Prevention of free fatty acid-induced lipid accumulation, oxidative stress, and cell death in primary hepatocyte cultures by a Gynostemma pentaphyllum extract

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ABSTRACT

Hepatocytes of a primary cell culture that are exposed to high glucose, insulin, and linoleic (LA) acid concentration respond with lipid accumulation, oxidative stress up to cell death. Such alterations are typically found in patients with non-alcoholic fatty liver disease (NAFLD). We used this cellular model to study the effect of an ethanolic Gynostemma pentaphyllum (GP) extract in NAFLD. When hepatocytes were cultured in the presence of high insulin, glucose, and LA concentration the extract completely protected the cells from cell death. In parallel, the extract prevented accumulation of triglycerides (TGs) and cholesterol as well as oxidative stress. Our data further demonstrate that GP stimulates the production of nitric oxide (NO) in hepatocytes and affects the molecular composition of the mitochondrial phospholipid cardiolipin (CL). We conclude that GP is able to protect hepatocytes from cell death, lipid accumulation, and oxidative stress caused by diabetic-like metabolism and lipotoxicity. Therefore, GP could be beneficial for patients with diabetes mellitus and NAFLD.

Introduction

NAFLD is one of the most spread metabolic disorders in highly developed industrial countries (Preiss and Sattar 2008). With the prevalence of 20–30%, NAFLD has a high impact in clinical practice. First, individuals with NAFLD are asymptomatic. In a later stage of the disease, patients develop elevated serum levels of TGs and transaminases. Lipid droplets accumulate in liver cells and the size of the organ increases (hepatomegaly). NAFLD often progresses to steatohepatitis, cirrhosis and finally to hepatocellular carcinoma.

Diabetes and obesity are factors that are known to trigger NAFLD (Videla et al. 2006; Lockman and Nyirenda 2010). Changes in lipid metabolism originating from insulin resistance and/or excessive fat intake cause increase in serum free fatty acid (FFA), TG and cholesterol concentration. The challenge of liver cells with high concentrations of FFA can result in abnormal fat accumulation as TG rich lipid droplets and oxidative stress (Müller et al. 2010). Both processes contribute to the damage of liver cells (Feldstein et al. 2004). The pathological potential of high FFA concentration depends on the type of the fatty acid. The major part of studies state lipotoxic properties of saturated FFA such as palmitate (PA) resulting in oxidative stress and apoptosis, whereas unsaturated FFA have no or low lipotoxic effects (Ricchi et al. 2009; Malihi et al. 2006). However, there are controversial reports demonstrating lipotoxic effects of unsaturated FFA (Videla et al. 2004; Turpeinen et al. 1999). In our previous study (Müller et al. 2010) we have shown that hepatocytes of a primary culture from rat liver exposed to LA, but not PA, caused decrease in the number of surviving cells, increase in oxidative stress, and lipid accumulation in the presence of high glucose and insulin concentrations. In this context, the observed oxidation of the mitochondrial phospholipid CL has been discussed to mediate mitochondrial dysfunction and apoptosis.

The first choice to prevent NAFLD in obese and diabetic individuals is the intervention of the basic illness by body weight reduction and the administration of anti-diabetic drugs, respectively. Currently, the application of plant extracts as nutritional supplements for prevention of metabolic disorders have been come into the focus of interest. GP, a wild growing herb, has been used in Asian countries in traditional medicine. Extracts of GP are composed of a complex mixture of compounds. Main components that mediate biological effects are gypenosides. More than 82 different gypenosides have been identified in GP (Cui et al. 1999). A broad spectrum of beneficial effects of GP extracts have been reported including antioxidative activity (Lin et al. 2000; Shang et al. 2006), anti-hyperlipidemic and hypoglycaemic activity (Megalli et al. 2005,
There are also reports demonstrating inhibition of NAFLD progression (Chou et al. 2006). However, the mechanism of these GP effects has not been completely identified.

Here we report about the effect of an ethanolic extract from GP on FFA-dependent cell death, oxidative stress, and lipid accumulation in hepatocytes of a primary cell culture from rat liver. We exposed hepatocytes to LA or PA in the presence of high glucose and insulin concentration and analyzed the effect of an ethanolic GP extract on cell viability, oxidative stress, total lipid (TL) accumulation, TG and cholesterol accumulation, nitric oxide (NO) metabolism, and the distribution of molecular CL species.

Materials and methods

Materials

Porcine insulin was from Nova Biolabs (Bagsvaerd, Denmark). Collagenase A used for the perfusion was from Biochrom AG (Berlin, Germany). GP extract powder was from Herbasin (Shenyang) Co., Ltd. (China). The HPLC-fingerprint analysis of the extract is published in Liu et al. (2008) and Schild et al. (2010). M199 medium and bovine serum albumin were from Roche Molecular Biochemicals (Mannheim, Germany). All other chemicals and reagents were from Sigma (Munich, Germany).

Cell culture

Hepatocytes from adult male Wistar rats (180–250 g) were isolated by collagenase perfusion in situ (Seglen 1973) and purified by centrifugation applying Percoll for better separation (Oetjen et al. 1990). Isolated hepatocytes were cultured with M199 in 35 mm dishes. The dishes were coated with collagen prepared from rat tail tendons (1 g/300 ml) which have been dissolved in 0.1% acetic acid for 24 h. The M199 culture medium additionally contained 4% new-born calf serum, 15 mM Hepes, 10 mM glucose, 0.2% BSA and 10⁻⁷ M insulin. Every dish was filled with 10⁶ cells/ml and 2 ml of culture medium. The medium was changed after 4 h and 24 h. The hepatocytes were cultured for 48 h in gas atmosphere containing 78% N₂, 17% O₂ and 5% CO₂ at 37 °C. The cells were incubated with and without GP in the absence of FFA (i), with 100 µM palmitate (ii), and with 100 µM linoleic acid (iii), respectively.

Determination of protein

The protein was determined according to the method of Lowry (Lowry et al. 1951). The cells were dissolved in 1% dodecylsulfate in the presence of 0.1 M NaOH. For quantification BSA was used as the standard.

Determination of viability

Viability was estimated using the trypan blue exclusion method. Cells were harvested from the dishes and incubated with trypan blue for 10 min. Afterwards, viable and dead cells (blue) were counted using a Neubauer chamber. Viability is expressed in percent of total (quotient of the number of trypan blue positive cells to the sum of vital and dead cells × 100%).

Determination of total lipid content (TL)

Lipids were extracted by the method of Bligh and Dyer (1959) with some modifications. A sample of 500 µl was mixed with 5 µl 0.01 M BHT for antioxidant protection, 2 ml methanol, and 1 ml chloroform. The mixture was incubated for 20 min at 37 °C. Afterwards, 0.4 ml 0.01 M HCl, 1 ml chloroform and 1 ml H₂O₂ were added. The samples were centrifuged for 10 min at 2600 × g. The lower lipidl phase was collected and dried at 45 °C under nitrogen atmosphere. Finally, the lipid contents of the samples were determined gravimetrically.

Determination of cellular cholesterol content

To determine the cellular content of cholesterol we applied the method described by Gallo et al. (1978). The reaction mixture contained 0.01 M potassium phosphate buffer (pH 7.0), 5.2 mM sodium taurocholate, 2.0 mM sodium azide, 14.0 mM phenol, 0.82 mM 4-amino-antipyrine, 0.5 mM Triton X100, 90 units of cholesterol oxidase, and 33,000 units of horseradish peroxidase.

80 µl of cell suspension was mixed with 3 ml of reaction mixture and subsequently incubated for 10 min at room temperature. The absorbance was measured at 500 nm. Cholesterol standards (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg of cholesterol per ml) were prepared in isopropanol.

Determination of TG content

For the determination of cellular TG content, we used a sample of 20 µl cell suspension per dish. The concentration of TGs was determined by using a Hitachi 912 analyzer (Bosch, Germany).

Estimation of NO

NO content of the hepatocytes was estimated indirectly by using the method for nitrite detection as described by Giess and modified by Ding et al. (1988). The supernatants of the dishes were used for analysis. Therefore, the supernatants were mixed with 1 ml of Griess reaction containing one part of sulfanilamide and one part of N-(naphthyl)ethylenediamine dihydrochloride in 5% aqueous phosphoric acid and incubated for 10 min at room temperature. Afterwards, the absorption was measured at 540 nm. For quantification nitrite standard solutions were used.

Quantification of CL species

Extraction of CL: 50 ng of tetra-myristoyl-CL([C14:0]₄-CL; Avanti Polar Lipids Inc., Alabaster, AL) was added as internal standard to 10 µl cell suspension. For extraction of CL chloroform/methanol (2/1, v/v) containing 0.05% BHT as antioxidant was added. The lipid and aqueous phases were separated by adding 0.01 M HCl, intensive shaking and centrifugation. After centrifugation, the lower lipid phase was collected and dried under nitrogen atmosphere and acidified. Ice-cold methanol (2 ml), chloroform (1 ml) and 0.1 M HCl (1 ml) were added. The solution was intensively mixed. After 5 min of incubation on ice the samples were separated by the addition of chloroform (1 ml) and 0.1 M HCl (1 ml). The chloroform/methanol phase was recovered as CL-containing sample. The samples were dried under nitrogen and dissolved in 0.8 ml chloroform/methanol/water (50/45/5, v/v/v/v). After mixing and filtering of the mixture over 0.2 µm PTFE membranes the samples were ready for analysis.

HPLC–MS/MS analysis: For measurement of molecular CL species a TSQ Quantum Discovery Max (Thermo Fisher Scientific GmbH, Dreieich, Germany) was used in the negative ion electrospray ionization (ESI) mode. The HPLC system consisted of a Surveyor MS quaternary narrow bore pump with integrated vacuum degasser and a Surveyor auto sampler. Auto sampler tray temperature was held at 8 °C. In partial loop mode a sample of 10 µl of lipid extract dissolved in chloroform/methanol/water (50/45/5, v/v/v/v) was injected and CL was separated by using a LiChroCart column (125 mm × 2 mm), LiChrophase Si60 (5 µm particle diameter;
Merck, Darmstadt, Germany) and a linear gradient between solution A (chloroform) and solution B (methanol/water 9:1, v/v). All solutions contained 25% aqueous ammonia (0.1 m\text{M}). The gradient was as follows: 0–0.2 min 92% solution A, 8% solution B; 0.2–4.5 min 92–30% solution A, 8–70% solution B; 4.5–6 min 30% solution A, 70% solution B; 6–6.5 min 30–92% solution A, 8% solution B. The flow rate was 300 \mu\text{L/min}. Total time of analyses was 11 min. The eluate between 0.3 and 6 min was introduced into the mass spectrometer. Nitrogen was used as the nebulizing gas and argon as collision gas at a pressure of 1.5 m\text{Torr}. The spray voltage was 3.5 kV, the ion source capillary temperature was set at 375 °C and the cone-voltage was 30 V. Daughter fragments from the doubly charged parent derived from \text{[C18:2]_{4-CL}} with \text{m/z} 723.6 ([M–2H]^2–/2) were obtained using a collision energy of 36 eV. This molecular CL species and the internal standard (\text{m/z} 619.6) were analyzed by mass transfer reaction monitoring their doubly charged ions and their respective fatty acids LA \text{m/z} 279.2 and myristic acid \text{m/z} 227.2 using the selected reaction monitoring (SRM) mode. The same approach was used for parent and daughter fragments of other molecular species of CL. The quantity of these molecular species was related to the content of \text{[C18:2]_{4-CL}}.

**Determination of F2-isoprostanes (F2-IsoPs)**

The content of F2-IsoPs was determined as sum of esterified and non-esterified compounds. For the determination of F2-IsoPs concentrations hepatocyte samples of 150 \mu\text{L}, containing about 0.5–1.0 x 10^6 cells/mL plus 1.5 \mu\text{M} 10 mM BTH to prevent autoxidation were incubated with 125 \mu\text{M} 1 M KOH, at 45 °C for 30 min. Afterwards, the solutions were neutralized by the addition of 4 \text{mL} 0.01 M \text{HCl}. The pH was adjusted to about 2 with 1 M \text{HCl}. 9a,11α-\text{PGF}_{2α}-d_4 (Axxora GmbH, Lörrach, Germany; 0.5 ng in 10 \mu\text{L} ethanol) was added as internal standard. The samples were applied onto a C18-cartridge (Vac 3cc 500 mg, Waters Corporation, USA), pre-washed with 5 mL methanol and 5 mL water (\text{pH} 2). The cartridge was washed with 10 mL water (\text{pH} 2) and 10 mL of acetonitrile/water (15/85, v/v). Subsequently, isoprostanes were eluted from the columns with 5 mL of n-hexane/ethylacetate/2-propanol (30/65/5, v/v/v). The extract was dried under argon stream at 45 °C. The residue was reconstituted with 40 \mu\text{L} pentafluorobenzyl-bromide (10% in acetonitrile) and 20 \mu\text{L} of N,N-diisopropylethylamine (10% in acetonitrile) and incubated at 45 °C for 30 min. After incubation 50 \mu\text{L} bis-(trimethylsilyl) trifluoroacetamide (BSTFA) and 5 \mu\text{L} of N,N-diisopropylethylamine were added and the solution incubated at 45 °C for 60 min. The solvents were removed and the samples were reconstituted in 60 \mu\text{L} isooctane containing 0.1% BSTFA. F2-IsoPs were separated and measured by gas chromatography–mass spectrometry/negative-ion chemical ionization (DSQ/Trace GC Ultra, Thermo Fisher Scientific, Dreieich, Germany) using a DB-5 MS column (50 m x 0.25 mm inner diameter; 0.25 \mu\text{m} film thickness; J&W Scientific, Folsom, USA) with the following program: the initial temperature was 80 °C for 2 min; with a rate of 30 °C/min to final temperature of 280 °C holding for 19 min; total run time: 27.7 min. The quantitative analysis was performed with ammonia as reagent gas using selected ion monitoring (SIM) of the carboxylate anion [M–181]– at m/z 569 for F2-IsoPs and 573 for 9a,11α-\text{PGF}_{2α}-d_4 (internal standard).

**Statistics**

Data are presented as mean ± SEM from at least 6 individual cell cultures. Statistical analysis of the variation among the groups was performed with Mann–Whitney-U test using SPSS 18.0. A value of \text{p} < 0.05 was considered as statistically significant.

**Results**

**Effect of GP on viability of cultured hepatocytes**

First, we investigated whether the presence of GP in the culture medium affects the viability of hepatocytes. Therefore, we subjected the cultures to different concentrations of GP and determined cell viability after 48 h of cultivation. As depicted in Fig. 1, GP did not influence the viability of hepatocytes that were cultured in the presence of 10 mM glucose and 10⁻² M insulin. The additional presence of FFA in the culture medium had a significant impact on cell viability depending on the type of FFA. LA, an essential unsaturated fatty acid with two double bounds, decreased viability in viability from 80.4 ± 1.1% of total (without LA) to 67.6 ± 1.0% of total (with LA) whereas PA did not influence the viability in comparison to control. GP prevented LA-mediated decrease in viability in a dose dependent manner. Moreover, GP protected hepatocytes from decrease in viability during 48 h of cultivation in the presence of palmitate (PA).

**Effect of GP on cellular lipid content**

FFA-induced accumulation of lipids in hepatocytes is a process that significantly contributes to cellular injury. In our previous work, we have demonstrated that LA causes massive accumulation of TGs (Müller et al. 2010). To study the effect of GP on lipid accumulation hepatocytes were incubated at different concentrations of the extract in the presence of PA or LA and the content of TG and cholesterol was analyzed. In the absence of FFA in the culture medium the cellular content of lipids remained unchanged 1.47 ± 0.08 mg/mg protein (after 4 h of cultivation) to 1.31 ± 0.07 mg/mg protein (after 48 h of cultivation; control). LA caused massive increase in TG content to 301 ± 21% of control within 48 h of cultivation. In contrast, PA led to a smaller increase in TG content to 137 ± 5% of control. GP generally protected hepatocytes from excessive lipid accumulation in a dose dependent manner.

A significant part of accumulated lipids in hepatocytes consists of TGs that form droplets within the cell (Müller et al.
In parallel to TL, the TG content in hepatocytes slightly increased in the presence of high glucose and insulin within 48 h of cultivation from 0.20 ± 0.05 μg/mg protein to 0.243 ± 0.05 μg/mg protein. The presence of LA resulted in massive increase in TG content to 434.16 ± 17.20% of control. Under all conditions, GP lowered TG accumulation. In particular, the ethanolic extract of GP protected hepatocytes from massive LA-mediated TG accumulation in a dose dependent manner (Fig. 2A). A third essential component of hepatocellular lipids is cholesterol. In the presence of high glucose and insulin concentration the cellular cholesterol content slightly increased from 0.113 ± 0.004 μmol/mg protein to 0.146 ± 0.006 μmol/mg protein (control). FFA caused moderate increase in cholesterol content. The presence of LA resulted in an increase to 172.23 ± 9.82% of control and PA to 110.94 ± 6.55% of control, respectively. In general, GP diminished cholesterol accumulation in hepatocytes within 48 h of cultivation in a dose dependent manner. Most impressing, GP was able to prevent significant cholesterol accumulation in the presence of LA (Fig. 2B).

**Effect of GP on F2-IsopPs formation as indicator of oxidative stress**

F2-IsopPs are generated by non-catalytic oxidation of arachidonic acid in all cell compartments by reaction with ROS such as hydroxyl radicals (Morrow et al. 1990). The concentration of F2-IsopPs increased in hepatocytes within 48 h of cultivation from 0.629 ± 0.043 ng/mg protein (after 4 h of cultivation) to 1.313 ± 0.146 ng/mg protein (after 48 h of cultivation; control). LA and PA caused further increase in F2-Isoprostane concentration to 615.68 ± 28.14% of control and to 116.53 ± 33.23% of control, respectively. GP effectively diminished generation of F2-IsopPs at low and high concentration (Fig. 3). We did not observe a strong dose dependency of the antioxidative activity of the extract. The middle concentration of the extract was less effective than the lowest and highest concentration in preventing oxidation of arachidonic acid.

**Effect of GP on hepatocellular nitrite content**

In our previous paper we reported about alterations in NO metabolism by PA and LA and concluded that NO prevents lipid accumulation and oxidative stress in hepatocytes (Müller et al. 2010). Thereby, significant increase in nitrite concentration was observed when hepatocytes were cultured in the presence of PA. Here we show that GP is able to cause significant elevation in nitrite concentration in the presence of PA and LA (Fig. 4). In the absence of FFA, nitrite concentration was not affected by high insulin and high glucose (106 ± 3% of initial). GP also caused no significant change in nitrite concentration.

**Effect of GP on CL structure**

The adequate content and assembling of the mitochondrial phospholipid CL is essential for normal cell function. Oxidation of CL or changes in CL structure may result in mitochondrial and subsequently cellular dysfunction. On the contrary, changes
in CL composition may be beneficial by reducing the vulnerability against oxidative attack possibly triggering apoptosis. To search for GP-dependent CL alterations in hepatocytes, we analyzed seven molecular CL species by HPLC–MS/MS. Over all, some GP-dependent changes were found in particular CL species showing the tendency to increase the degree of saturation in fatty acid residues. GP caused decrease in \( (C18:2)_n-CL \) concentration the most abundant and highly unsaturated molecular CL species in hepatocytes with four linoleic acid residues each carrying two double bounds (eight double bounds per molecule; Fig. 5A). In compensation, GP caused significant increase in the content of \( [(C18:1)_2(C18:2)(C16:1)-CL] \) or \( [(C18:2)_2(C18:1)(C16:0)-CL] \) that carries only 5 double bounds per molecule (Fig. 5B).

**Discussion**

In this work, we used hepatocytes of a primary cell culture to study effects of an ethanolic extract from GP in NAFLD. Although these cells were cultured in the presence of high insulin and glucose concentration, insulin signaling in glycolysis is completely abolished and glycogen synthesis is restricted to 30% of normal activity (Klein et al. 2002). Such changes in cellular glucose metabolism also occur in individuals with diabetes mellitus type II. Moreover, high concentrations of FFA accompany diabetes and obesity (Boden 2008). In our previous work (Müller et al. 2010) we have demonstrated that, in particular, LA caused NAFLD-like changes within hepatocytes including lipid accumulation, oxidative stress and cell death. Thus, our experimental approach represents essential aspects of NAFLD.

There is no information available about toxic effects of GP on primary cultures of hepatocytes. Moreover, long time studies with Wistar rats fed with high doses of GP displayed no side effects (Attawish et al. 2004). Therefore, it is reasonable to assume that GP has no toxic effect in hepatocytes of primary cell cultures. In fact, our data support this assumption. GP rather protected hepatocytes from damage during 48 h of cultivation (Fig. 1). Most important, our data provide for the first time evidence that GP has the potency to almost completely protect hepatocytes from LA-dependent cell death.

Main factors that mediate cell damage are lipid accumulation and oxidative stress (Müller et al. 2010). It has been shown, that the unsaturated fatty acid LA triggers the deposition of TGs in hepatocytes when the cells are incubated at high glucose and high insulin concentrations in vitro (Müller et al. 2010) or when high concentrations of LA are fed to rats (Rustan et al. 1992). In contrast, the saturated fatty acid PA did not cause lipid accumulation (Müller et al. 2010; Ricchi et al. 2009). It has been demonstrated in metabolically altered rats that GP is able to lower TG and cholesterol concentration in serum (Megalli et al. 2005, 2006; Tan et al. 2011). A possible mechanism might be GP-dependent alteration of NO metabolism. It has been shown that GP affected NO synthase levels in bovine aortic endothelial cells (Tanner et al. 1999). There is a body of evidence showing a closed link between NO and lipid metabolism. NO donors can cause accelerated β-oxidation and transport of free fatty acids (Marra et al. 2007). Another point of interaction is the NO-dependent fatty acid synthesis (Roediger et al. 2010).
et al. 2004). NO regulates enzyme activities of fatty acid metabolism directly (Tanner et al. 1999) and indirectly by inhibiting the expression of citrate synthase (Schild et al. 2008, 2006). On the other side, alterations in the expression of nitric oxide synthase isoforms have been reported for the liver of obese rats (Fujimoto et al. 2005; Wan et al. 2000). In humans with NAFLD changes in iNOS protein levels have also been detected. On the other side, dietary fats have been shown to affect NO metabolism (Tipoe et al. 2009). The experiments reported here demonstrate that NO possess lipid reducing effects in hepatocytes cultured in the presence of high glucose and insulin concentration and that PA stimulates NO production. We have further demonstrated that GP affects NO generation in hepatocytes. However, GP caused significant increase in nitrite content only at the highest concentration whereas TG and cholesterol content was affected already at lower GP concentrations. Thus, the finding that GP prevents massive accumulation of TGs and cholesterol in the presence of high insulin, glucose and LA concentration points toward a direct influence of GP on lipid metabolism in hepatocytes. One mechanism could be the interaction between LA, GP and peroxisome proliferator activating receptor (PPARx). It has been demonstrated that GP caused increase in PPARx mRNA and protein in murine macrophages (Huang et al. 2006).

The mitochondrial phospholipid CL plays a crucial role for cellular fate. It is essential for proper transport of electrons within the respiratory chain and subsequently for sufficient ATP production. Oxidation and/or loss of CL are involved in the induction of apoptosis (McMillin and Dowhan 2002). Even changes in the distribution of molecular CL species are associated with mitochondrial dysfunction as has been demonstrated in patients with Barth syndrome (McKenzie et al. 2006). Here, we demonstrate for the first time that GP influences the distribution of molecular CL species in hepatocytes. These changes are characterized by increasing the degree of saturation of fatty acid residues. The final distribution of molecular CL species is the result of CL synthesis and remodeling of the acyl-chain composition (Schlae et al. 2000; Xu et al. 2003). Apparently, GP affects at least one of these metabolic processes. It is generally assumed that unsaturated fatty acid residues are vulnerable against the attack of reactive oxygen species (ROS) (Wiswedel et al. 2010). Therefore, the GP-dependent switch toward a higher degree of saturation within fatty acid residues of CL could protect hepatocytes from mitochondrial dysfunction and induction of apoptosis. It might be suggested that increased stability of CL by GP contributes to the protection of hepatocytes cultured in the presence of high insulin, glucose and free fatty acid concentration.

Reactive oxygen and nitrogen species can damage biological molecules including DNA molecules, proteins and lipids. We have found that LA causes oxidative stress in hepatocytes cultured in the presence of high insulin and glucose concentration as indicated by increased oxidation of arachidonic acid to F2-IsopPs. Elevated generation of F2-IsopPs has been also observed in other studies related to NAFLD (Zhu et al. 2008; Turpeinen et al. 1998). GP reduced F2-IsopPs production in hepatocytes subjected to high glucose, insulin, and LA concentration. It is known that GP possesses antioxidative activity. The complex mixture of saponines and flavonoids within the ethanolic extract from GP acts directly as an antioxidant (Lin et al. 2000; Shang et al. 2006). Furthermore, it has been shown that GP induces the expression of enzymes of the antioxidative defense system such as superoxide dismutase and glutathione peroxidase (Shang et al. 2006; Schild et al. 2010; Wang et al. 2010). Additionally, GP stimulates the expression of nitric oxide synthases (Tanner et al. 1999). Besides direct effects of NO on mitochondrial energy metabolism NO reacts with reactive oxygen species. It can cause the production of the reactive peroxynitrite by reaction with superoxide anion radicals (Halliwell et al. 1999). NO itself can act as antioxidant (Wink et al. 1994). All of these processes may contribute to the over all antioxidative effect of the ethanolic extract from GP.

From our results we conclude that GP is able to protect hepatocytes from cell death caused by diabetic-like metabolism and lipotoxicity. In this context, main effects of GP are prevention of lipid accumulation and oxidative stress. These properties of GP could be beneficial for patients with diabetes mellitus and NAFLD.

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References


