Effects of Glycyrrhetic Acid (GE) on Some Gluconeogenic Enzymes, Lipoprotein Lipase and Peroxisome Proliferator-Activated Receptors Alpha and Gamma

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Abstract: The aim of this study was to examine the role of glycyrrhetic acid (GE) as a potential compound in the amelioration of metabolic syndrome. Rats given intraperitoneal injection of GE were sacrificed after 24 hours. Blood was collected for the determination of glucose, insulin and lipid profiles; while tissues were used for 11β-HSD1, gluconeogenic enzymes activities, PPAR-α/γ and LPL expression by RT-PCR. Intraperitoneal injection of 50mg/kg GE to normal rats significantly lowered blood glucose while insulin level and HOMA-IR showed no significant changes. H6PDH activities increased in the liver, kidney, subcutaneous and visceral adipose tissues and quadriceps femoris but decreased in the abdominal muscle. PEPCK activities were significantly reduced in the kidney and decreased in the liver but showed an increase in the subcutaneous and visceral adipose tissues. G6Pase activities were found to be reduced in both the liver and kidney. 11β-HSD1 activities increased in the liver but decreased in all other tissues. There were improvements in lipid profiles in GE-treated rats. Up-regulation of LPL activity was seen in all tissues except quadriceps femoris. PPAR-α expression was up-regulated in the liver, heart and abdominal muscle while down-regulated in the kidney and quadriceps femoris but were undetectable in the subcutaneous and visceral adipose tissues. PPAR-γ expression was up-regulated in all tissues except the kidney. GE prevented hyperglycaemia and improved lipid profiles possibly through 11β-HSD1 inhibition instead of via PPAR agonism.

Keywords: Glycyrrhetic acid, glucose-6-phosphatase (G6Pase), hexose-6-phosphate dehydrogenase (H6PDH), lipoprotein lipase, phosphoenolpyruvate kinase (PEPCK), peroxisome proliferator-activated receptors (PPAR), 11β-hydroxysteroid dehydrogenase (11β-HSD1).

INTRODUCTION

The metabolic syndrome (MetS) is a cluster of risk factors which are dyslipidemia, hyperglycaemia, hypertension, central obesity and insulin resistance (IR) [1]. When an individual has three out of these five factors, he/she will be considered to have MetS and is at risk of getting cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [1]. The adaptation of obesogenic diet and sedentary lifestyle has been recognized as the major contributor to MetS which can be related to rapid urbanization and improved socioeconomic status [2]. Obesity, especially increased accumulation of fat in the visceral region, has been shown to have a strong link with dyslipidemia, IR, hypertension, T2DM and CVD [3,4]. This can be associated with increased free fatty acids (FFA) release into the portal circulation, thus impairing hepatic metabolism followed by systemic IR and metabolic derangements [5].

Lipoprotein lipase (LPL) is an enzyme involved in lipid metabolism. It hydrolyzes the triacylglycerol (TAG) store within lipoproteins and facilitates uptake of fatty acids by the peripheral tissues [6]. Low LPL activity inhibits chylomicrons and very-low-density lipoprotein (VLDL) lipolysis. This contributes to excessive TAG level in the circulation and can lead to dyslipidemia [7]. LPL expression is regulated by the peroxisome proliferator-activated receptor (PPAR) under different physiological conditions [8].

Glucocorticoids (GC) are important regulators of carbohydrate and lipid metabolism [9]. Alterations of GC level have been related to obesity and IR which could finally lead to MetS [10]. 11β-hydroxysteroid dehydrogenase (11β-HSD) is an enzyme that catalyzes the inter-conversion of active GC and inactive GC [10]. One of the major effects of GC is the increase in hepatic glucose production due to their opposing actions on insulin that counteracts the increased level of gluconeogenic enzymes which include phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [11,12]. Hexose-6-phosphate dehydrogenase (H6PDH), an enzyme found in the endoplasmic reticulum lumen, is involved in the pentose phosphate pathway. H6PDH catalyzes the i.) conversion of glucose-6-phosphate (G6P) to 6-phosphogluconolactone (6PGL) and ii.) generation of reduced adenine dinucleotide phosphate (NADPH) from NADP+ [13,14]. 11-βHSD1 converts inactive GC to active GC. This reaction utilizes NADPH produced from the reaction catalyzed by H6PDH, which in turn depend on the
availability of G6P [10,15]. Overexpressions of H6PDH, PE PKCK and G6Pase have been shown in T2DM patients where GC were in excess [11,12].

The peroxisome proliferator-activated receptors (PPAR) is a group of ligand-activated transcription factors from the nuclear receptor superfamily that controls the expression of various genes involved in glucose and lipid metabolism e.g. PPAR-α, PPAR-β/δ and PPAR-γ [16]. PPAR-α is predominantly expressed in tissues with a high capacity for fatty acid oxidation (FAO) which include the liver, heart, kidney and skeletal muscle [17]. PPAR-γ is mainly involved in adipogenesis and storage of FFA in the adipose tissues [16]. PPAR-γ can be found abundantly in the adipose tissues and large intestine while existing at intermediate levels in the kidney, liver as well as small intestine [18].

Fibrates (a PPAR-α agonist) are a widely used class of lipid-modifying agents for dyslipidaemia while thiazolidinediones (TZDs) (a PPAR-γ agonist) are a class of insulin-sensitizing agents used in T2DM patients [19]. Combined therapy of fibrates and TZDs is often used since most patients diagnosed with T2DM also suffer from dyslipidaemia [20]. Several PPAR-α/γ agonists with dual actions had been tested on subjects which showed significant anti-hyperglycaemic and anti-hyperlipidaemic effects. However, some of these dual PPAR-α/PPAR-γ agonists were withdrawn at the late-stage of drug development due to safety concerns, for example, in 2004, ragaglitazar was discontinued as it was found to induce anaemia and urothelial cancer [20].

Despite the effectiveness of fibrates and TZDs in the treatment of dyslipidaemia and T2DM, both classes of these drugs have been associated with unwanted side effects. Cases of rhabdomyolysis and cholesterol gall stone formation have been reported with the use of fibrates while the most common side effects of TZDs are weight gain and fluid retention [21,22]. Therefore, potential PPAR-α and PPAR-γ ligands from natural sources e.g. glycyrhizic acid (GE) are being looked into.

GE, a triterpenoid aglycone, is an active metabolite of glycyrhizic acid (GA) which is the primary active ingredient of the root extract of licorice plant, Glycyrrhiza glabra [23,24]. GA has been found to lower blood glucose in rats [6,25,26]. Both GA and GE are known inhibitors of both isoforms of 11β-HSD i.e. 11β-HSD1 and 11β-HSD2 [27,28], thus resulting in reduced GC level. Such alteration in GC action could be utilized as the therapeutic strategy for T2DM. Furthermore, triterpenoids have been found to be PPAR agonists [29,30]. GE, as a triterpenoid compound with reported inhibitory effects 200-1000 times more potent than GA on 11β-HSD1 [31] may exert anti-dyslipidaemic and anti-diabetic effects at a lower concentration.

As GE has been reported to be 200-1000 times more potent than GA [31], the roles of GE in modulating glucose and lipid metabolism were being looked into in this study. The parameters measured include:

i.) blood glucose, serum insulin and Homeostatic-Model Assessment for insulin resistance (HOMA-IR),

ii.) lipid profiles

iii.) LPL, PPAR-α and PPAR-γ expression

iv.) PEPCK, H6PDH, G6Pase activities

v.) 11β-HSD1 activities.

MATERIALS AND METHODS

Animal Sampling and Treatment

All animal procedures were approved by Monash University School of Biomedical Sciences Animal Ethics Committee (AEC Approval number MARP/2012/043). Sixteen male Sprague-Dawley rats (180-200g) were supplied by Monash University Sunway Campus Animal House. The rats were divided into two groups (8 rats per group) i.e. the control and treated groups. All rats were kept in individual polypropylene cages in a room maintained at 24°C and exposed to 12h light- 12h dark cycle. The rats from the control group were given intraperitoneal (IP) injection of 99% dimethyl sulfoxide (DMSO) while rats from the treated group were given IP injection of 50mg/kg GE in 99% DMSO. After injection, the rats from both groups were fed ad libitum with standard rat chow (Gold Coin, Malaysia) and drinking water.

Sample Collection

Upon completion of 24 hours treatment, the rats were subjected to overnight fast and anaesthetized by intraperitoneal injection of pentobarbital sodium (Nembutal) (150mg/kg). The blood and plasma were collected and plasma was stored at -80°C. Liver, kidney, heart, subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), abdominal muscle (AM) and quadriceps femoris (QM) were harvested and stored at -80°C until required for further analysis.

Blood and Serum Biochemical Analysis

Blood glucose was measured using Trinder’s glucose oxidase reaction while serum insulin was determined using Rat/Mouse Insulin ELISA (Milipore, USA). Serum FFA, TAG and total cholesterol were determined using the Randox FA115 Non-Esterified Fatty Acids kit (Randox, UK), Randox Triglycerides Kit (Randox, UK) and Randox CH200 Cholesterol Kit (Randox, UK) respectively. HDL-Cholesterol and LDL-Cholesterol were determined using Randox CH203 HDL Precipitant (Randox, UK) and Friedewald formula [32], respectively.

RNA Extraction and cDNA Synthesis

Qiagen RNeasy Mini Kit (Qiagen, USA) and Qiagen RNeasy Lipid Tissue Mini Kit (Qiagen, USA) were used for extraction of RNA from the liver, kidney, heart, AM and QF; and SAT and VAT, respectively. The concentration and purity of RNA were determined by measuring the absorbance at 260 and 280nm. cDNA synthesis was done using the Qiagen Omniscript Reverse Transcriptase Kit (Qiagen, USA).

Real Time Reverse Transcription Polymerase Chain Reaction qRT-PCR of Lipoprotein lipase (LPL), Peroxisome proliferator-Activated Receptor-α and -γ (PPAR-α and PPAR-γ) Gene

The comparisons of LPL, PPAR-α and PPAR-γ expressions between control and GE-treated rats were performed...
using the Comparative Ct (ΔΔCt) Method, with BAC as reference, GE-treated group as target and control group as calibrator [33]. Agarose gel electrophoresis was performed on the amplicons obtained from qRT-PCR to ensure primer specificity. The expression of LPL, PPAR-α and PPAR-γ was determined by qRT-PCR using the probe, forward and reverse primers that are specific for Rattus norvegicus as listed below:

### Table 1. The Probe, Forward and Reverse Primers for LPL, PPAR-α and PPAR-γ Specific for Rattus norvegicus

<table>
<thead>
<tr>
<th>Primer/probe</th>
<th>Primer sequence (5’ → 3’)</th>
</tr>
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<tbody>
<tr>
<td>PPAR-α forward primer</td>
<td>TGTGGAGATCGGCCCTGGGCTT</td>
</tr>
<tr>
<td>PPAR-α reverse primer</td>
<td>CCGGATGGTTGCTCTGAGGT</td>
</tr>
<tr>
<td>PPAR-α probe</td>
<td>(6-FAM) TGCAG-GAGGGATGGTCAGTGCTCA (BHQ1)</td>
</tr>
<tr>
<td>PPAR-γ forward primer</td>
<td>CCCTGGCAAAAGCATTTGTAT</td>
</tr>
<tr>
<td>PPAR-γ reverse primer</td>
<td>GGTTATTTGCTGTCCTTTCC</td>
</tr>
<tr>
<td>PPAR-γ LNA probe</td>
<td>(6-FAM) TTCCTCCCGCTGACCA (BHQ1)</td>
</tr>
<tr>
<td>BAC forward primer</td>
<td>GTATGGGTCAGAAGGACTCC</td>
</tr>
<tr>
<td>BAC reverse primer</td>
<td>GTTCAATTGGGATCTTCCG</td>
</tr>
<tr>
<td>BAC LNA probe</td>
<td>(TET) CCTCTCTGCTGTCAGACCA (BHQ1)</td>
</tr>
<tr>
<td>LPL forward primer</td>
<td>CAGCAAGGCATACAGGT</td>
</tr>
<tr>
<td>LPL reverse primer</td>
<td>CGAGTCTTCAGGTACATCTTAC</td>
</tr>
<tr>
<td>LPL LNA probe</td>
<td>(6-FAM) TTCTCTGGCTTGACCA (BHQ1)</td>
</tr>
</tbody>
</table>

### Protein Determination

Tissue samples were homogenized using Heidolph DIAx 900 rotor stator homogenizer (Sigma-Aldrich, U.S.A) prior to enzyme activity determination and centrifuged to obtain the appropriate fraction. A modified Lowry’s method was used to determine the protein concentration [6].

### Determination of 11β-Hydroxysteroid Dehydrogenase (11β-HSD1) Activities

The 11β-hydroxysteroid dehydrogenase (11β-HSD1) activities were determined using high performance liquid chromatography (HPLC) following methods described by Chandramouli et al. [6]. A unit of 11β-HSD1 activity is defined as one pmole of 11-dehydrocorticosterone produced/50 mg of tissue protein used/ hour [34].

### Hexose-6-Phosphate Dehydrogenase (H6PDH) Activities

There was no significant difference for H6PDH activities in the liver between the control (0.58 ± 0.07 units) and GE-treated groups (0.90 ± 0.18 units) (P>0.05) as well as the kidney between the two groups with the control group being 3.22 ± 0.44 units and the GE-treated group being 3.29 ± 0.28 units respectively (P>0.05). In the SAT, the mean H6PDH activities in the control and GE-treated groups were 6.04 ± 0.50 units and 7.76 ± 0.84 units respectively while in the VAT, the mean activities in control and GE-treated groups were 4.18 ± 0.61 units and 4.99 ± 0.66 units respectively (P>0.05) (Fig. 4). In the abdominal muscle (AM), the mean H6PDH activities in the control and GE-treated groups were 4.79 ± 0.51 units and 4.12 ± 0.36 units respectively while in the quadriceps femoris muscle (QF), the mean H6PDH activities in the control and GE-treated groups were 5.12 ± 0.33 units and 6.15 ± 0.58 units respectively (P>0.05) (Fig. 4).

### Glucose-6-Phosphatase (G6Pase) Activities in the Liver and Kidney

The mean hepatic G6Pase activities in the control and GE-treated rats were 140.45 ± 5.33 units and 137.24 ± 7.77
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Fig. (1). Mean blood glucose concentration (mmol/L) of control and GE-treated groups (** indicates P<0.01 between groups).

Fig. (2). Mean serum insulin (ng/mL) of the control and GE-treated groups (P>0.05).

Phosphoenolpyruvate Carboxykinase (PEPCK) Activities in the Liver, Kidney, Subcutaneous and Visceral Adipose Tissues

The mean hepatic PEPCK activities in the control and GE-treated groups were 3.04 ± 0.45 units and 2.35 ± 0.44 units respectively (P>0.05) while the mean renal activities in the control group were 5.69 ± 0.67 units and the GE-treated group were 3.66 ± 0.28 units. A significant reduction of 55.71% was seen in the GE-treated group (P<0.05). In the SAT, no significant difference (P>0.05) was seen between the control (6.88 ± 1.05 units) and the GE-treated groups (7.23 ± 1.25 units) while in the VAT, similar observation was seen for the control (10.79 ± 1.75 units) and the GE-treated (11.68 ± 1.27 units) groups (Fig. 6).
Administration of GE to Rats did not Improve $11\beta$-HSD1 Activities in all Tissues Except the Liver

In the liver, the control and GE-treated groups had mean $11\beta$-HSD1 activities of $261.54 \pm 34.14$ units and $166.26 \pm 25.78$ units, respectively with a significant reduction of $36.43\%$ in the GE-treated group ($P<0.05$). However, the reduction of renal PEPCK was not significant in the GE-treated group ($P>0.05$). Renal $11\beta$-HSD1 activities were $44.93 \pm 3.83$ units and $35.75 \pm 2.91$ units, respectively. In the SAT, the control and GE-treated groups had mean $11\beta$-HSD1 activities of $27.50 \pm 3.76$ units and $24.27 \pm 2.60$ units, respectively. No significant difference was seen between the two groups ($P>0.05$). No significant difference ($P>0.05$) was observed between the control ($24.03 \pm 2.14$ units) and the GE-treated group ($23.42 \pm 3.97$ units) in the VAT. The activities in SAT were observed to be $16.25 \pm 4.47$ units for the control and $13.01 \pm 3.51$ units for the GE-treated group while in the QF, the values were $10.43 \pm 3.82$ units and $9.48 \pm 1.71$ units respectively. No significant difference was seen between the two groups ($P>0.05$) (Fig. 7).
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Fig. (5). Mean glucose-6-phosphatase (G6Pase) activities in the control and GE-treated rats (P>0.05).

Fig. (6). Mean phosphoenolpyruvate carboxykinase (PEPCK) activities in the control and GE-treated rats (* indicates P<0.05). [SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

Positive Shift in Lipid Profile in GE-Treated Rats

Consistent improvements in all the lipid parameters were observed in the treated group relative to the control group. A comparison of the lipid parameters and FFA between control and GE-treated groups is shown in (Fig. 8). The mean serum TAG concentration of control and GE-treated groups were 0.51 ± 0.05 mmol/L and 0.29 ± 0.02 mmol/L respectively, representing a significant 43% decrease in the GE-treated group (P<0.01). The mean serum FFA concentration of control and GE-treated groups were 0.38 ± 0.06 mmol/L and 0.27 ± 0.04 mmol/L respectively. Although insignificant, the GE-treated group showed a 29% decrease (P>0.05). As for the mean total cholesterol concentration, the value in the GE-treated group was significantly lower than the control group being 1.29 ± 0.12 mmol/L and GE-treated group being 0.95 ± 0.09 mmol/L indicating a significant decrease of 27% in the GE-treated group (P<0.05). Overall, intraperitoneal administration of 50mg/kg of GE to rats showed significant improvement in lipid profiles with a significant decrease in TAG, total cholesterol and LDL-cholesterol and a non-significant decrease in FFA and an increase in HDL.

Increased Lipoprotein Lipase (LPL) Expression in all Tissues With GE Treatment

It was observed that LPL expression was insignificantly up-regulated in all tissues except QF in the GE-treated rats. The LPL expression was up-regulated by 1.63 ± 0.66 fold in the liver and 1.32 ± 1.18 in the kidney. The up-regulation
Activities of 11β-HSD type 1 in all tissues from both the control and GE-treated groups (* indicates P<0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

Mean concentration of serum triglycerides (TAG), free fatty acids (FFA), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in rats from the control and GE-treated groups (** indicates P<0.01; * indicates P<0.05).

trend was also observed in the heart (1.14 ± 0.04 fold) and the AM (1.21 ± 0.75 fold). In adipose tissues, i.e. SAT and VAT, LPL expression was up-regulated by 1.19 ± 0.47 fold and 2.67 ± 0.52 fold respectively. On the other hand, LPL expression was hardly detectable in the QF (0.01 ± 0.16 fold). However, all the observations were statistically insignificant (P>0.05) (Fig. 9).

**PPAR-α and PPAR-γ Expression in the GE-Treated Rats**

The PPAR-α expression was insignificantly up-regulated in the liver (1.02 ± 0.14 fold), heart (1.17 ± 0.41 fold) and AM (1.16 ± 0.30 fold) (P>0.05). However, non-significant down-regulation was observed in the kidney (-1.31 ± 0.29 fold) and QF (-1.36 ± 0.34 fold) (P>0.05). The PPAR-α expression was not detectable in both the SAT and VAT of all rats (Fig. 10). The PPAR-γ expression was insignificantly up-regulated in the liver (1.12 ± 0.18 fold), heart (1.12 ± 0.53 fold), AM (1.45 ± 0.80 fold), QF (1.02 ± 0.47 fold), SAT (1.23 ± 0.58 fold) and VAT (1.29 ± 0.95 fold) (P>0.05). However, non-significant down-regulation was observed in the kidney (-1.21 ± 0.41 fold) (P>0.05) (Fig. 11).

**DISCUSSION**

The GE-treated rats had significantly lower blood glucose level than the control group. Previous studies conducted by Chia et al. [38] with 50mg/kg GA showed the same anti-hyperglycaemic effect on rats. GE, the pharmacologically active compound of GA, has been reported to be 200-1000
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Fig. (9). Fold difference of LPL expression in different tissues with BAC as the endogenous reference, tissues from GE-treated group as target and tissues from control group as calibrator. LPL was up-regulated in all tissues except QF (P>0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

Fig. (10). Fold difference of PPAR-α expression in different tissues with BAC as the reference, GE-treated group as target and control group as calibrator (P>0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

The improved serum lipid parameters in GE-treated rats can also be related to GE-mediated inhibition of 11β-HSD1. The reduced serum FFA may be related to the increased expression of plasma membrane fatty acid binding protein (FABP) that facilitates uptake of FFA into cells following inhibition of 11β-HSD1, thus lowering circulating FFA level [40]. The significant reduction in serum TAG may be related to reduced VLDL secretion following inhibition of 11β-HSD1. The rate of VLDL secretion depends on the amount of lipid available for VLDL assembly [41]. Adipose tissues which act as a reservoir for unlimited TAG storage have times more potent than GA [31] and it is postulated to improve glucose and lipid metabolism at a lower concentration than GA. The lowering of blood glucose level can be related to the antiglucocorticoid effect of GE. GC, as an antagonist of insulin, (i) inhibits insulin secretion from the pancreatic β-cells of Langerhans; (ii) inhibits peripheral glucose uptake by preventing translocation of glucose transporter to the plasma membrane and (iii) promotes gluconeogenesis in the liver, skeletal muscles and adipose tissues [39]. Inhibition of 11β-HSD1 decreases concentration of active GC that antagonizes insulin activities and inhibits the events that contribute to elevated blood glucose level [27]. It was also found that inhibition of 11β-HSD1 promotes hepatic glucose uptake by increasing translocation of glucose-transporter 2 (GLUT-2) to the membrane of hepatocytes hence resulting in the lowering of blood glucose level [39].
been the primary target of intense investigations in obesity and MetS [42]. Nevertheless, increased evidence has shown that other tissues, particularly the liver, are able to store significant amount of fat, mostly as TAG in obesity and T2DM [43]. Hence, FAO plays a crucial role in energy homeostasis where maintenance of a high FAO rate is expected to aid in the reduction of fat accumulation despite excess energy consumption [44]. The ability of the tissues to up-regulate the FAO enzyme system in response to the increased fat uptake becomes critical to prevent the development of MetS [42,45]. It was found that the rate of lipid catabolism increases following increased expression of enzymes involved in fatty acid catabolism (e.g. carnithine palmitoyltransferase-I) following 11β-HSD1 inhibition [41]. Since GC is known to promote lipogenesis and VLDL secretion, the increased channeling of lipid to the oxidative pathway reduce the amount of lipid available for VLDL production, hence leading to reduced TAG level [41]. The increased HDL level may be related to the increase in synthesis of apo-AI, the main component of HDL-cholesterol. Morton et al. [41] found a significant elevated apo-AI level in 11β-HSD1 knockout mice. Followed by reduced hepatic TAG production and increased FFA oxidation as induced by 11β-HSD1 inhibition, there is reduced generation of small, dense LDL from TAG-rich VLDL by hepatic lipase [40]. This contributes to the significant reduction in serum LDL-cholesterol.

Some gluconeogenic enzymes like H6PDH, PEPCK and G6Pase were shown to be affected by the GE. Most tissues showed slight elevation in H6PDH activities. However, the increase was found to be non-significant which could be related to the increased availability of G6P. GE, as an insulinotopic agent, may increase the substrates i.e. G6P for H6PDH via induction of glucose transport, thereby enhancing the H6PDH activities. Chandramouli et al. [6] demonstrated that GA administration at 100mg/kg in rats showed parallel reduction in 11β-HSD1 and H6PDH activities while Walker and Andrew [46] commented that the relationship between 11β-HSD1 activity and the physiological variations in H6PDH remains elusive.

PEPCK is the key regulatory enzyme involved in gluconeogenesis (in the liver and kidney) and glyceroneogenesis (in the adipose tissues) [27]. The liver and kidney of GE-treated rats displayed decreased PEPCK activities (only significant in the latter tissue) which could be related to the GE-mediated reduction in 11β-HSD1. This leads to decreased production of active GC that mediate PEPCK gene transcription, thereby decreasing PEPCK activities and glyceroneogenesis rate. The decreased PEPCK activities in the liver and kidney could also be related to the slight increase in insulin as insulin has been related to inhibition of PEPCK transcription via the activation of PI3K pathway [47]. In addition, PPAR-γ activation was also found to down-regulate PEPCK gene expression in the liver and kidney, thereby decreasing gluconeogenesis. GE, as a potential PPAR-γ agonist, would lead to down-regulation of PEPCK gene transcription and hence lower PEPCK activities in the GE-treated rats. Similarly, the transcription of G6Pase is subjected to control by GC [48]. Elevated circulating FFA is also known to exert allosteric simulation on G6Pase through the PPAR-mediated pathway, resulting in increased G6Pase expression and glycoeneogenesis [49]. The non-significant decrease in G6pase activities could hence be related to the lowering effect of GE in both the activating compounds of G6pase- FFA and GC.

In the present study, PPAR-γ expression was detected in both SAT and VAT, suggesting their importance in both adipogenesis and adipocyte differentiation. The PPAR-α expression in both SAT and VAT was too low to be detected, which corresponded to the low FAO activity in the adipose tissues [50]. This is because the adipose tissues act as the primary energy storage site instead of energy utilization. The non-significant down-regulation of PPAR-α expression in quadriceps femoris may be due to fiber-type-
selective transcriptional response of PPAR-α agonists. De Souza et al. [51] showed that administration of PPAR-α agonists, fenofibrate and Wy-14,643 to rats selectively induced PPAR-α activation in the type 1 (slow twitch) but not type 2 (fast twitch) skeletal muscle fibers. Abdominal muscle contains higher composition of type 1 muscle fibers as compared to quadriceps femoris (QF) which is mainly composed of type 2 muscle fibers [52]. Hence, as a potential PPAR-α agonist, GE may selectively induce PPAR-α transcriptional activity in the AM instead of QF.

CONCLUSION

The current study has shown that GE administration at dosage of 50mg/kg for 24 hours could lower blood glucose level significantly. GE also modulates serum lipid towards the beneficial side as shown by the improvement in each lipid parameter which includes TAG, FFA, total cholesterol, HDL-cholesterol and LDL-cholesterol following inhibition of 11β-HSD1. Furthermore, selective induction of PEPCk activities took place in parallel with the lowering of blood glucose level upon GE administration. This suggests that GE could be a potential compound in ameliorating hyperglycaemia and dyslipidaemia mediated through its antiglucocorticoid effect instead of via PPAR activation. However, even though GE has shown to be 200-1000 times more potent than GA [31,34], the potency of GE at 50mg/kg was not shown in the present study as it did not exert much significant effect on most of the parameters studied compared to the earlier work with GA [9,35,36].

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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Declared none.

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