Phytopreventative Anti-Hyperlipidemic Effects Of Gynostemma Pentaphyllum In Rats

Samer Megalli[a], Fugen Aktan[b], Neal M. Davies[c], Basil D. Roufogalis[a]

[a]Faculty of Pharmacy and Herbal Medicines Research and Education Centre University of Sydney, Sydney NSW, Australia
[b]Faculty of Pharmacy, Ankara University, Tadogan, Ankara, Turkey
[c]College of Pharmacy, Department of Pharmaceutical Sciences, Washington State University, Pullman, Washington, U.S.A.

Received May 7, 2005; Revised September 12, 2005; Accepted September 13, 2005; Published September 16, 2005

Abstract Purpose: *Gynostemma pentaphyllum* is widely used in traditional Chinese medicine. Preliminary studies indicate *Gynostemma* isolated triterpine glycosides lower cholesterol. Our studies examine anti-hyperlipidemic effects of gypenosides. **Methods:** Gynostemma activity was examined in poloxamer P407 induced hyperlipidemia in rats. **Results:** 1 g/kg P407 induced plasma triglyceride (25 fold), total cholesterol (6 fold), low density lipoprotein cholesterol (LDL) (7 fold), high density lipoprotein cholesterol (HDL) (1.6 fold), and nitrite (8 fold). After acute (4 days) and chronic (12 days) oral administration the gypenoside extract (250 mg/kg) reduced triglyceride (53% and 85%, respectively) and total cholesterol levels (10% and 44%, respectively). No significant effects on LDL or HDL cholesterol were observed. The gypenosides reduced nitrite ~80%. Similar results were obtained with atorvastatin (75 mg/kg for 4 days); except that LDL cholesterol was reduced (17%) and HDL cholesterol increased. 50% of lipoprotein lipase (LPL) plasma activity was inhibited by ~20 μM P407. Gynostemma had no effect on LL, however, it reversed the P407 inhibition of LPL activity in a concentration-dependent manner, with a 2-fold increase at ~10 μg/ml. **Conclusions:** These studies demonstrate efficacy of *Gynostemma pentaphyllum* in lowering triglyceride, cholesterol and nitrite in acute hyperlipidemia. The results suggest further investigations of Gynostemma gypenosides are warranted to examine the mechanisms of this activity.

Corresponding Author: Dr. Sam Megalli PhD, Faculty of Pharmacy, Pharmacy building A15, The University of Sydney, NSW 2006, Australia. smegalli@pharm.usyd.edu.au

INTRODUCTION

Gynostemma pentaphyllum (Thumb.) Makino (GP) (Jiaogulan-Chinese name) is an herbaceous vine plant of the cucurbitaceous family and is distributed naturally in shaded and humid places (1). In traditional Chinese medicine it is indicated for heat clearing, detoxification, and as an anti-tussive and expectorant for relieving cough and chronic bronchitis. In Japan it is indicated as a diuretic, antipyretic, anti-inflammatory and tonic. GP contains saponins (triterpene glycosides or gypenosides); more than 100 dammarane-type glycosides, also called gypenosides, have been isolated and identified (2). A general structure of dammarane-type gypenoside is illustrated in Figure 1. Some of these saponins are the same as those from Panax ginseng (ginsensides) (3-4). Flavonoids such as rutin are also found. The medicinal properties of GP have been mainly attributed to the saponins (2).

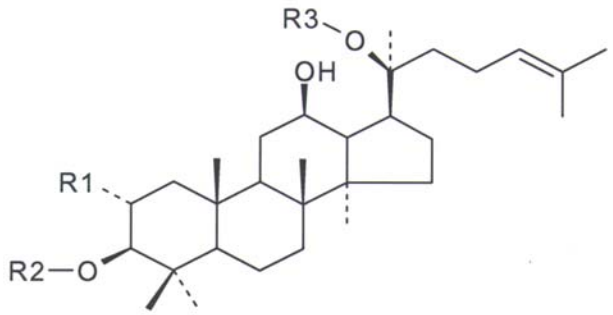

Figure 1: General Structure of Dammarane-Type Gypenosides. Gypenoside consists of both the hydrophobic sapogenin part and the hydrophilic sugar part in the molecule (where R1 and R2 = glucose, rhamnose; R3 = glucose, xylose).

Poloxamer 407 (Pluronic RF-127) has been used to induce hyperlipidemia in rats. P407 is a biocompatible, non-ionic surfactant (5) is considered non-toxic and safe in animal chronic administration for long term studies (6). In the rat, once daily intraperitoneal (ip) injections of 0.33 mg/kg P407 for four consecutive days resulted in the increase of monocyte numbers with no other toxicological complications (7). In another study, 0.5 g/kg of P407 was given to C57BL/6 mice every third day for 200 days, without any significant weight loss or alterations in the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (8). The increase in triglycerides (TG) seen following P407 injection is mainly due to the inhibition of TG degradation, due

to a direct inhibitory effect on lipoprotein lipase (LPL), the enzyme responsible for TG degradation. However, the effect of P407 on hypercholesterolemia suggests that the elevations of cholesterol could be mediated through indirect activation on HMG-CoA reductase (6).

Herbal medicines and nutraeuticals are being increasingly accepted and utilised in the Western medicine to treat and/or prevent various diseases. In this present study, we investigate the effect of gypenosides isolated from G*ynostemma pentaphylumm* on hyperlipidemia and nitrite levels in an acute model of hyperlipidemia. In addition, *in vitro* and *in vivo* studies have been utilised to further explore the possible modes of action of *Gynostemma pentaphyllum.*

MATERIALS AND METHODS

Purified gypenoside extract was provided by Ankang Pharmaceutical Institution of Beijing Medical University, People's Republic of China. The Ankang extract in a capsule formulation was dissolved in 90% ethanol and thoroughly mixed with a magnetic stirrer then filtered twice using filter paper and evaporated down using a Buchi Rotavapor R-114. The extract was then dried in a vacuum oven and kept in a bell jar with silica gel. A final purity of approximately 90% gypenosides was obtained.
Poloxamer 407, also called Pluronic RF-127, was donated by BSAF Australia. The poloxamer 407 was made at a final concentration of 30% (w/w) by dissolving the powder in distilled cool water; the solution was then kept refrigerated overnight to facilitate its dissolution. Needles and syringes used to administer P407 were cooled prior to administration to prevent P407 gelation within the syringe. Poloxamer was administered ip to the rats. Atorvastatin was purchased in a tablet form at strength 80 mg through Australian Pharmaceutical Industries in Northmead, Sydney. After crushing the tablets, powder was then dissolved in 1% CMC for oral dosing via gavage.

For the assay of lipoprotein lipase (LPL), Glycerol tri [9, 10(n)-^{3}H] oleate ([^{3}H] TO) was obtained from Amersham Biosciences, Sydney (Code TRA191). Lipoprotein enzyme from bovine milk and cold glyceryl trioleate, also know as Triolein (TO), and lecithin stored in chloroform (100 mg/ml) were obtained from Sigma-Aldrich (Australia). Glycerol and 0.2 M Tris-HCl (pH 8.0) containing 3% bovine serum albumin (BSA) were also obtained from Sigma-Aldrich (Australia). Ethanol was obtained from Asia Pacific Specialty Chemicals Ltd. Sydney, Australia. Other materials including ketamine hydrochloride and carboxymethylcellulose (CMC) were purchased from Sigma-Aldrich (Australia). Total cholesterol (TC), total triglyceride, (TG) low density lipoprotein cholesterol (LDL) and high density lipoprotein cholesterol (HDL) measuring kits were obtained from Sigma-Aldrich (Australia) and Trace Scientific Ltd.(Australia) and used according to manufacturers instructions after precipitation techniques and modified enzymatic procedures to plasma samples.(9-11) All other reagents and chemicals were of analytical grade.

Animal Grouping and Treatments

Experiments were conducted in accordance with the Animal Ethics Committee at The University of Sydney guidelines, Approval number L24/5-2001/2/3369.

Sprague-Dawley (S-D) rats were purchased from Animal Services at the University of Sydney. The rats obtained were males, 4-8 weeks old and average weight 250-300 g. Rats were housed 3-4 per cage in a temperature controlled (22 ± 1)˚C room, with a light/dark cycle of 12 hr. For a week following their receipt, the animals were allowed free access to a standard rat chow diet and tap water while they were acclimating to the environment. During the experimentation all rats had free access to standard rat chow and water at all times unless otherwise stated in the methods section. *Gynostemma pentaphyllum* and atorvastatin samples were well mixed in 1% carboxymethylcellulose. Treatments were administered to rats using oral gavage via a curved feeding needle (Harvard Apparatus). At the start of the experiment animals were randomly distributed so that body weights, initial TG, TC and other parameters were similar in all the experimental groups.

At the end of each study, animals were sacrificed and blood collected via cardiac puncture for analysis. Rats were sacrificed after the induction of anaesthesia using 1 ml of ketamine injection (1 g /10 ml) and sacrificed using a lethal injection of 0.5 ml concentrated solution of potassium chloride (70%) solution directly into the heart.

Effect of Acute and Chronic Gynostemma pentaphyllum

Sprague-Dawley rats were divided into 3 groups (Figure 2). A control (C) group (12 rats), did not receive any treatment apart from 1% CMC as an oral

gavage, a second group treated with P407 1 g/kg alone (P407) (12 rats) and a group receiving GP 250 mg/kg once daily as an oral gavage for 4 days for acute experiments (12 rats) and 12 days for chronic experiments. In addition, atorvastatin was used as a positive control. Rats were divided into 3 groups (Figure 2). A control group (12 rats), not receiving active treatment (C) apart from 1% CMC as an oral gavage, a group treated with P407 1g/kg alone (P407) (15 rats) and a group treated with 75 mg/kg atorvastatin once daily for 4 days as an oral gavage (atorvastatin) (9 rats). In the latter 2 groups in each of the above experiments, hyperlipidemia was induced by injecting rats with P407 intraperitoneally 48 hr prior to blood collection. All groups had free access to food and water. Pharmacological endpoints measured were TG, TC, HDL cholesterol, LDL cholesterol and nitrite levels.

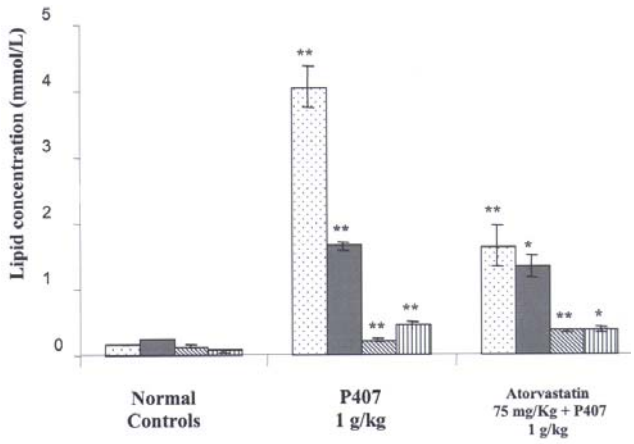

Figure 2: Effects of Atorvastain on Lipids in P407 Hyperlipidemia Three groups are compared; a normal control group received ip saline injection, a P407 group, received 1 g/kg P407. Rats in these 2 groups were given an oral gavage of 1% CMC daily for 4 days. In the third group (atorvastatin 75 mg/kg group) rats were administered atorvastatin 75 mg/kg for 4 days and an injection of P407 1 g/kg ip 48 hr prior to blood collection. Mean ± SEM, n=12 for normal rat group, n=15 for P407 group and n=9 for atorvastatin group. Lipids measured are triglyceride ⁘ total cholesterol 1 ▒, high density lipoprotein cholesterol ▨ and low density lipoproteins ▥.*= $P < 0.05$ and **= $P < 0.01$ (all relative to normal controls in the P407 group and relative to P407 group in the atorvastatin group).

In vitro Effect of Gynostemma pentaphyllun and P407 on Lipoprotein Lipase Activity

To determine the effects of P407 and GP on LPL *in vitro*, two rats were anaesthetized with ketamine. Each rat received an intravenous injection into the tail vein of 1000 IU of heparin in a volume of 0.3 ml. Two minutes following the heparin injection, blood was drawn using cardiac puncture. Blood was then pooled centrifuged and plasma frozen until further experimentation.

To determine the effect of P407 on the activity of LPL contained in post-heparin plasma from rats, increasing concentrations of P407 (2.5, 5, 10, 20 μM) were incubated (total volume of 0.2 ml) with substrate enzyme for 15 min at 37ºC. To determine the effect of *Gynostemma pentaphyllum* (GP) on the activity of LPL, increasing concentrations of GP (5, 10, 25, 50, 100 and 200 μg/ml) were incubated in the assay mixture for 15 min at 37ºC. This experiment was performed in the presence or absence of 20 μM P407.

A method to measure LPL established in 1976 using a stable, radioactive substrate emulsion was employed (12). Fatty acid-labelled trioleoyglycerol was emulsified by homogenisation in glycerol with lecithin as detergent. This anhydrous emulsion was stable for 6 weeks. Substrate solutions for enzyme assay were prepared by diluting the emulsion with buffer containing serum and albumin. The fatty acid produced on hydrolysis was isolated in a one-step liquid-liquid partition system (13).

Data Analysis

All data are expressed as the standard error of the mean (± SEM). Comparisons among the control and treatment groups will be made using one-way analysis of variance followed by a Student-Newman-Keuls *t*-test using the GraphPad Instat statistical program. With all analyses, an associated probability (*P* value) of less than 5% ($P < 0.05$) was considered significant.

RESULTS

Hyperlipidemia

The dose of P407 chosen from a preliminary dose response study in rats was 1 g/kg and the optimum time for measurement of P407 induced hyperlipidemia was determined to be 48 hours. In acute studies, lipid values in normal rats were compared with P407 (1 g/kg) treated rats 48 hr post hyperlipidemia induction. TG levels were increased by 25 fold (from 1.51 ± 0.1124 mmol/L to 38.24 ± 3.0541 mmol/L), TC levels increased by more than 6 fold (from 2.52 ± 0.1512 mmol/L to 16.32 ± 0.6321 mmol/L), HDL levels increased by 1.6 fold from (1.44 ± 0.1511 mmol/L to 2.33 ± 0.1537 mmol/L)

and LDL levels increased by 7 fold (from 0.8249± 0.1465 mmol/L to 5.42 ± 0.4368 mmol/L). All of these increases in plasma lipids were statistically significant ($P < 0.05$).

Effect of Atorvastatin Administered Acutely on P407 Treated Rats

Atorvastatin administered by oral gavage (75 mg/kg for 4 days) was used as a positive control (Figure. 2). Atorvastatin significantly decreased the elevation of triglycerides; cholesterol and LDL induced by P407 treatment 48 hr before blood collection, and also increased HDL levels.

Three groups are compared; a normal control group received ip saline injection, a P407 group, received 1 g/kg P407. Rats in these 2 groups were given an oral gavage of 1% CMC daily for 4 days. In the third group (atorvastatin 75 mg/kg group) rats were administered atorvastatin 75 mg/kg for 4 days and an injection of P407 1 g/kg ip 48 hr prior to blood collection. Mean ± SEM, n=12 for normal rat group, n=15 for P407 group and n=9 for atorvastatin group. Lipids measured are triglyceride, total cholesterol, high density lipoproteins and low density lipoproteins.*= $P < 0.05$ and **= $P < 0.01$ (all relative to normal controls in the P407 group and relative to P407 group in the atorvastatin group).

Effect of Gynostemma pentaphyllum Administered acutely and chronically on P407 Treated Rats

To determine the acute effect of GP on lipid levels, rats received GP for 4 consecutive days, after which hyperlipidemia was induced by injecting P407 48 hr prior to blood collection. GP was found to be effective in significantly reducing both TG and TC levels after 4 days of pre-treatment at a dose of 250 mg/kg (Figure 3). GP significantly reduced TG levels by 53% (from 38.24 ± 3.0512 mmol/L to 18.04 ± 3.4241 mmol/L) and TC by 10% (from 16.32 ± 0.6348 mmol/L to 14.81 ± 1.5633 mmol/L). No significant changes were seen on HDL cholesterol or LDL cholesterol levels but there was a trend towards a reduction in LDL cholesterol levels.

To determine the effect of a more chronic pre-treatment of GP on lipid levels, SD rats received GP for 12 days; subsequently hyperlipidemia was induced by injecting P407 ip 48 hr prior to blood collection. Chronic administration of GP 250 mg/kg over a twelve day period significantly reduced TG and TC levels in plasma of P407 treated rats (Figure 4).

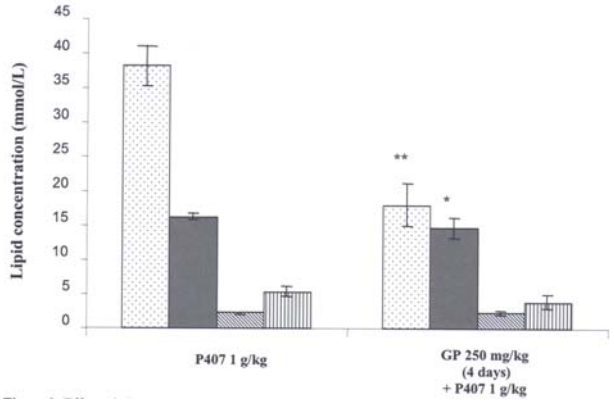

Figure 3: Effect of *Gynostemma pentaphyllum* on Lipids in P407 Hyperlipidemia. Acute administration. Two groups are compared; a P407 group received an oral gavage of 1% CMC daily for 4 days followed by an ip injection of 1 g/kg P407 48 hr prior to blood collection. The second group (GP 250 mg/kg) received a daily oral gavage of GP 250 mg/kg for 4 days and an injection of P407 (1 g/kg) ip 48 hr prior to blood collection. Mean ± SEM, n=12 for all groups. Lipids measured are triglyceride ⋰⋱ total cholesterol 1 ▓, high density lipoprotein cholesterol ▨ and low density lipoproteins ▥.*= $P < 0.05$ and **= $P < 0.01$ relative to P407 controls.

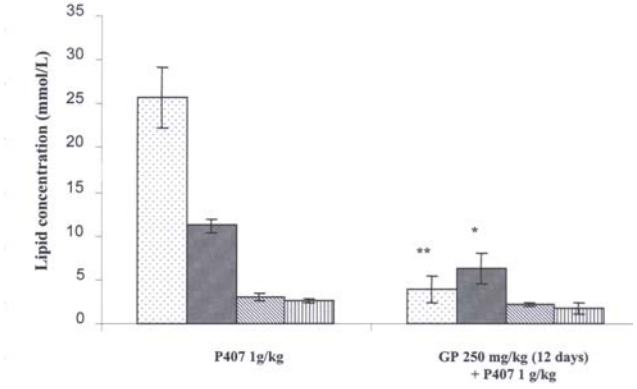

Figure 4. Effect of *Gynostemma pentaphyllum* on Lipids in P407 Hyperlipidemia Chronic administration Two groups are compared, a P407 group which received an oral gavage of 1% CMC daily for 12 days followed by an ip injection of 1 g/kg P407 48 hr prior to blood collection. The second group (GP 250 mg/Kg) received a daily oral gavage of GP 250 mg/kg for 12 days and an injection of P407 (1 g/kg) ip 48 hr prior to blood collection. The data are expressed as mean ± SEM, n=8 for all groups. Lipids measured are triglyceride ⋰⋱ total cholesterol 1 ▓, high density lipoprotein cholesterol ▨ and low density lipoproteins ▥.*= $P < 0.05$ and **= $P < 0.01$ relative to P407 controls.

GP significantly reduced TG levels by 85% (from 25.73 ± 3.5422 mmol/L to 3.98 ± 1.4214 mmol/L), and TC levels by 44% (from 11.27 ± 0.7235 mmol/L to 6.31 ± 1.7044 mmol/L), a 35% reduction in LDL levels (from 2.63 ± 0.4115 mmol/L to 1.72 ± 0.6038 mmol/L), was not statistically significant. No significant changes in HDL levels were noted.

Effect of Gynostemma pentaphyllum on Plasma Nitrite in P407 Treated Rats

The effect of GP on nitrite levels in plasma was examined in rats with enhanced nitrite levels induced by P407. Twenty-four h following injection of 1 g/kg P407 ip to rats, plasma nitrite levels significantly increased by more than 8 fold (from 8.05 ± 0.02 μM to 68.03 ± 6.51 μM). At 48 and 72 h time points, levels increased significantly by more than 18 fold, to 149.81 ± 3.93 μM and 149.60 ± 5.51 μM, respectively. In acute studies, where GP 250 mg/kg was administered to SD rats for 4 days, and P407 (1 g/kg) was injected ip 48 hours prior to plasma collection, a significant reduction in plasma nitrite levels were observed. Nitrite levels were reduced by 74% (from 144.63 ± 9.92 μM to 38.21 ± 8.53 μM) (Figure 5). In chronic studies, where GP 250 mg/kg was fed to SD rats for 12 days, and P407 (1 g/kg) was injected 48 hours prior to plasma collection, significant reduction in plasma nitrite levels were observed. Nitrite levels were reduced by 86% (from 144.63 ± 9.92 μM to 20.01 ± 5.93 μM).

Three groups are compared, a control group received saline injection ip and 1% CMC as an oral gavage, a P407 group received an oral gavage of 1% CMC daily for 4 days and an injection of 1 g/kg P407 ip 48 hr prior to blood collection. The third group (GP 250 mg/kg) received a daily oral gavage of GP 250 mg/kg for 4 days and an injection of P407 1 g/kg ip 48 hr prior to blood collection. Mean ± SEM, n=12 for all groups. *= $P < 0.05$ and **= $P < 0.01$, relative to controls for P407 group and relative to P407 controls for GP 250 group.

Effect of Gynostemma pentaphyllum on Lipoprotein lipase (LPL) Activity in vitro

The *in vitro* effect of P407 on LPL enzyme activity was determined. Increasing concentrations of P407 were incubated with the enzyme as described in Methods section. As the dose of P407 was increased, the activity of LPL was progressively reduced, 50% of the LPL enzyme activity being inhibited at a poloxamer concentration of approximately 20 μM. LPL activity was almost abolished at P407 concentration of 100 μM (Figure 6). The effect of incubation of various concentrations of GP on LPL enzyme activity was examined (see Methods section). GP, at concentrations from 5 to 100 μg/ml in the absence of P407 had no effect on LPL enzyme activity (Figure 7). In the presence of 20 μM P407 (see Methods section) GP reversed the P407 inhibitory effect on LPL activity in a dose-dependent manner (Figure 8). At a GP concentration of 10 μg/ml, LPL enzyme activity increased by 2 fold compared to the control. Further increases of LPL enzyme activity were observed as GP concentrations were increased further, however, enzyme activity remained reached a ceiling effect and remained almost unchanged at 100-200 μg/ml.

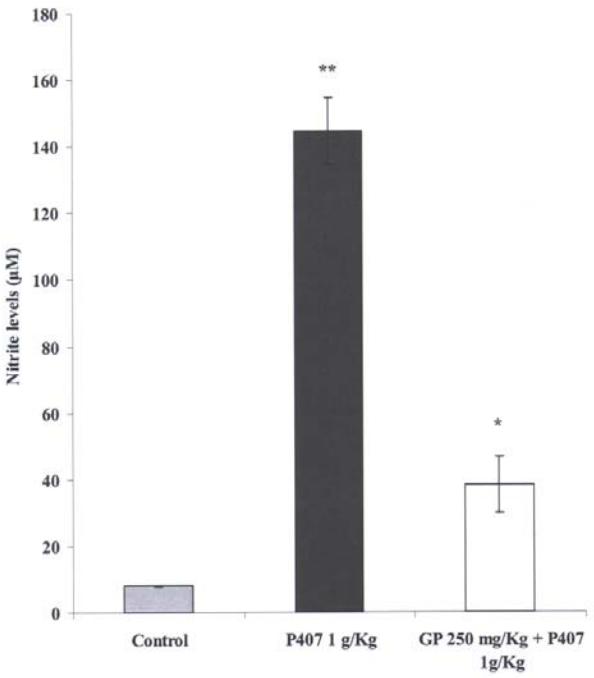

Figure 5: Effect of *Gynostemma pentaphyllum* On Plasma Nitrite in P407 Hyperlipidemia. Three groups are compared, a control group received saline injection ip and 1% CMC as an oral gavage, a P407 group received an oral gavage of 1% CMC daily for 4 days and an injection of 1 g/kg P407 ip 48 hr prior to blood collection. The third group (GP 250 mg/kg) received a daily oral gavage of GP 250 mg/kg for 4 days and an injection of P407 1 g/kg ip 48 hr prior to blood collection. Mean ± SEM, n=12 for all groups. *= $P < 0.05$ and **= $P < 0.01$, relative to controls for P407 group and relative to P407 controls for GP 250 group.

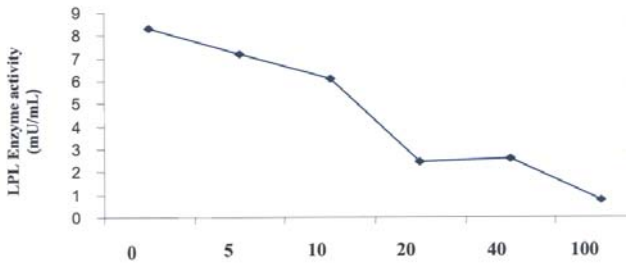

Figure 6: The Effect of P407 on the Activity of Lipoprotein Lipase. Values are means of duplicate samples assayed twice. The error bars represent the range of the two replicates.

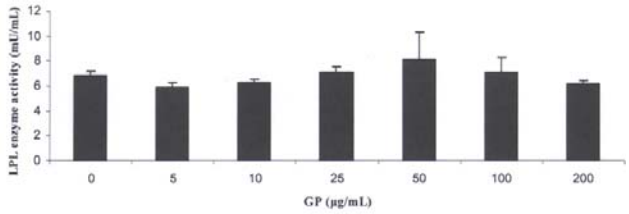

Figure 7 The Effect of Gynostemma *pentaphyllum* on Lipoprotein Lipase Activity in the Absence of P407 Values are means of duplicate samples assayed twice. The error bars represent the range of the two replicates,

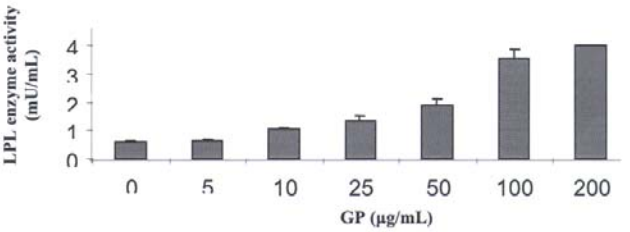

Figure 8. The Effect of *Gynostemma pentaphyllum* in the Presence of 20 µM P407 on the Activity of Lipoprotein Lipase Incubations were carried out as above in the presence of 20 µM of P407.Values is means of duplicate samples assayed twice. The error bars represent the range of the two samples.

DISCUSSION

The first aim of this study was to reproduce in our laboratory a reliable and reproducible hyperlipidemic rat model, suitable for rapidly screening the effects of *Gynostemma pentaphyllum* on lipid levels. Investigations of antihyperlipidemic effects of xenobiotics have previously required long-term feeding studies that are often prohibitive to researchers as they are time consuming and costly. This current model appears to be reproducible, sensitive cost-effective, and may have applicability for screening of various sub-fractions of *Gynostemma* and other herbs, traditional medicines, nutraceuticals, and other xenobiotics for anti-hyperlipidemic activity.

The results obtained with the poloxamer model are comparable to those in the literature (6, 14-19). Collection of blood at 48 hours post P407 ip injections was considered optimal, since TG, TC and LDL cholesterol levels were at peak levels. In addition, 1 g/kg of P407 was considered a suitable dose, since the elevation of lipid levels were significantly higher than the control, however, they were below the near maximum levels obtained at 2 g/kg of P407. Thus 1 g/kg P407 and blood collection at 48 hours following induction of P407 were considered suitable conditions for inducing hyperlipidemia, and sufficient for accurate measurement and of a suitable magnitude to detect both reductions and increases in lipid levels following treatment protocols. The effect of P407 on lipid sub-fractions (LDL and HDL cholesterol) and nitrite levels was determined in the rat model. P407 increased TG, TC, LDL and HDL cholesterol and nitrite levels in plasma. However, the fold increase in TG was significantly greater than all the other lipids measured. This could possibly be due to the activating effect P407 on endothelial heparin-releasable LPL (19). Furthermore, the novel finding that injection of P407 ip increased plasma nitrite levels by more than 10 fold is consistent with hypercholesterolemic patients where in severe hypercholesterolemic the mean basal value of nitrites was statistically higher than that of the controls (20). In the P407 model there may be elevated nitric oxide concentrations due to the increase of LDL consistent with the hypothesis of a stimulating effect of LDL upon NO endothelial synthesis (20). These nitrite plasma levels are consistent with what has been demonstrated in patients with acute coronary heart disease and cholesterolemia. (21) It appears that significantly enhanced synthesis of plasma nitrites in hypercholesterolemic patients and the P407 model may involve NO and endothelium-damaging substances such as LDL. Therefore, this model appears to be a useful model for acute screening of potential lipid-lowering drugs and also to study the pathophysiological importance of NO in hyperlipidemia, since hyperlipidemia and NO are both rapidly induced in this model.

The present study was designed to examine

whether GP would attenuate the hyperlipidemia response observed in P407 treated rats. The effect of 90% pure saponin fraction *Gynostemma pentaphyllum* on lipid levels was tested using the P407 model. The dose of *Gynostemma pentaphyllum* at 250 mg/kg was found in preliminary studies to be a suitable dose for producing near maximum decreases in lipid increases and therefore was used in all studies *Gynostemma pentaphyllum* was effective following both acute (4 day) and chronic (12 day) administration to reduce TG and TC levels. However, chronic treatment yielded significantly greater reductions in both TG and TC levels.

LDL cholesterol levels were not reduced by acute administration of GP, and although LDL cholesterol levels were reduced in the chronic studies by more than 30%, due to variability statistical significance was not achieved. Our finding in relation to effect of atorvastatin on LDL cholesterol levels, where LDL cholesterol declined by 17% in rats, is qualitatively similar to results demonstrating that LDL cholesterol was reduced by more than 37% in human studies (22). The current finding is supported by a study conducted by Johnston and co-workers (14), testing various HMG-CoA reductase inhibitors in P407 induced hyperlipidemia in C57BL/6 mice. In this study the reduction in TG levels by atorvastatin was more profound than reductions in TC levels, with TC levels being reduced by 19% and TG levels by 36%. In another study, with P407 in normal rats the effect of pravastatin also yielded similar results (18).The increase in TG seen following P407 ip injection is considered to be mainly due to the inhibition of TG degradation, due to a direct inhibitory effect on lipoprotein lipase (LPL) bound to capillary endothelium (7). Hence, the effect on LPL could be a possible mode of action of *Gynostemma pentaphyllum*, since it was significantly effective in reducing TG levels in this model. The hypothesis that GP acts on LPL activity was therefore examined. LPL is vital in the metabolism of triglycerides and is involved in several pathological disorders, including atherosclerosis and obesity. The *in vitro* studies demonstrated that *Gynostemma pentaphyllum* reversed the inhibitory effect of P407 on heparin-releasable LPL, whereas it had no effect on LPL in the absence of P407 inhibition.

LPL is organised into two structurally distinct regions, consisting of a larger amino-terminal domain and a smaller carboxy terminal end, connected by a flexible peptide. Various studies have shown that the catalytic triad occurs in a groove that consists of hydrophobic chains of three sites, with access of the substrate to the active site pocket being blocked by a polypeptide lid. Binding of the lipoprotein substrate to LPL produces a conformational change that leads to the opening of the lid and enhancement of LPL activity (23-25). Our *in vitro* studies have shown that P407 inactivates LPL enzyme activity when the two are incubated concomitantly. P407 could possibly achieve this by closing this polypeptide lid, hence inactivating LPL, without denaturing the enzyme. This effect is consistent with previous literature where both *in vitro* and *in vivo* studies indicated that P407 induced hypertriglyceridemia was due to reversible inactivation of LPL bound to capillary endothelium (7). The reversal of the inhibitory action of P407 on LPL by GP could possibly have been achieved by an effect of gypenosides on opening of the polypeptide lid, previously closed by P407 treatment, hence activating the enzyme. The current finding of GP activated LPL enzyme activity is of considerable interest, as LPL plays a central role in the overall degradation of TG. The fact that GP alone had no effect on LPL enzyme activity might suggest GP that it does not act directly on the LPL enzyme, but on its inactivated form. It is tempting to speculate that GP will also activate LPL inhibited by other metabolic imbalances, but this remains to be determined in future work. The possible effect of GP on LPL *in vivo* is the subject of our ongoing investigations.

Maintaining a balance of production of nitric oxide is important for the cardiovascular system. At low levels nitric oxide acts a vasodilator, thereby playing an important role in the regulation of vessel tone in the cardiovascular system. Lower than normal production of nitric oxide can be associated with vasoconstriction and may contribute to atherosclerosis. Overproduction of NO, or cytotoxic NO metabolites contributes to numerous pathological processes (26). In atherosclerotic lesions, inflammatory processes up regulate iNOS production and macrophages, resulting in excessive NO production and vascular damage (27). Furthermore, excess NO induces oxidation of LDL within the arterial walls and up regulation of intracellular cell adhesion molecule expression (28). In one study, the direct release of nitric oxide by gypenosides derived from *Gynostemma pentaphyllum* was examined *in vitro*. In this study, nitric oxide production was observed in bovine aortic endothelial cells. It was concluded that *Gynostemma pentaphyllum* directly stimulated nitric oxide release in these vascular cells (29). More recently, our laboratory has explored

additional mechanisms of action of *Gynostemma pentaphyllum*. It was concluded that GP suppresses NO synthesis in murine macrophages by inhibiting iNOS enzymatic activity to a small extent and effectively attenuating NF-κB-mediated iNOS expression, implicating these mechanisms in the GP therapeutic effects (29). In the current study, the new finding that GP at 250 mg/kg reduced P407 induced elevation of nitrite levels, in both acute and chronic studies, indicates that GP may also have anti-inflammatory effects. It is plausible that GP may have cardio protective or anti-atherosclerotic properties and if this is indeed the case its use in controlling pathological conditions, including inflammation and cardiovascular disease warrants further research to examine this hypothesis.

The rat model of P407 hyperlipidemia may have certain limitations. Lipoprotein metabolism in rat differs from man in two ways. Firstly, the rat has a highly efficient mechanism for clearance of chylomicron and VLDL remnants from the circulation; hence rats have lower levels of LDL. Secondly, the absence of cholesteryl ester transfer proteins (CETP) in the rat may lead to high levels of HDL, which may act as the main cholesterol carrier (30). However in one study, CETP-like mRNA was detected by RNase protection analysis in several rat tissues, namely, heart, skeletal muscle, adipose tissue and small intestine (31). An interesting observation from a recent study is the detection of CETP activity and CETP protein in P407 treated rat plasma (19). In this study it was postulated that administration of P407 to rats causes post-transcriptional up regulation of CETP protein as well as the corresponding protein activity, which would facilitate increased transfer of cholesteryl esters between plasma lipoproteins (19). This latter finding should also be considered in the overall effects of GP in hyperlipidemia. The testing of saponin activities *in vivo* is more relevant than *in vitro* assays. If saponins have sufficient fat solubility they can be absorbed unchanged in significant quantities in the small intestine. However, if saponins are not absorbed they will pass to the large intestine where gut flora will convert them to sapogenins. Sapogenins have improved lipid solubility and will be absorbed to a greater extent; hence in these cases saponin acts as a prodrug with the bioactivity of saponins being due mainly to their sapogenins. Thus extrapolation of *in vitro* results alone for saponins could be unreliable and the use of animal models in these cases are essential.

Findings in this present study are important for the further characterization of this novel model of hyperlipidemia and in exploring a potentially effective lipid-lowering herbal medicine with traditional use and promising clinical significance. Utilising the poloxamer P407 model, GP was shown to be effective in significantly lowering TG and TC levels, and showed a trend in lowering LDL cholesterol levels in chronic studies. Importantly, it was determined for the first time that plasma nitrite levels were also elevated in this model. These findings are of potential importance in the treatment and/or prevention of cardiovascular diseases. However, more work is needed to investigate possible mechanisms of action of GP. With the growing interest of the Western world in complementary and alternative medicines investigations such as these that scientifically examine traditional beliefs and experience are required and are ever increasingly forthcoming in the literature. Overall, the use of an effective herbal drug to supplement other drug treatments in controlling hyperlipidemia and enhancing cardiac functions could be potentially of clinical value if these models are translatable to human clinical studies and outcomes.

ACKNOWLEDGEMENTS

Dr. S. Megalli was supported by a post-graduate scholarship from the Faculty of Pharmacy at the University of Sydney. *Gynostemma pentaphyllum* extract was supplied by Ankang Pharmaceutical Institute of the Beijing University, People's Republic of China.

REFERENCES

1. Li L, Lau B. Protection of vascular endothelial cells from hydrogen peroxide-induced oxidant injury by Gypenosides, Saponins of Gynostemma pentaphyllum. Phytother Res 7: 299-304, 1992.

2. Cui J, Eneroth P, Bruhn J. *Gynostemma pentaphyllum*: identification of major sapogenins and differentiation from Panax species. Eur J Pharm Sci 8:187-191, 1999.

3. Joo C, Cho Y, Koo J, Kim C, Lee S. The effect of ginseng saponins on the absorption of rats and cholesterol in rats. K Biochem J 3:1-17, 1980.

4. Joo C, Kim D, Koo J. The effect of ginseng saponin on hypercholesterolemia induced by prolonged cholesterol feeding in rabbits. K Biochem J 13:51-58, 1980.

5. Wout Z, Maggiore J, Palicharla P, Johnston T. P407 mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats. J Parent Sci Tech 46: 192-200, 1992.

6. Palmer W, Emeson E, Johnston T. P407 induced hyperlipidemic atherogenic animal model. Med Sci Sp Ex

29:1416-1421, 1997.

7. Johnston T, Plamer W. Mechanism of Poloxamer induced hypertriglyeridemia in rat. Biochem Pharmacol 46: 1037-1042, 1993.

8. Johnston T, Barker J, Hall D, Jamal S, Plamer W, Emerson E. Potential downregulation of HMG-CoA reductase after prolonged administration of P407 in C57BL/6 mice. J Cardiovas Pharmacol 34: 831-842, 1999.

9. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 20: 470-475, 1974.

10. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19: 476-482, 1973.

11. Heuck CC, Erbe I, Mathias D. Cholesterol determination in serum after fractionation of lipoproteins by immunoprecipitation. Clin Chem 31: 252-256, 1985.

12 Nilsson-Ethle P, Schotz M. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. J Lipid Res 17: 536-550, 1976.

13. Belfrage P, Vaughan M. Simple liquid-liquid partition system for isolation of labelled oleic acid from mixtures with glycerides. J Lipid Res 10: 341-344, 1969.

14.Johnston T, Nguyen L, Chu W, Shefer S. Potency of select statin drugs in a new model of hyperlipidemia and atherosclerosis. Int J Pharm 229: 75-86, 2001.

15.BrocksDR, Wasan KM. The influence of lipids on stereoselective pharmacokinetics of halofantrine: Important implications in food-effect studies involving drugs that bind to lipoproteins. J Pharm Sci 91: 1817-1826, 2002.

16. Eliot LA, Jamali F. Pharmacokinetics and pharmacodynamics of nifedipine in untreated andatorvastatin-treated hyperlipidemic rats. J Pharmacol Exp Ther. 1999 Oct;291(1):188-93.

17. Johnston TP, Palmer WK. The effect of pravastatin on hepatic 3-hydroxy-3-methylglutaryl CoA reductase obtained from poloxamer 407-induced hyperlipidemic rats. Pharmacotherapy. 17:342-347, 1997.

18. Porter J, Carter B, Palmer W. Effects of pravastatin plasma lipid concentrations in P407 induced hyperlipidemic rats. Pharmacotherapy 15:92-8, 1995.

19. Wasan K, Kwong M, Goldberg I, Wright T, Johnston T. P407 mediated alterations in the activities of enzymes regulating lipid metabolism in rats. J Pharmacol Sci 6:189-197, 2003.

20. Ferlito S, Gallina M, Catassi S, Bisicchia A, Di Salvo MM.Nitrite plasma levels in normolipemic and hypercholesterolemic patients with peripheral occlusive arteriopathy. Panminerva Med 41:307-309, 1999.

21. Ferlito S, Gallina M. Nitrite plasma levels in acute and chronic coronary heart disease. Minerva Cardioangiol 45: 553-558, 1997.

22. Jacobson T. Clinical context: Current concepts of coronary heart disease management. Am J Med 110: 3-11, 2001.

23.Mead, J., Irvine, S. and Ramji, D. (2002). Lipoprotein lipase: structure, function, regulation and role in disease. Journal of Molecular Medicine 80:753-769.

24.Tilbeurgh, V., Roussel, A., Lalouel, J. and Cambillau, C. (1994). LPL. Molecular model based on the pancreatic lipase X ray structure: consequences for heparin binding and catalysis. Journal of Biological Chemistry 269:4626-4633.

25.Yang, Gu, Z., Yang, H., Rohde, M., Gotto, A. and Pownall, H. (1989). Structure of bovine milk LPL. Journal of Biological Chemistry 264:16822-27.

26.Hobbs, A., Higgs, A. and Moncada (1999). Inhibition of nitric oxide synthase as a potential therapeutic target, Annual Review of Pharmacology and Toxicology. 39:191-220.

27.Bogdan, C. (2001). Nitric oxide and the immune response. Natural Immunity 2:907-916.1.

28.Tanner, M., Bu, X., Steimle, J. and Myers, P. (1999). The direct release of nitric oxide by gypenosides derived from the herb *Gynostemma pentaphyllum*. Nitric oxide 3:359-365.

29.Aktan, F., Henness, S., Roufogalis, B. and Ammit, A. (2003). Gypenosides derived from *Gynostemma pentaphyllum* suppress NO synthesis in Murine macrophages by inhibiting iNOS enzymatic activity and attenuating NF-κB-mediated iNOS protein expression. Nitric oxide 8:235-242.

30.Tsi, D. and Tan, B. (2000). The mechanism underlying the hypocholesterolemic activity of aqueous celery extract, its butanol and aqueous fractions in genetically hypercholesterolemic Rico rats. Life Sciences 66:755-767.

31.Jiang, X., Moulin, P., and Quinet, E. (1991). Mammalian adipose tissue and muscle are major sources of lipid transfer protein mRNA. Journal of Biological Chemistry 266:4631-9.