Effect of Three Plant Extracts and Atorvastatin on Serum Lipids and Lipoproteins in Rats Fed High Cholesterol Diet

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Abstract
Introduction: To evaluate the relative efficacy of Gloriosa superba, Valeriana wallichii and Odenlandia corymbosa extracts and atorvastatin in correcting the dyslipidemia induced by feeding high cholesterol diet to rats.

Methods: The study was carried out on healthy male adult rats of Wistar strain. Dyslipidemia was induced by feeding them fructose-rich high fat diet (F-HFD). Gloriosa superba extract, Valeriana wallichii extract, Odenlandia corymbosa extract and atorvastatin were given orally for 30 days along with F-HFD to four different groups. Serum lipids and lipoproteins were measured.

Results: Feeding F-HFD for 30 days led to dyslipidemia in the form of raised serum cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol and free fatty acids. In the rats fed F-HFD + Valeriana wallichii extract, serum cholesterol, triglycerides and VLDL-cholesterol were significantly lower as compared to rats fed F-HFD alone. No such effect was seen in the rats given F-HFD + Gloriosa superba extract and F-HFD + Oldenlandia corymbosa extract. In the rats fed F-HFD + atorvastatin, serum lipids were significantly lower as compared to rats fed F-HFD alone and the effect was stronger than that of Valeriana wallichii.

Conclusion: Valeriana wallichii possesses anti-dyslipidemic activity but is not as effective as atorvastatin.

Keywords: Anti-dyslipidemic, Gloriosa superba, Valeriana wallichii, Odenlandia corymbosa.

Introduction
Dyslipidemia is an abnormality of plasma lipids in which plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) are elevated and high-density lipoprotein cholesterol (HDL-C) is lowered, either singly or in combination¹. Detection and correction of this condition is important for prevention and control of cardiovascular disease (CVD) and its sequelae. In 80% cases, lipid abnormalities are related to diet and lifestyle while heredity plays a role in the remaining cases². The prevalence of dyslipidemia is increasing worldwide³,⁴. Studies have shown increased prevalence of dyslipidemia and have attributed it to increasing prevalence of obesity and type 2 diabetes mellitus, which are linked to changes in lifestyle associated with modernization and socioeconomic development⁵.

A number of anti-dyslipidemic drugs are available in modern medicine to treat abnormal plasma lipids. The drugs used for controlling dyslipidemia include fibrates, statins and bile acid-binding resins. The statins are the most widely used out of these. However, long-term use of these drugs is not free from undesirable effects. Atorvastatin, a widely used lipid-lowering agent, is an inhibitor of HMG-CoA reductase, which catalyzes the conversion of HMG-CoA to
mevalonate. Inhibition of HMG-CoA reductase decreases cholesterol synthesis and leads to upregulation of LDL cholesterol (LDL-C) receptors in the liver, mediated by activation of sterol regulatory element binding protein resulting in enhanced clearance of LDL from the circulation, thus playing an important role in preventing atherosclerosis.

Some indigenous plants are supposed to possess cardio-protective properties e.g. Gloriosa superba, Valeriana wallichii and Odenlandia corymbosa. Gloriosa superba is commonly known as Glory Lily (Bachnag in Hindi) and belongs to family Liliaceae. It is found commonly in forest and is under cultivation in fairly large areas of India. Valeriana wallichii belongs to Valerianeaceae family and grows in temperate Himalayas from Kashmir to Bhutan. Ithas been used in the Ayurvedic system of medicine for centuries. Its native names include Tagara, Nata, Vakra etc. Odenlandia corymbosa belongs to family Rubiaceae and is commonly known as Diamond Flower (Daman Pappar in Hindi). It is found all over India.

Since plant products are generally safe, the present study was undertaken to evaluate the effectiveness of Gloriosa superba, Valeriana wallichii and Odenlandia corymbosa extracts in correcting the dyslipidaemia resulting from feeding fructose-rich high fat diet to rats and to compare their effect with that of atorvastatin.

Material and Methods
Authenticated samples of Gloriosa superba, Valeriana wallichii and Odenlandia corymbosa were procured. The plant samples were dried under shade. They were ground by an electric blender, and the resulting powder (200 g) was extracted with one liter of 70% alcohol for 72 hours at room temperature. The extract was filtered and the solvent was evaporated to dryness. The yield of extract was calculated and stored at 2-8°C for further use.

The study was carried out on healthy male adult rats of Wistar strain (200-225 g) obtained from the Animal House of King George’s Medical University, Lucknow (U.P.). The study was started after obtaining permission from the Animal Ethics Committee of the University. All the rats were initially maintained on Hindustan Lever Food Pellets diet and water ad libitum. The cages were kept in a temperature and humidity controlled room with a 12-hour light-dark cycle. The rats were divided into six groups of six rats each and were fed as follows for 30 days:

Group 1 - Control rats fed normal pellet diet.
Group 2 - Rats fed fructose-rich high fat diet (F-HFD).
Group 3 - Rats fed F-HFD and given extract of Gloriosa superba (250 mg/kg of body weight orally once a day).
Group 4 - Rats fed F-HFD and given extract of Valeriana wallichii (250 mg/kg of body weight orally once a day).
Group 5 - Rats fed F-HFD and given extract of Odenlandia corymbosa (250 mg/kg of body weight orally once a day).
Group 6 - Rats fed F-HFD and given atorvastatin (10 mg/kg of body weight orally once a day).

After 30 days, serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-C) were quantified by CHOD-PAP, GPO-POD and PEG-PAP methods respectively, and low density lipoprotein cholesterol (LDL-C) by immuno-turbidimetric assay. Very low density lipoprotein cholesterol (VLDL-C) was calculated by Friedewald’s formula. Serum free fatty acids (FFA) were measured by the method of Monsinger et al and phospholipids (PL) by malachite green method.

Plasmalecithin cholesterol acyl transferase (LCAT) activity was measured according to Albers et al and post heparin lipolytic activity (PHLA) according to Wind and Robinson.

Statistical Analysis
Student’s unpaired t-test was used for comparison. The p value<0.05 was considered significant. Analysis was carried out on SPSS 16.0 version.
**Results**

The rats fed F-HFD for 30 days had significantly higher serum TC, TG, LDL-C, VLDL-C, HDL-C, and FFA than the rats fed normal pellet diet. No significant difference was seen in serum PL, LCAT, and PHLA (Table 1). Thus, feeding F-HFD for 30 days produced significant dyslipidemia.

**Table 1:** Final levels of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), free fatty acids (FFA), phospholipids (PL), lecithin cholesterol acyl transferase (LCAT) and post heparin lipolytic activity (PHLA) in rats fed normal diet and rats fed fructose-rich high fat diet (F-HFD) for 30 days (all the values are mean±SD).

|                      | Rats on normal diet | Rats on F-HFD       |
|----------------------|---------------------|---------------------|
| TC (mg/dL)           | 62.6±9.72           | 128±18.2**          |
| TG (mg/dL)           | 48.1±11.2           | 110±16.6**          |
| HDL-C (mg/dL)        | 28.3±8.61           | 40.5±6.86**         |
| LDL-C (mg/dL)        | 24.6±8.2            | 65.5±19.2**         |
| VLDL-C (mg/dL)       | 9.6±2.2             | 22±3.3**            |
| FFA (mg/dL)          | 62.5±18.9           | 90.1±11.6*          |
| PL (mg/dL)           | 102.3±23.7          | 128.1±26.3          |
| LCAT (nmol/mL/h)     | 36.4±14.7           | 44.1±13.4           |
| PHLA (nmol/mL/h)     | 21.0±3.8            | 21.6±5.50           |

*p<0.05, **p<0.001 and ***p>0.05 as compared to normal diet group.

**Table 2:** Final levels of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), free fatty acids (FFA), phospholipids (PL), lecithin cholesterol acyl transferase (LCAT) and post heparin lipolytic activity (PHLA) in rats fed fructose-rich high fat diet (F-HFD), F-HFD + G. superba extract (250 mg/kg BW/day), F-HFD + V. wallichii extract (250 mg/kg BW/day), F-HFD + O. corymbosa extract (250 mg/kg BW/day) and F-HFD + atorvastatin (10 mg/kg BW/day) for 30 days (all the values are mean±SD).

|                      | F-HFD                           | F-HFD + G. superba extract | F-HFD + V. wallichii extract | F-HFD + O. corymbosa extract | F-HFD + atorvastatin |
|----------------------|---------------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------|
| TC (mg/dL)           | 128±18.2                        | 120±16.6                    | 91.5±14.6**                  | 124.6±17.0**               | 68.3±11.6***          |
| TG (mg/dL)           | 110±16.6                        | 90±11.8*                    | 70.5±12.1***                 | 99.1±12.8*                 | 57±4.73***            |
| HDL-C (mg/dL)        | 40.5±6.86                       | 31.6±5.00**                 | 30.1±6.61**                  | 38.1±2.85*                 | 29.1±6.79*            |
| LDL-C (mg/dL)        | 65.5±19.2                       | 71±14.9*                    | 47.2±11.2*                   | 70±17.6*                   | 27.7±12.3***          |
| VLDL-C (mg/dL)       | 22.0±3.3                        | 18±2.3*                     | 14.1±2.4***                  | 19.8±2.5*                  | 11.4±0.94***          |
| FFA (mg/dL)          | 90±11.6                         | 84.1±12.4*                  | 71.5±11.0*                   | 86.6±11.8*                 | 59±17.1*              |
| PL (mg/dL)           | 128.1±26.3                      | 102.6±17.8*                 | 92.1±15.7*                   | 120±27.1*                  | 80.6±3.14**           |
| LCAT (nmol/mL/h)     | 44.1±13.4                       | 50±12.9*                    | 54.1±11.5*                   | 49.1±9.96*                 | 59.8±10.4*            |
| PHLA (nmol/mL/h)     | 21.6±5.00                       | 16.8±5.07*                  | 13±3.14*                     | 17.8±4.16*                 | 11.8±2.63*            |

*p<0.05, **p<0.01, ***p<0.001 and p>0.05 as compared to F-HFD group.

**Discussion**

Dyslipidemia in the form of elevated total cholesterol, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol and triglycerides and decreased high density lipoprotein (HDL) cholesterol is one of the...
major coronary risk factors. There is a strong pathophysiological association between raised LDL cholesterol level and initiation and progression of coronary atherosclerosis\(^{14}\).

Currently available therapies for dyslipidemia in modern medicine, e.g. fibrates, statins and bile acid sequestrates, correct lipid abnormalities to a significant extent but can also produce adverse effects. Therefore, there is a need to develop safe and effective treatment modalities for dyslipidemia. Medicinal plants can provide drugs that are less toxic, have fewer side effects and are cost effective. Some plants are supposed to have cardio-protective effects. If we can find plant products with lipid-lowering effect, they can provide a new option for the treatment of dyslipidemia.

We explored three plants viz. *Gloriosa superba*, *Valeriana wallichii* and *Oldenlandia corymbosa* for their possible anti-dyslipidemic effect in rats. For comparison, we took a standard drug i.e. atorvastatin. *Gloriosa superba* and *Oldenlandia corymbosa* did not show an anti-dyslipidemic effect but *Valeriana wallichii* was found to have a significant anti-dyslipidemic effect. When *Valeriana wallichii* extract and atorvastatin were compared, it was seen that the effect of atorvastatin was stronger. This finding is in agreement with the report of Zanguei and Jowhary who had investigated the effect of a different species of the same genus i.e. *Valeriana officinalis*\(^{15}\).

The results of the present study show that *Valeriana wallichii* extract can favorably modify the dyslipidemia resulting from feeding high cholesterol diet to rats. The extract was somewhat less effective than atorvastatin but if we can isolate the active compound from the plant, it may turn out to be more effective. One of the limitations of the present study was small sample size. Studies on larger samples are warranted for robust results.

**Conclusion**

*Valeriana wallichii* extract lowers serum lipid levels but is not as effective as atorvastatin.

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