based diet therapy therefore, has a major role in reducing the risk of atherosclerosis, which can lead to development of effective and better management.
of hyperlipidemia thereby reducing the risk of cardiovascular diseases.

*Asparagus racemosus* (AR) willd. (Family: Liliaceae), commonly known as shatavari, satawar or satmuli, is found at low altitudes throughout India. The dried root of the plant is used in Ayurveda as a tonic, galactogogue, aphrodisiac, rejuvenator, antispasmodic, antitumor and anti-inflammatory and the medicinal/pharmacological value of AR root is attributed to the presence of steroidal saponins and sapogenins (10,11). The root of AR is also used in the treatment of nervous disorders, dyspepsia, diarrhea, dysentery, tumors, hyperdipsia, neuropathy and hepatopathy (11). This plant is reported to have immunostimulant, antihepatotoxic and antioxytocic activities (11,12). Recent reports on AR indicate that the root extracts have antioxidant and antidiarrheal activities in laboratory animals (13,14). The present study was undertaken to investigate the potential of *Asparagus* root (powder) as a dietary supplement in normalizing the hyperlipidemic/hypercholesteremic and oxidative stress conditions induced by chronic intake of lipid-rich diets with regard to plasma and hepatic lipid profiles, cholesterol metabolism and antioxidant status. Additionally, the effect of *Asparagus* root was assessed on normocholesteremic animals with a view to investigate and to compare its influence on lipid metabolism in both normal and hyperlipidemic rats.

**Methods**

**Plant Material**

Roots of AR were collected from university botanical garden, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India. The roots were washed thoroughly with tap water and dried at 37°C in an incubator, diced into small pieces, powdered in a mixer grinder and used for the experiments. Phytosterol and saponin content of the root were estimated using ferric chloride—sulphuric acid and vanillin-sulphuric acid methods, respectively (15,16). The polyphenol and flavonoid content of the root were analyzed using Folin–Ciocalteu and vanillin-sulphuric acid reagents, respectively (17,18). The total ascorbic acid (TAA) content was estimated using 2,4-dinitrophenyl hydrazine reagent (19) (Table 1).

**Albino Rats**

Male albino rats (Charles Foster) weighing 150–200 g were used for the experiment. Rats were housed individually with *ad libitum* access to water in a well-ventilated animal unit (26 ± 2°C, humidity 62%, 12 h light/dark cycle). Rats were fed standard Chow (Pranav Agro-Industries Ltd., Baroda, India). The care and procedures adopted for the present investigation were in accordance with the approval of Institutional Animal Ethics Committee.

**Experimental Design**

After a 10-day adaptation period, 48 rats were divided into 6 groups of 8 rats each (NC-normal control; NAR-I-normal animals administered with 5 g% AR root powder; NAR-II- normal animals administered with 10 g% AR root powder; HC- hypercholesteremic animals; HAR-I- hypercholesteremic animals administered with 5 g% AR root powder; HAR-II- hypercholesteremic animals administered with 10 g% AR root powder). Hypercholesteremia was induced by addition of 0.5 g% cholesterol and 1.0 g% sodium taurocholate to the basal diet. The basal diet contained carbohydrates (56 g%), proteins (22 g%), fat (4 g%), fiber (4 g%) and mineral mixture (6 g%).

Their body weight and food intake were recorded every third day during the experimental period (4 weeks). Fecal samples of the last 24 h of the experimental period were collected from individual animals from each group (5 g/rat), dried, powdered and extracted for fecal sterols. At end of experiment, animals were deprived of food overnight, sacrificed under light anesthesia and blood was collected immediately by cardiac puncture; plasma was separated by centrifugation. Liver was excised and, both plasma and liver were kept frozen until analyzed.

**Biochemical Analysis**

**Plasma and Hepatic Lipid Profiles**

Plasma total lipid (TL) content was estimated by sulphophosphovanillin method (20). Total cholesterol (TC) and triglycerides (TG) were estimated by ferric perchlorate-sulphuric acid and L-α-glycerophosphate oxidase (GPO) methods, respectively (21,22). HDL-cholesterol (HDL-C) was extracted using phosphotung-ate-magnesium chloride reagent from plasma (23) and estimated (21). LDL-C, very low-density lipoprotein cholesterol (VLDL-C) and Atherogenic Index (AI) was calculated (24). The liver TL was extracted in chloroform: methanol (2:1) (25) and estimated by

<table>
<thead>
<tr>
<th>Phytoconstituents mg/g dry tissue</th>
<th>AR root</th>
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<tbody>
<tr>
<td>Phytosterols</td>
<td>7.90±0.90 (0.79%)</td>
</tr>
<tr>
<td>Saponins</td>
<td>88.33±1.61 (8.333%)</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>16.92±1.10 (1.692%)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>4.76±0.32 (0.476%)</td>
</tr>
<tr>
<td>Total ascorbic acid</td>
<td>7.62±0.06 (0.762%)</td>
</tr>
</tbody>
</table>

Table 1. Phytoanalysis of AR root (Triplicate values: mean ± standard deviation)
gravimetric analysis. TC and TG were extracted (25) and estimated (21,22).

**Hepatic HMG-CoA Reductase and Bile Acid Profile**

Hepatic HMG-CoA reductase (EC 1.1.1.34) activity was measured in terms of the ratio of HMG-CoA to mevalonate (26). Colorimetric assays were carried out for both HMG-CoA and mevalonate using hydroxylamine reagent at alkaline and acidic pH, and the ratio of HMG-CoA to mevalonate determined. It is inversely proportional to the enzyme activity i.e. the increase in ratio corresponds to a decrease in enzyme activity. The alkaline-ethanolic extract of hepatic bile acid was acidified and estimated using vanillin-phosphoric acid reagent (27).

**Fecal Cholesterol, Neutral Sterol and Bile Acid Content**

The fecal cholesterol, neutral sterols and bile acid were extracted using alkaline methanol medium (28) for fecal cholesterol and neutral sterols estimation (21,29). A portion of the extract was acidified and used for bile acid estimation (27).

**Hepatic Antioxidant Profile**

Catalase (EC 1.11.1.6) was assayed spectrophotometrically as decomposition of H₂O₂ at 240 nm (30) and superoxide dismutase (SOD; EC 1.15.1.1) activity was measured using nitrobluetetrazolium reduction method (31). TAA contents were estimated (19).

**Statistical Evaluation**

Results are expressed as means ± SEM. Significant differences among the groups were determined by one-way ANOVA using 10th version of SPSS with Duncan’s test as post hoc analysis. Differences were considered significant if $P < 0.05$.

**Results**

**Body Weight, Food Intake and Liver Weight**

There were no significant differences in final body weights; food intake or liver weights in all AR-treated groups except HAR-II group. This group registered a significant decline in liver weight ($P < 0.05$, 22%) as compared to HC group (data not presented).

**Plasma and Hepatic Lipids**

When normal animals administered with AR root powder at 5 and 10 g% dosages, the plasma and hepatic lipid profiles showed no significant differences although NAR-II animals registered a significant increase in plasma HDL-C fraction ($P < 0.05$, 11%) as compared to NC animals. However, AR administered hypercholesteremic groups (HAR-I and HAR-II) revealed significant decreases ($P < 0.05$) in TL (14%, 21%), TC (25%, 35%), LDL-C (32%, 45%) and AI (37%, 49%) with increase in HDL-C ($P < 0.05$, 19%, 27%) as compared to HC group. The TG and VLDL-C content however, remained unaffected in these groups (Figs. 1–7). The HAR-I and HAR-II groups also registered a significant reduction ($P < 0.05$) in hepatic TL (13%, 24%) and TC (21%, 36%) while the TG level showed no significant change (Figs. 8–10).
Cholesterol Metabolism

The NAR-I and NAR-II groups revealed no significant changes in hepatic HMG-CoA reductase activity and bile acid production when compared to those of NC animals. The HAR-I and HAR-II groups however, exhibited a significant rise ($P < 0.05$) in HMG-CoA reductase activity (25%, 35%) and bile acid production (28%, 36%) as compared to HC group (Figs. 11 and 12). There was no significant variation in fecal cholesterol, neutral sterol and bile acid content of NAR-I and NAR-II groups as compared to NC group. However, both HAR-I and HAR-II groups revealed significantly higher ($P < 0.05$) amounts of cholesterol (29%, 56%), neutral

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Figure 4. Effect of AR feeding on plasma HDL-C levels. Results are mean ± SEM, $n = 8$. Values with different letters are significantly different from each other ($P < 0.05$).

Figure 5. Effect of AR feeding on plasma LDL-C levels. Results are mean ± SEM, $n = 8$. Values with different letters are significantly different from each other ($P < 0.05$).

Figure 6. Effect of AR feeding on plasma VLDL-C levels. Results are mean ± SEM, $n = 8$. Values with different letters are significantly different from each other ($P < 0.05$).

Figure 7. Effect of AR feeding on plasma AI. Results are mean ± SEM, $n = 8$. Values with different letters are significantly different from each other ($P < 0.05$).

Figure 8. Effect of AR feeding on liver TL levels. Results are mean ± SEM, $n = 8$. Values with different letters are significantly different from each other ($P < 0.05$).

Figure 9. Effect of AR feeding on liver total cholesterol levels. Results are mean ± SEM, $n = 8$. Values with different letters are significantly different from each other ($P < 0.05$).

Figure 10. Effect of AR feeding on liver TG levels. Results are mean ± SEM, $n = 8$. Values with different letters are significantly different from each other ($P < 0.05$).

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Cholesterol Metabolism

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sterol (30%, 55%) and bile acid (28%, 37%) in fecal matter when compared to HC group (Figs. 13–15).

Antioxidant Status

The antioxidant status (activities of catalase, SOD and ascorbic acid content) did not vary significantly in NAR-I and NAR-II groups from that of NC. However, HAR-I and HAR-II groups registered a significant increase ($P < 0.05$) in catalase (33%, 35%) and SOD (21%, 29%) activities and TAA content (20%, 23%) as compared to HC group (Figs. 16–18).
In plasma cholesterol levels (8,33), the circulation of bile acids making it unavailable for cholesterol from micelles and interfere with enterohepatic (7,32) and on the other hand, saponins precipitate bile acid micelles and decrease the cholesterol circulation to compete and displace cholesterol from the intestinal excretion and decreased cholesterol levels in the hypercholesteremic animals. This observation indicates that the effect of AR root on body cholesterol metabolism is strongly influenced by hyperlipidemia and hypercholesteremia. It is well documented that while a low level of HDL-C is indicative of high risk for cardiovascular disease, an increase in HDL-C level could potentially contribute to antiatherogenicity, inhibit LDL oxidation and protect endothelial cells from the cytotoxic effects of oxidized LDL (44,45). In the present context, a significant increase in plasma HDL-C levels with a concurrent decline in plasma cholesterol level and an improvement in AI of HAR-I and HAR-II animals clearly indicate the beneficial role of AR root administration to hypercholesteremic animals.

An increased production of superoxide anions and metabolites and/or a reduced bioavailability of antioxidants causes oxidative damage to cells and tissues. An imbalance between pro-oxidant and antioxidant levels in the body gives rise to cellular oxidative stress that plays an important role in the genesis of cardiovascular diseases (46). Cholesterol-rich diets are known to increase both LDL-C levels and oxidative stress and result in increased oxidized LDL levels leading to atherosclerotic plaque formation (3). Several studies suggest that naturally occurring antioxidants such as polyphenols, flavonoids and vitamin C in diet may play a role as antiatherogenic agents (3,9,47,48). The polyphenols and flavonoids are known to scavenge free radicals, including hydroxyl and...
superoxide anions, inhibit lipid peroxidation and improve lipid profiles (47–50). Both these are also known to stimulate catalase and SOD gene transcription and decrease malondialdehyde concentration (51,52). Several lines of evidence suggest that ascorbic acid is a powerful antioxidant in biological systems as an electron donor, readily scavenging the reactive oxygen and nitrogen species to prevent cellular oxidative damage (53) and to reduce genotoxic effects of lipid peroxidation products (54). Further, ascorbic acid can also stimulate SOD activity (55). Wiboonpun et al. (56) identified that roots of AR possess an in vitro antioxidant property against DPPH and the antioxidant compound was identified as racemofuran along with two known compounds—asparagamine A and racemosol. Furan fatty acids possess a radical-scavenging ability and are generated in large quantities in both plants and algae, and when animals consume foods containing these furan fatty acids, they contribute to the body’s overall antioxidant activity owing to their affinity to react with peroxyl radicals (57). Additionally, Asparagus root extract possesses in vivo antioxidant activity in rats although the principles responsible for this antioxidant activity were not reported (13). Presently noted higher levels of hepatic catalase and SOD activities in HAR-I and HAR-II groups indicate the possible role of polyphenols and flavonoids of AR root, in modulating the expression of both catalase and SOD activities. While the hypercholesterolemia reduced the hepatic ascorbic acid content in HC group, both HAR-I and HAR-II groups registered significantly higher amounts of ascorbic acid in hepatic tissues. Taken together, these observations indicate that AR root powder administration to HC animals can reduce lipid levels in both blood and liver, increase bile acid production and modulate antioxidant enzyme activities. We conclude that AR root administration could ameliorate hyperlipidemic/hypercholesteremic and oxidative stress in hypercholesterolemic conditions.

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References


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