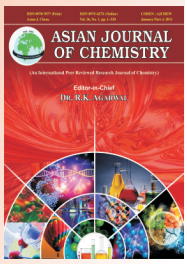
Asian Journal of Chemistry; Vol. 26, No. 9 (2014), 2617-2620

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15759



Evaluation of Chrysin as an Effective Antilipidemic Agent

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Received: 11 May 2013; *Accepted*: 18 September 2013; *Published online*: 28 April 2014; AJC-15081

Chrysin is an flavone which is ubiquitously found in the flowers and honey of many plants. Chrysin has been found to possess anxiolytic and chemopreventive property in cardiovascular disease and cancer and it has been thus far used by male body builders. However, its role in ameliorating other diseased conditions is not yet properly investigated. The aim of the present study was to determine whether chrysin would lower the lipid levels when compared to quercetin and atorvastatin. Our results demonstrate that chrysin not only lowers total cholesterol and low density lipoprotein levels, but also triglycerides. Thus it is a potential anti-hyperlipidemic agent that could replace the currently prescribed statins that produce adverse effects on prolonged usage.

Keywords: Chrysin, Atorvastatin, Hyperlipidemia.

INTRODUCTION

Statins belong to a class of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG CoA reductase) inhibitors[1]. HMG CoA reductase is an enzyme that catalyzes the formation of mevalonate-a precursor in the cholesterol biosynthetic pathway[2,3]. Atorvastatin is a synthetic lipid soluble statin introduced in the nineties to treat hyperlipidemic conditions[1-3]. Atorvastatin has shown to lower cholesterol, low density lipoprotein (LDL) and triglycerides but like its other statin counterparts, has also shown adverse effects on prolonged use as well as at high doses. The major side-effects associated with statin therapy are myopathy and rhabdomyolysis[3-14]. Therefore, research is still on to identify an ideal replacement for statin therapy.

Many reports have indicated the efficacy of plant extracts such as silymarin, sunflower, polygala, *etc*., to lower lipid levels in animal models[15-19]. It is a well known fact that flavonoids are a class of compounds that are ubiquitously present in plants which may be responsible for their medicinal properties. Therefore, lipid lowering effects of many plant extracts could be attributed to the presence of flavonoids[15,16,20]. Though there are more than six thousand flavonoids identified from plants, not many have been investigated for their anti-hyperlipidemic effects.

Chrysin (5, 7-dihydroxy flavone) is an flavone which is found in passion flower, pine species, sunflower and in the flowers and honey of many plant species[21]. It has been widely used by male body builders as it prevents the action of the cytochrome P450 enzyme aromatase from converting androgens to estrogens[21]. Chrysin also possesses antioxidant[18,21], antiinflammatory[18,21-23], vasodilatory[24], chemoprotective[25] and anxiolytic[26] properties. No studies have been carried out to evaluate the efficacy of chrysin against hyperlipidemic conditions. Thus the aim of this study was to investigate the anti-hyperlipidemic properties of chrysin and compare it with atorvastatin.

EXPERIMENTAL

Animals and diet: Male Wistar rats weighing 150 g were divided randomly into five groups of four each. Group 1 was fed with standard laboratory diet while groups 2-5 were fed with a high cholesterol diet containing 1 % w/w cholesterol and 2 % w/w ghee for 60 days. All the groups were maintained at standard laboratory conditions with free access to water. Chrysin was procured from Sigma-Aldrich Chemicals, Bangalore. 20 mg/kg of chrysin was gavaged perorally to group 3 for ten days after induction of hyperlipidemia. Groups 4 and 5 were treated with 20 and 80 mg/kg of atorvastatin, respectively for the same period.

After overnight fasting, the rats were anesthesized with diethyl ether and blood was collected by retro-orbital puncture. The animals were euthanized by cervical dislocation. The liver and kidney were collected and fixed immediately in 10 % neutral buffered formalin for 24 h. Then the organs were trimmed and processed using ascending compositions of alcohol, xylene for clearing followed by paraffin embedding. The paraffin embedded sections were cut into thin sections of

3-4 micron thickness and were stained using Mayer's hematoxylin, eosin and phloxine stains. The sections were mounted with Canada balsam and qualitatively observed under the microscope. All animal experiments were carried out after obtaining clearance from the Institutional Animal Ethics Committee (4/SASTRA/IAEC/RPP).

Lipid analysis: Total cholesterol, high density lipoprotein and triglycerides were assayed using standard enzymatic kits from M/s.Beacon Diagnostics, India. The low density and very low density lipoprotein levels (LDL + VLDL) were calculated using the relationship:

Total cholesterol = HDL + (LDL+ VLDL)

The colorimetric analysis was performed using UV-visible spectrophotometer (Lambda 25, Perkin Elmer Instruments, USA).

Statistical analysis: Statistical significance of the data were analyzed using one way ANOVA with confidence limits $p < 0.05$.

RESULTS AND DISCUSSION

Fig. 1 shows the cholesterol profile for the various groups after ten days of study. The concentration of cholesterol was significantly low in animals treated with chrysin and atorvastatin when compared to control ($p < 0.05$) and was comparable to the normal group. Both chrysin and the higher dose of atorvastatin (80 mg) had lowered the total cholesterol level by nearly 75 % when compared to the positive control. The reduction in total cholesterol levels by atorvastatin is due to its inhibition of HMG CoA reductase that catalyzes the formation of mevalonate-a precursor in the cholesterol pathway[27]. Hence it is hypothesized that chrysin may also have a similar effect but it has to be confirmed by further experiments.

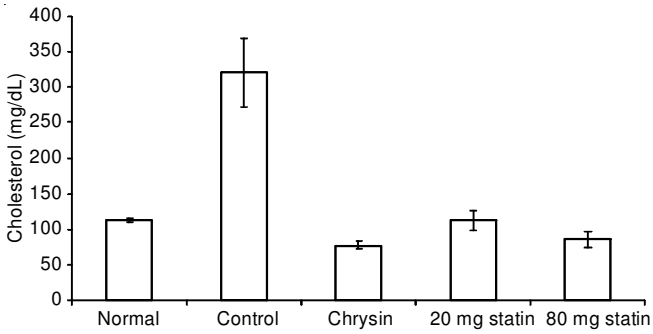

Fig. 1. Cholesterol profiles of various groups ($p < 0.05$)

Fig. 2 shows the high density lipoprotein profile for the various groups. It is seen that both atorvastatin as well as chrysin have not increased the high density lipoprotein levels when compared with to control. Atorvastatin has been observed to show a negative dose response to high density lipoprotein levels[27-30] and lowers the high density lipoprotein levels[28-31] and we observe similar results in the present study.

Fig. 3 shows the LDL + VLDL profile for the various groups. The groups administered with chrysin and atorvastation showed significantly lower levels of LDL ($p < 0.05$) and was comparable to the normal group. It is interesting to note that while atorvastatin had lowered the low density lipoproteins by nearly 75-80 %, chrysin has brought down the LDL and

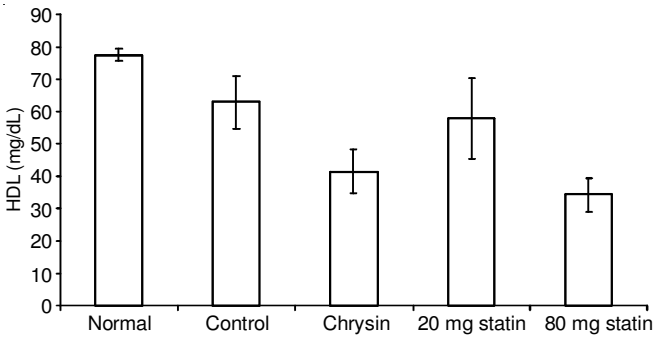

Fig. 2. HDL profiles of various groups ($p < 0.05$)

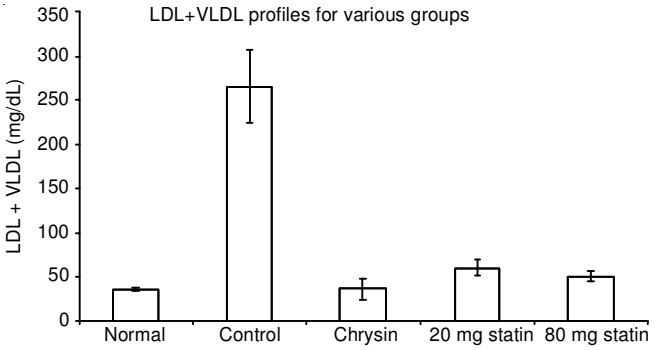

Fig. 3. LDL + VLDL profiles of various groups ($p < 0.05$)

VLDL values to nearly normal over the period of study (86 % reduction) in a statistically significant manner ($p < 0.05$). Earlier reports have indicated that the LDL lowering effect of atorvastatin is dose dependent[32-34]. In the present study the atorvastatin doses used do not show any statistically significant difference. But it is significant to note that chrysin reduces LDL+VLDL at a lower dose. It has to be determined in further studies whether the reduction in LDL levels by chrysin and atorvastatin is due to reduced LDL synthesis or increased LDL clearance.

The total cholesterol (TC) to high density lipoprotein (HDL) ratio is more indicative of cardiovascular disease than total cholesterol levels[16,35]. A higher value indicates more risk as it indicates an increase in total cholesterol values and a decrease in high density lipoprotein thus increasing the possibility of atherosclerosis and corresponding cardiac arrests. Though the total cholesterol values are not significantly different among the groups treated with chrysin and atorvastain, there is a significant difference in the. TC/HDL ratios ($p < 0.05$). Among the treated groups, the chrysin group (1.86) had the TC/HDL ratio closest to the normal value (1.46). This reflects a 63 % reduction from the positive control (5.08). The lower dose of atorvastatin shows a TC/HDL ratio close to chrysin (1.94) while the higher dose of atorvastatin does not show a favourable ratio (2.48).

The LDL/HDL ratios also show a similar trend. The LDL/HDL ratio is considered a more correct indicator of cardiac risk than the TC/HDL ratio because low ratio indicates lower risk while a higher ratio indicates higher risk[16,35]. The LDL/HDL ratio is lowest in the case of chrysin (0.86) followed by the lower dose of atorvastatin (1.04) and 1.48 for the higher dose of atorvastatin. These are higher that those obtained for

the normal group (0.46) but much lower than those for the positive control (4.21). However these ratios are not statistically significant between the chrysin and atorvastatin groups indicating that both compounds are effective in reducing cardiac risk.

Fig. 4 shows the triglyceride profiles of the various groups. It is seen that while chrysin has reduced the triglyceride levels to normal value (65 % reduction), both doses of atorvastatin have only moderately reduced the triglyceride level by 21-26 %. Atorvastatin has been reported to reduce triglyceride levels but whether the reduction is dose-dependent or dose-independent is a matter of further study. Previous studies have shown that the triglyceride levels are reduced from 9 to 45 % while using atorvastatin[2]. In the present study, we have observed that chrysin has significantly reduced the triglyceride concentration when compared to the groups treated with 20 and 80 mg atorvastatin and control ($p < 0.05$) and the values obtained were comparable to the normal group. However, the concentration of triglycerides in the groups treated with 20 and 80 mg atorvastatin was significantly higher than that of the normal group. This implies that chrysin might have a different mechanism for lowering triglycerides which requires further experimental validation.

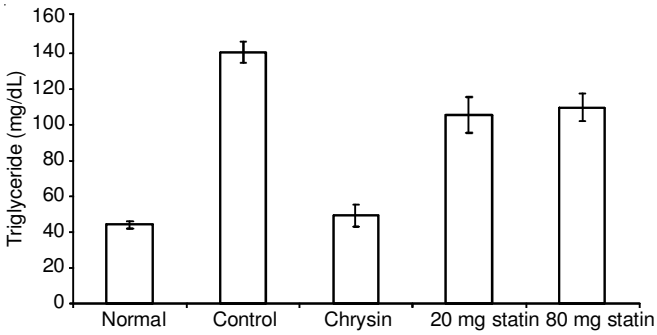

Fig. 4. Triglyceride profiles of various groups ($p < 0.05$)

Histopathological analysis of liver samples from each of the study groups are shown in Fig. 5. It is observed that the positive control liver section (Fig. 5B) shows marked hepatocellular hypertrophy with practically no sinusoidal spaces. There are oval swollen hepatocytes seen with practically no hepatic architecture. The liver section from the chrysin group (Fig. 5C) shows moderate hepatocyte hypertrophy and the presence of sinusoidal spaces. The liver section from the atorvastatin group (Fig. 5D) also showed moderate hypertrophy indicating partial recovery but with thinner sinusoidal spaces when compared with the chrysin and normal groups.

Kidney sections from the various groups indicate no toxic effects in the chrysin group. Thus, it can be inferred that chrysin has caused no systemic toxicity or adverse effects during the treatment period. It was observed that while the animals treated with atorvastatin were lethargic and showed a loss of appetite, whereas, the chrysin group was active and had an increased appetite during the treatment period. One of the factors that may have promoted this behaviour could be the fact that chrysin inhibits aromatase-a cytochrome P450 enzyme, that prevents the conversion of testosterone into estrogen[23]. This could result in better muscle development, increased

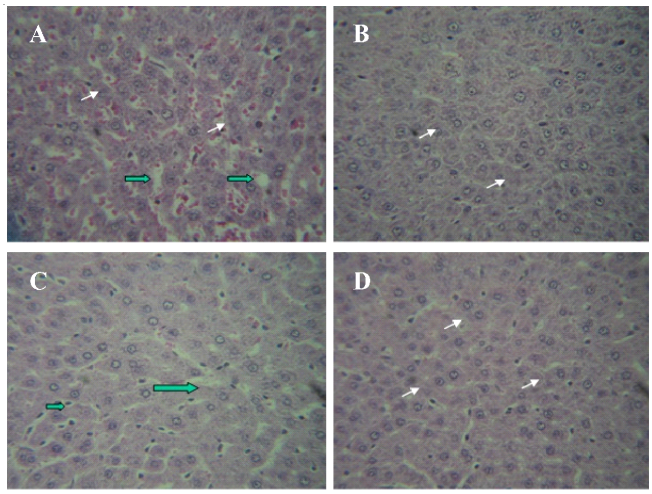

Fig. 5. Liver sections of various groups: (a) Normal (b) Postive control (c) Chrysin treated (d) Atorvastatin treated

metabolism and increased activeness. This phenomenon has been implicated by earlier researchers and has lead to the widespread use of chrysin supplements by male body builders. One of the possible effects that might have supplemented chrysin action is the alkaline medium (sodium carbonate) in which it has been solubilized. Acidosis associated due to hyperlipidemia could be reduced in alkaline medium but this alone cannot lower the lipid levels significantly. Therefore, it could be concluded that chrysin by itself possesses excellent lipid lowering properties.

Conclusion

Though a lot of side effects have been reported for the use of statins, they have been used extensively for a variety of reasons. Our results have demonstrated that chrysin shows significantly lower levels of HDL, LDL + VLDL and triglycerides when compared to the control. Moreover, these values were comparable to the normal group. This proves that chrysin is a more potent antilipidemic agent than atorvastatin without any adverse effects and hence could be used to replace statins. Further studies are underway to understand the mechanism of action of chrysin on chrysin and to determine the optimal dose and duration of treatment.

ACKNOWLEDGEMENTS

The authors thank the TRR Research cell of SASTRA University for funding the project.

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