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Effect of Quercetin, Genistein and Kaempferol on Glutathione and Glutathione-Redox Cycle Enzymes in 3T3-L1 Preadipocytes

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Abstract

Context and objective—Many studies have shown that cellular redox potential is largely determined by glutathione (GSH), which accounts for more than 90% of cellular non-protein thiols. The aim of this study was to delineate the effect of three flavonoids- namely quercetin, kaempferol and genistein and exogenous GSH on oxidative damage by the Fenton's pathway through the GSH and GSH-redox cycle enzymes in 3T3-L1 cells.

Materials and methods—3T3-L1 preadipocytes were exposed to each flavonoid and GSH at concentrations of 0, 5, 10, 15, 20 and 25 μM and then GSH levels and activities of glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx) and superoxide dismutase (SOD) were measured.

Results—Exogenous GSH did not have significant effect on intracellular GSH although slight decrease was observed at 15–25 μM doses. However, each of the three flavonoids sustained intracellular GSH levels in the cells as compared to the respective controls. Quercetin had the most profound effect, followed by kaempferol and genistein in that order. GSH-Px, GSH-Rx and SOD activities increased for all the doses tested compared to their respective controls. Again, quercetin had the maximum increase in enzyme activities followed by kaempferol and genistein for the enzymes tested.

Discussion and conclusion—These findings suggest that the flavonoids play an important role in diminishing oxidation-induced biochemical damages. The enhancement of these enzymes may increase the resistance of the organism against oxidative damage by the Fenton's pathway.

Keywords

Flavonoids; Glutathione peroxidase; Glutathione reductase; Superoxide dismutase; Oxidative damage; Fenton's pathway

INTRODUCTION

Glutathione (GSH) is a major antioxidant that maintain*s* the redox equilibrium of a cell (Schafer and Buettner, 2001). Oxidative stress results when this redox equilibrium is altered in favor of oxidized GSH (GSSG), which can either be due to a decrease in the reducing

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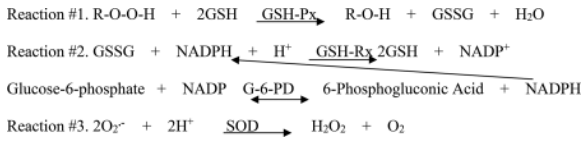
capacity of the cell or an increase in the reduction potential (Genestra, 2007). The former is a result of decreased levels of cellular antioxidants, predominantly GSH, while the latter comes from increased generation of reactive oxygen species (ROS). The sum of these changes is increased oxidative stress. Oxidative stress can damage most of the cellular macromolecules, leads to protein and DNA adducts formation and lipid peroxidation. Such markers of oxidative stress are increased in Parkinson's disease (PD) patients, with raised levels of both 8-hydroxyguanine and 4-hydroxynonenal, indicative of oxidative DNA damage and lipid peroxidation, detected in the substantia nigra (SN) (Alam et al., 1997; Yoritaka et al., 1996). The cell regenerates reduced GSH from GSSG using reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the enzyme glutathione reductase. It also has important roles in maintaining the intra- and extracellular redox environments and modulating intracellular transport of copper (Cu^{2+}) into metalloproteins immediately after uptake, preventing toxicity from unbound intracellular redox active Cu^{2+} (White and Cappai, 2003). When the ratio of GSH to GSSG drops, it serves as one important indicator of oxidative stress in cells (Lakritz et al., 1997). For example, GSH depletion can activate neuronal 12-lipoxygenase (12-Lox), which, in turn, generates increased intracellular peroxide levels through catabolism of arachidonic acid (Li et al., 1997). The subsequent oxidative damage from H_2O_2 and hydroxyl radicals ($^{\cdot}OH$) may play an important role in neuronal dysfunction and/or death in neurodegenerative diseases. Thus, GSH plays a vital role in combating oxidative stress in cells.

Several enzyme systems have also been identified as protective against oxygen radicals and their resulting damage (Boadi et al., 1991). Of primary importance is the enzyme defense mechanism through the GSH redox cycle, in which GSH acts as a substrate for glutathione peroxidase (GSH-Px), thereby converting hydrogen peroxide to water and organic peroxides (R-O-O-H) to the corresponding stable alcohols (Boadi et al., 1991) (see reaction #1 below). The GSSG, is replenished by glutathione reductase (GSH-Rx) (see reaction #2 below) by reducing GSSG at the expense of NADPH, generated by NADPH generating enzymes such as Glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6PGD) (Das et al., 1993). Similarly, superoxide dismutase (SOD), an enzyme found in all oxygen-metabolizing organisms catalyzes the dismutation of the superoxide radical ($O_2^{\cdot -}$) to less toxic products (Boadi et al., 1991; Das et al., 1993) (see reaction #3 below).



The average daily (Western) diet contains about 1 g of mixed flavonoids, an amount that might be sufficient to achieve pharmacologically significant concentrations in tissues. The ability of flavonoids to act as antioxidants depends on their free radical-scavenging and metal-chelating capabilities (Di Carlo et al., 1999; Yano et al., 1993). Di Carlo et al. (1999) have suggested that vitamins A, C and E have been traditionally regarded as antioxidants in fruits and vegetables. However, plant flavonoids have been shown to offer more protective health benefits against oxidative deoxyribonucleic acid (DNA) damages caused by γ-ray

radiation, UV irradiation, chemicals, and endogenous oxidative stress. Secondly, these authors indicated that flavonoids are capable of modulating the activity of enzymes, and affect the behavior of many cell systems. These compounds may possess significant anti-hepatoxic, anti-allergic, anti-inflammatory, anti-osteoporotic, and even antitumor activity (Di Carlo et al., 1999; Aherne and O'Brien, 2000). While this may account for the anti-mutagenic activities of flavonoids in experimental systems, relatively little is known about the mechanisms of protection of these compounds in preventing oxidative damage in obese subjects. We have demonstrated and reported elsewhere that, single treatments of quercetin, genistein, or the two in combination can inhibit lipid peroxidation induced by Fe^{2+} and Cu^{2+} in U937 cells (Boadi et al., 2003). We speculated that the flavonoids probably enhance the cell's capabilities to better cope with oxidation through the GSH and its antioxidant enzymes to bring about the reduced lipid peroxidation. Thus, the purpose of the current study is to use the flavonoids quercetin, genistein and kaempferol to prevent oxidative damage in 3T3-L1 cells through the GSH/GSH-Redox system. The 3T3-L1 cell model was chosen because many reports suggest that this cell line is one of the most well-characterized and reliable *in vitro* models for studying the differentiation of preadipocytes to adipocytes (Takahashi et al., 2004). Our study will test two hypotheses: (1) that the above-mentioned flavonoids can maintain GSH in 3T3-L1 cells under oxidative stress; and (2) that the flavonoids might be helpful in obesity to better cope with oxidative damage by enhancing the levels of the cell's GSH, and it's antioxidative properties through the GSH-PX, GSH-Rx, and SOD pathways.

METHODS

Chemicals

Quercetin dihydrate (3,3',4',5,7-pentahydroxy flavone), isoflavone kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one), genistein (4',5,7-trihydroxy isoflavone), and ferrous chloride tetrahydrate ($FeCl_2.4H_20$), were purchased from Sigma-Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO) and hydrogen peroxide (H_2O_2) were purchased from Fisher Scientific, Suwanee, GA. 3T3-L1 preadipocyte fibroblast cells were procured from the American Type Culture Collection (ATCC), Manassas, VA. All chemicals were of high purity according to manufacturer's instruction and used without further purification.

Culturing of 3T3-L1 mouse fibroblast Cells

Mouse 3T3-L1 fibroblast cells from ATCC were maintained at 37°C under 5% CO_2 atmosphere in Dulbecco's Modified Eagle's Medium (DMEM) containing 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, 1500 mg/L sodium bicarbonate, 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin (Takahashi, 2009). At 80% confluence in T-75 flasks, cells were trypsinized with 3 ml of trypsin-EDTA solution and later sub-cultured in 6-well plates before treatment with the flavonoids.

Antioxidant activity testing of flavonoids and exogenous GSH in 3T3-L1 Cells

The Fe, H_2O_2 and flavonoid solutions for the experiment were prepared as described previously (Boadi et al., 2003). Antioxidative activity as described by Stinson et al. (1992) was used with the following modifications. 1×10^6 cells were seeded in a total volume of 1

ml of DMEM medium per well in 6 well plates. They were incubated with Fe (50 μM) ions (Kennedy et al., 1997) and H_2O_2 (0.01 mM) with and without flavonoid sample and exogenous GSH at 5, 10, 15, 20 and 25 μM for each of the three flavonoids tested in this study for 24 h at 5% CO_2. Following the incubation, cells in each well were trypsinized with 1 ml trypsin-EDTA solution, neutralized with 2 ml of DMEM medium and centrifuged at 3000 × *g* for 10 min at 4°C in a refrigerated table top Eppendorf centrifuge (Fisher Scientific, Suwanee GA). Cells were treated in separate experiments to determine whether DMSO, H_2O_2 and Fe^{2+} ions were affecting cell growth and viability that might compromise GSH levels and enzyme activities. Cells (1×10^6) per well were incubated with 0.5% DMSO, 0.01 mM H_2O_2, and 50 μM Fe^{2+} ions, as described above. Furthermore, viability (measured by the trypan blue exclusion test) and cell number, determined by cell counting using a Neubauer improved hemocytometer, were determined before and after the addition of the reagents under investigation.

Analysis of Reduced Glutathione (GSH) in 3T3-L1 Cells

Reduced GSH was analyzed as described (Boadi et al., 2005) with some modifications as prescribed by the Calbiochem GSH Assay Kit (No. 354102). Following incubation, cells (3.5×10^6 cells) were pelleted by low speed centrifugation as described above was re-suspended in 300 μl of 5% metaphosphoric acid (MPA). Cells were sonicated under ice for 30 seconds using a cell dismembrator (Fisher Scientific, Suwanee GA) at a setting of 3. The homogenate was then centrifuged at 3,000 × *g* for 10 minutes at 4°C. The resulting supernatant was removed and stored at −70°C until used for the analysis of GSH. To an aliquot (10–20 μl) of the supernatant, the volume was adjusted to 220 μl with a buffer solution (200 mM potassium phosphate, pH 7.8 (25°C), containing 0.2 mM diethylene triamine pentaacetic acid (DTPA) and 0.025% lubrol). 11 μl of 12 mM solution of chromogenic reagent in 0.2 N HCl was added and mixed followed by the addition of 11 μl of 30% NaOH solution. Samples were incubated at 25°C for 10 minutes in dark after which the absorbance was measured at 400 nm using a Synergy microplate reader (Winooski, VT). GSH levels in cells were calculated from the standard curve using GSH prepared in 5% MPA (freshly prepared) as standard and expressed in μM.

Analysis of Glutathione Peroxidase (GSH-Px) in 3T3-L1 Cells

GSH-Px activity was analyzed as previously described (Boadi et al., 1991) with some modifications by the Calbiochem GSH-Px Assay Kit (No. B3878). The cell pellet was sonicated using Sonic Dismembrator Model 100 in cold buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 1 mM DTT) and centrifuged at 10,000 × *g* for 20 minutes at 4°C and the supernatant was used for the assay. Activity was measured in Synergy microplate reader that has been set to the assay temperature of 25°C. A typical assay for the sample in the 96-well plate contained 66 μl assay buffer (50 mM Tris-HCl, pH 7.6, 5 mM EDTA), 66 μl of NADPH reagent (0.2 mM NADPH), 22 μl of the sample supernatant. 66 μl of diluted 0.22 mM *tert*-butyl hydroxyperoxide was finally added to the wells. The samples were mixed by pipetting up and down twice with care taken to avoid the creation of bubbles. The absorbance at 340 nm was recorded manually at every 30 seconds in the plate reader for 60 minutes. A blank sample (to assess nonspecific oxidation of NADPH) containing 66 μl of the assay buffer, 67 μl of the NADPH reagent, 22 μl of distilled water and 66 μl of the 0.22

mM *tert*-butyl hydroxyperoxide was analyzed under the same conditions. Blank reading was subtracted from the controls and treated samples and enzyme activity was expressed as milliunits (mU) of NADPH oxidized/min/mg protein.

Analysis of Glutathione Reductase (GSH-Rx) in 3T3-L1 Cells

GSH-Rx activity was analyzed as previously described (Boadi et al., 1991) with some modifications by the Calbiochem GSH-Rx Assay Kit (No. B40674). The cell pellet was homogenized in cold buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA) and centrifuged at 8,000 × *g* for 10 minutes at 4°C and the supernatant was used for the assay. Activity was measured in Synergy microplate reader that had been set to the assay temperature of 25°C. A typical assay for the sample in the 96-well plate contained 44 μl of diluted sample, 88 μl of 1 mM GSSG, pH 7.5. 88 μl of NADPH reagent (0.22 mM NADPH) was added and the sample mixed by pipetting up and down three times (avoiding the making of bubbles). The absorbance at 340 nm was recorded manually every 60 seconds for 60 minutes. A blank sample containing 44 μl of diluted sample and 88 μl of the assay buffer without GSSG and 88 μl of the NADPH reagent was analyzed under the same conditions. GSH-Rx was measured without pre-incubation with flavin adenine dinucleotide, since the enzyme was previously determined not be activated (Boadi et al., 1991). Blank reading subtracted from the controls and treated samples and enzyme activity was calculated by subtracting the rate observed for a blank (assay buffer instead of GSSG) from the sample rate and expressed as milliunits (mU) of NADPH oxidized/min/mg protein.

Analysis of Superoxide Dismutase (SOD) in 3T3-L1 Cells

SOD activity was analyzed as previously described (Boadi et al., 1991) with some modifications by the Calbiochem SOD Assay Kit (No. 574600). The cell pellet was homogenized in cold buffer (50 mM 2-amino-2-methyl-1,3-propanediol containing 3.3 mM boric acid and 0.11 mM diethylenetriaminepentaacetic acid (DTPA), pH 8.8 at 37°C) and centrifuged at 8,000 × *g* for 10 minutes at 4°C and the supernatant was used for the assay. Activity was measured in Synergy microplate reader that has been set to the assay temperature of 25°C. The plate reader was zeroed at 525±2 with deionized water. Each well in a total reaction volume of 220 μl contained the following reagents: 198 μl of buffer or sample, 8.8 μl of blank or sample, 6.6 μl of R2 (which is a mercaptan scavenger – 33.3 mM 1, 4, 6-trimethyl-2-vinylpyridinium trifluoromethanesulfonate in 1 mM HCl). Following incubation at 37°C for 1 min., 6.6 μl of reagent R1 (0.66 mM 5,6,6a, 11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorene, in 32 mM HCl containing 0.5 mM DPTA and 2.5 % ethanol was added and mixed briefly. The absorbance was measured over time for 30 sec. Care was taken to ensure that not more than 10 sec. elapsed between the addition of R1 and commencing of monitoring the absorbance. SOD activity was calculated and expressed as units (U)/mg protein.

Effect of reagents on Cell Growth and Viability in 3T3-L1 Cells

To check if the reagents used (i.e., H_2O_2 (0.01 mM), Fe^{2+} (50 μM) and DMSO (0.5%)) were affecting cell growth and viability that might compromise GSH and enzymatic activity, separate experiments were conducted in which the reagents in question were incubated with

the respective reagents as described above. Following the experiment, cell viability and number were determined as previously described (Boadi et al., 2012).

Soluble Protein Content of 3T3-L1 cells

The soluble protein content of the cell lysate was determined by the Coomassie blue protein assay method (Pierce Cat. No. 23200) using bovine serum albumin (BSA) as standard. Absorbance of the samples together with the standards were read in a microplate reader as described above.

Statistical Analysis

Results are expressed as means ± SD. Statistical significance was determined by two-way Analysis of Variance (2-Way ANOVA) followed by Duncan's test for multiple comparisons using SPSS 20. $P<0.05$ was considered statistically significant. Each value in all figures represents the mean for each dose level of flavonoid and GSH tested, which was assayed in triplicates.

RESULTS

Cell Growth and Viability

Cell number was determined before and after treatments with the reagents. Results indicate that none of the reagents affected cell growth and viability before the analyses of the GSH and enzymatic activities (data not presented).

Effects of Quercetin, Kaempferol and Genistein on GSH Levels in 3T3-L1 Cells

Figure 1 shows the effects of the single treatments of quercetin, kaempferol and genistein at 0, 5, 10, 15, 20 and 25 μM on GSH levels in 3T3-L1 cells following oxidation by the Fenton's pathway and incubation for 24 h. GSH levels decreased ($p<0.05$) for quercetin at the 5, 10 and 25 μM doses and remained the same for the 15 and 20 μM as compared to its control. Levels of GSH were sustained for the kaempferol and genistein treated samples in comparison to their respective controls. Despite the significant decreases ($p<0.05$) in GSH for quercetin at the said doses each of the flavonoids were capable of sustaining GSH levels in cells. Genistein appears to have the greatest effect as compared to its control.

Effects of Quercetin, Kaempferol and Genistein on GSH-Px Activity in 3T3-L1 Cells

Figure 2 shows GSH-Px activity in cells following oxidation and the treatment of cells with the individual flavonoids. Enzyme activity remained the same from 0–10 μM and increased significantly ($p<0.01$) from 15–25 μM doses of different flavonoids as compared to their respective controls ($p<0.001$). Quercetin caused the greatest increase in enzyme activity followed by kaempferol and genistein.

Effects of Quercetin, Kaempferol and Genistein on GSH-Rx Activity in 3T3-L1 Cells

GSH-Rx activity in 3T3-L1 cells following treatment with the flavonoids and oxidation is shown in Figure 3. GSH-Rx activity increased significantly ($p<0.05$) from 15–25 μM for all

the tested flavonoids in comparison to their respective controls. Again, quercetin showed the greatest effect in enzyme activity followed by genistein and kaempferol.

Effects of Quercetin, Kaempferol and Genistein on SOD Activity in 3T3-L1 Cells

SOD activity (Figure 4) increased significantly ($p<0.05$) for all the flavonoid-treated cells compared to their respective controls. Enzyme activity doubled for the 25 μM dose compared to the controls. Quercetin had a significant effect ($p<0.05$) on enzyme activity followed by kaempferol and genistein. Of all the enzymes tested, SOD activity was highly up regulated by the single and different doses of the tested flavonoids.

Effects of Exogenous GSH on GSH levels in 3T3-L1 Cells

Figure 5 shows GSH levels following incubation of 3T3-L1 cells for 24 h after treatment with exogenous GSH and oxidation by the Fenton's pathway. There was no effect on GSH levels at the 5 and 10 μM doses compared to the control. However, levels of GSH slightly decreased ($p<0.05$), for the 15 and 25 μM dose levels. Addition of exogenous GSH did not boost the intracellular levels of GSH in the cells.

DISCUSSION

The tripeptide GSH is the major nonenzymatic regulator of intracellular redox homeostasis, ubiquitously present in all cell types (Meister and Anderson, 1983). This cysteine-containing tripeptide exists either in reduced GSH or oxidized GSSG form—better referred to as glutathione disulfide—and participates in redox reactions by the reversible oxidation of its active thiol (Kalyanaraman et al., 1996; Briviba et al., 1999). GSH can directly scavenge free radicals or act as a substrate for GSH-Px and glutathione transferase (GST) during the detoxification of H_2O_2, lipid hydroperoxides and electrophilic compounds (Masella et al., 2005). To help understand or enhance the antioxidant potential of obese subjects we have embarked on the single treatment of 3T3-L1 cells to quercetin, kaempferol and genistein at different doses with study their effects on the GSH/GSH-redox system. The hypotheses tested in the current studies are (a) that the above-mentioned flavonoids can maintain GSH in 3T3-L1 cells under oxidative stress; and (b) that the flavonoids might be helpful in obesity to better cope with oxidative damage by enhancing the levels of the cell's GSH, and it's antioxidative properties through the GSH-PX, GSH-Rx, and SOD pathways. The results indicate that the reagents used (i.e., H_2O_2 (0.01 mM), Fe^{2+} (50 μM) and DMSO (0.5%)) did not affect cell growth and viability that might compromise GSH and enzymatic activity (Boadi et al., 2012). However, each of the flavonoids is capable of maintaining GSH levels following the oxidative process. Such an observation may indicate the ability of each of the flavonoids to prevent the significant loss of intracellular GSH levels in 3T3-L1 cells. Thus, the mechanisms of GSH sustenance by the various flavonoids are probably a combination of GSH-synthesis stimulation (Chung and Maines, 1981; Taniguchi and Hara, 1983; Costagliola et al., 1986) and a GSH-sparing effect of quercetin (Das and Ratty, 1986), kaempferol and genistein being alternative antioxidants. If indeed ROS formation is the starting point to this oxidative damage leading to the derangement of GSH levels, then the conditions prevailing in this study created severe enough stress, but did not overwhelm the cells treated with the flavonoids. It is important to mention that the GSH/GSSG system is

indeed called upon during oxidative damage by the Fenton pathway as shown by the gradual decrease but not significant differences for the three tested flavonoids. Determining whether the presence of the flavonoids might have resulted in the *de novo* synthesis of GSH is currently ongoing and yet to be studied and characterized. If the intracellular GSH system is called upon during oxidative stress, one could argue that the addition of exogenous GSH can help supplement and synergistically boost the cells ability to overcome the oxidative stress. To address this concern, we have, in comparative studies, tested the exposure of only the same doses of exogenous GSH as used in the flavonoid studies to 3T3-L1 cells to ascertain if GSH could act as an agonist or boost the cells capabilities of overcoming the oxidative damage. The results (Figure 5) indicate that GSH levels remained the same for doses 0–10 μM and decreased slightly for the 15 and 25 μM dose levels. Such a decrease may be due to the fact that there is a maximum intracellular level of GSH that the cell can tolerate and, therefore, the addition of any exogenous amount of GSH does not benefit the cells to combat the oxidative damage. Irrespective of the above findings, it has been postulated that GSH is synthesized in two sequential ATP-dependent reactions catalyzed by γ-glutamylcysteine synthetase (γ-GCS)–the rate-limiting enzyme-and GSH synthetase. Furthermore, other factors in the regulation of the *de novo* GSH synthesis have been attributed to the availability of cysteine and the concentration of GSH itself that inhibits, γ-GCS activity by a feedback mechanism (Lu, 2000). It can be speculated that the presence of the flavonoids might have contributed to GSH synthesis by other alternative pathways. Thus, it is reasonable to hypothesize that the gradual maintenance of GSH by the flavonoids under oxidative stress may, at least in part, act through the antioxidant responsive elements (AREs) present in the promoter regions of many of the genes inducible by oxidative and chemical stress (Masella et al., 2005). In fact, recent studies strongly suggest that dietary polyphenols can stimulate antioxidant transcription and detoxification defense systems through ARE (Masella et al., 2005). Thus, using the Fenton reaction, a well-characterized model of oxidative injury, the three flavonoids have shown some protection to the 3T3-L1 cells by directly affecting GSH metabolism or acting as antioxidants, and/or maintaining increased GSH related enzyme activities.

In fact, exposure of cells to quercetin, kaempferol and genistein caused elevation in enzyme activities for GSH-Px, GSH-Rx and SOD in 3T3-L1 cells as compare to their respective controls following the oxidative damage (see Figures 2, 3 & 4). Though the exact mechanisms for the elevation in enzyme activities by these flavonoids are not known, similar observations have been observed by other investigators following exposure to other nutrients such as selenium, riboflavin and vitamin E (Chung and Maines, 1981; Combs 1981; Taniguchi and Hara, 1983; Boadi et al., 1991). Our findings suggest that these flavonoids could help in protecting elective obese patients from repeated oxidative stress as demonstrated in the 3T3-L1 preadipocytes. Cells assays for enzyme activities in this study were performed after 24 h incubation with flavonoids in order to test for the regeneration of GSH (Boadi et al., 1991) and enzyme induction, since it has been reported that the induction period of these enzymes may be between 24–48 h following exposure to oxidative stress (Deneke and Fanburg, 1982). The increase in enzyme activity observed in this study were significantly higher than their respective controls. This may suggest an increased antioxidant capacity of 3T3-L1 cells exposed to these flavonoids and may be comparable and translated

to the observation in animals fed the similarly supplemented flavonoid diets (Andres-Lacueva et al., 2005; Catoni et al., 2008; Rendeiro et al., 2009). We have, observed increased GSH-Rx activity in cells and this observation may find support in earlier papers reporting increased capacity of cells and animals to regenerated GSH following exposure to the oxidative damage (Kimbal et al., 1976; Meister and Tate 1976; Meister and Anderson, 1983; Boadi et al., 1991). The exponential increase in activities for GSH-Px, GSH-Rx and SOD in the presence of the flavonoids may indicate a common mechanism by the flavonoids in up-regulating enzyme activities. The above study suggests that an increase in all the components of the GSH-related antioxidant enzyme system, rather than an increase in any one of them, is important in preventing Fenton-mediated oxidative injury in 3T3-L1 cells. Although the specific contribution of each of the supplemented flavonoids in elevating the activity of the enzymes tested is not certain, it seems likely that exposing preadipocytes 3T3-L1 to these flavonoids may afford increased resistance to oxidative damage. Despite the beneficial effects observed for the 3T3-L1 preadipocytes following exposure to the flavonoids in this study, it is also important to note that recent evidences suggest that some polyphenols and some derivatives of quercetin and rutin can act as prooxidants (Kessler et al 2003; Hatia et al. 2014). However, exposure to qurcetin, kaempferol and genistein at the tested doses sustained GSH levels in the 3T3-L1 preadipocytes. This sustenance together with whatever antioxidant enzyme enhancement achieved in ways related or unrelated to actual GSH content could prevent oxidative damage in obese subjects.

CONCLUSIONS

Exposure of 3T3-L1 preadipocytes to oxidation in the presence of several doses of quercetin, kaempferol and genistein did not cause significant decreases in intracellular levels of GSH compared to their respective controls. The addition of exogenous GSH did not act as an agonist to increase intracellular GSH levels in cells for the doses tested. Activities of GSH-Px, GSSG-Rx and SOD increased for all the doses of the flavonoids tested, suggesting the cell's ability to tolerate oxidative damage and the efficiency of such compounds in boosting 3T3-L1 cell's defense mechanisms through GSH and its redox enzymes.

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Declaration of Interest

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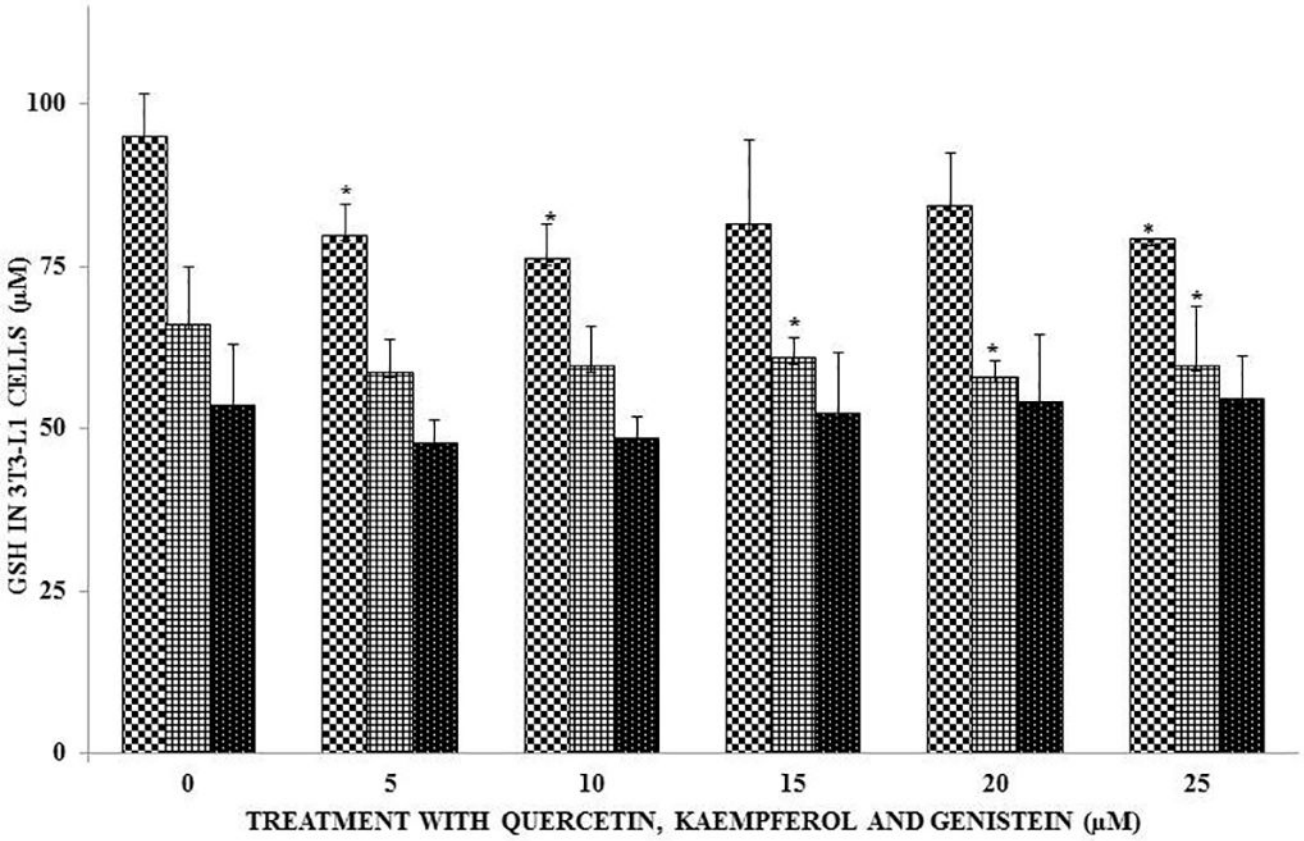

Figure 1.

The effects of several doses each of quercetin, kaempferol and genistein (0, 5, 10, 15, 20 and 25 μM) on GSH levels in Fe^{2+} induced oxidative damage in 3T3-L1 preadipocytes following incubation at 37°C and 5% CO_2 for 24 h. Each bar chart ± standard deviation in this and other figures in this paper represent mean for 4 different experiments for each dose level of flavonoid tested and, which was assayed in triplicates. Statistical significances denoted by asterisk in this Figure is shown as: comparison between the respective control for each flavonoid (i.e., without flavonoid) and flavonoid-3T3-L1 cells treated subgroups. * denotes $P<0.05$. Vertical bars in this and other figures denote standard deviation. The X-axis labels for Figures 1–4 are defined as follows: 0 – means control cells samples not treated with flavonoid; 5, 10, 15, 20 and 25 μM – means cell samples were each treated with the respective dose of quercetin, kaempferol and genistein for 24 h.

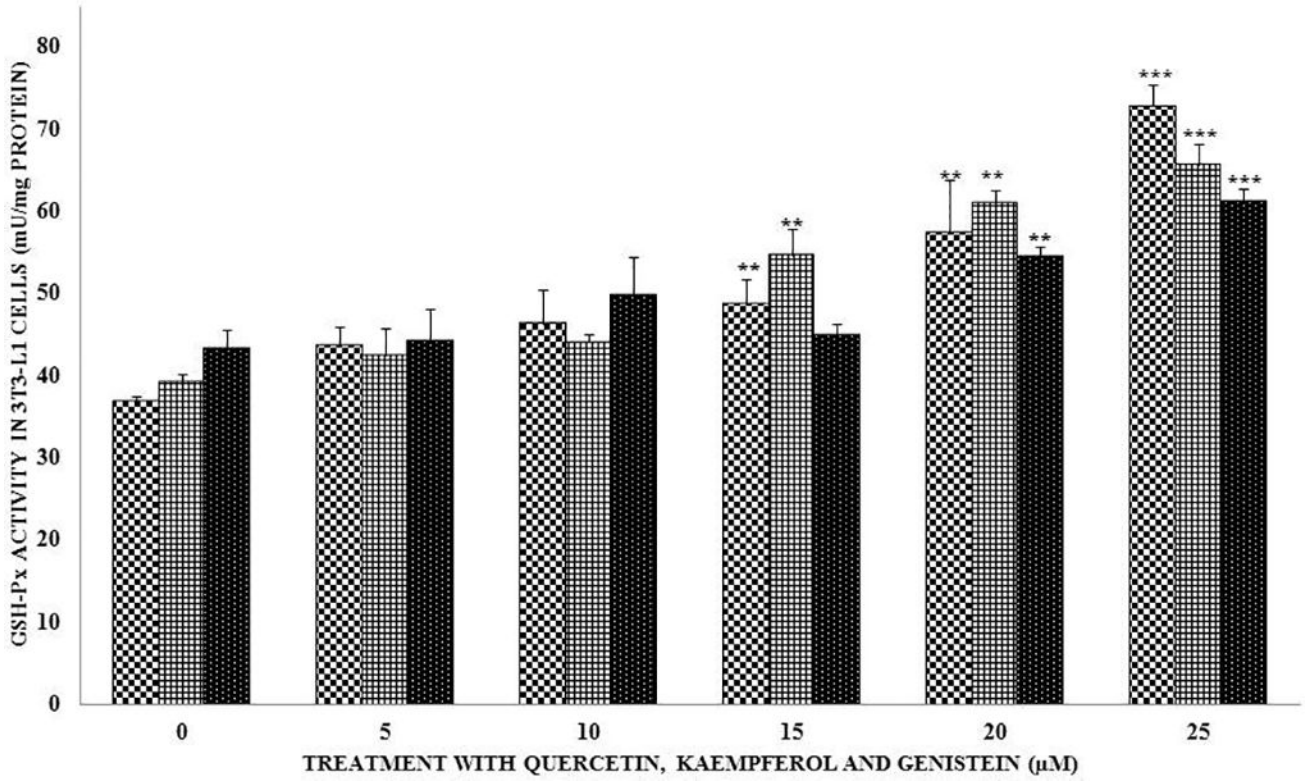

Figure 2.

The effects of several doses each of quercetin, kaempferol and genistein (0, 5, 10, 15, 20 and 25 µM) on GSH-Px activity in Fe^{2+} induced oxidative damage in 3T3-L1 preadipocytes following incubation at 37°C and 5% CO_2 for 24 h. Statistical significances denoted by asterisks in this Figure and Figures 3 and 4 are shown as: comparison between the respective control for each flavonoid (i.e., without flavonoid) and flavonoid-3T3-L1 cells treated subgroups. * denotes $P<0.05$; ** denotes $P<0.01$; *** denotes $P<0.001$ in this and other figures.

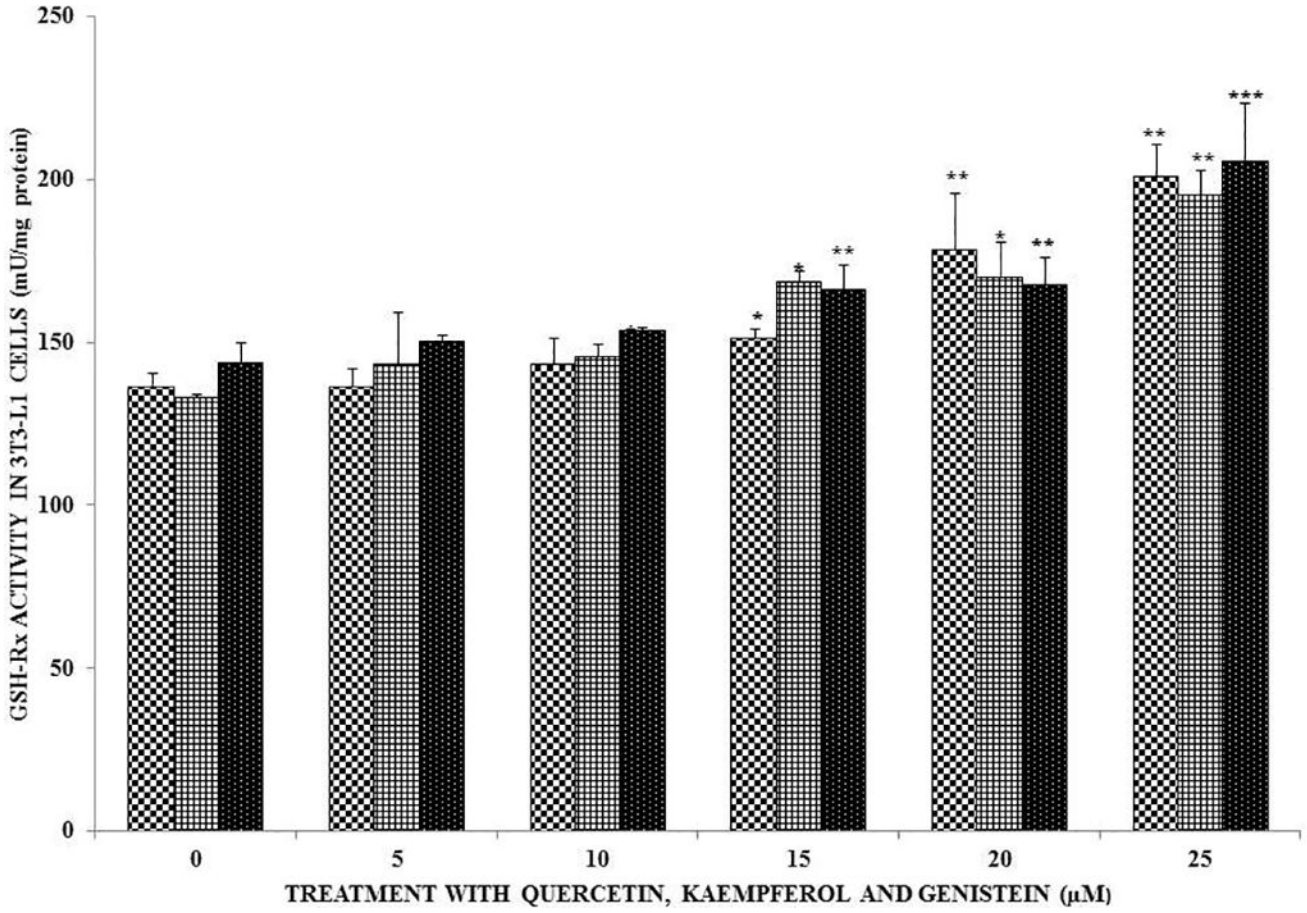

Figure 3.

The effects of several doses each of quercetin, kaempferol and genistein (0, 5, 10, 15, 20 and 25 μM) on GSH-Rx activity in Fe^{2+} induced oxidative damage in 3T3-L1 preadipocytes following incubation at 37°C and 5% CO_2 for 24 h.

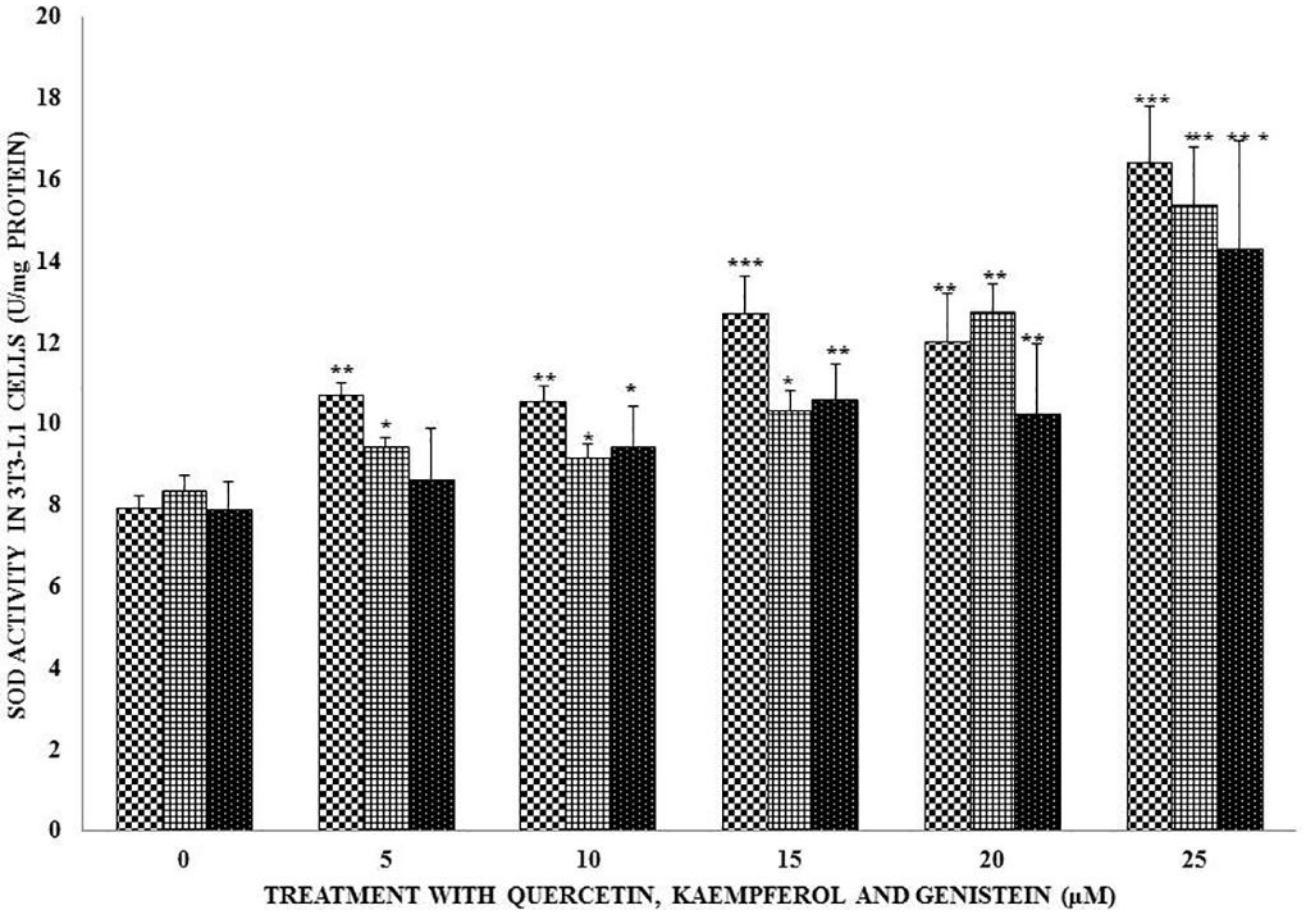

Figure 4.

The effects of several doses each of quercetin, kaempferol and genistein (0, 5, 10, 15, 20 and 25 μM) on SOD activity in Fe^{2+} induced oxidative damage in 3T3-L1 preadipocytes following incubation at 37°C and 5% CO_2 for 24 h..

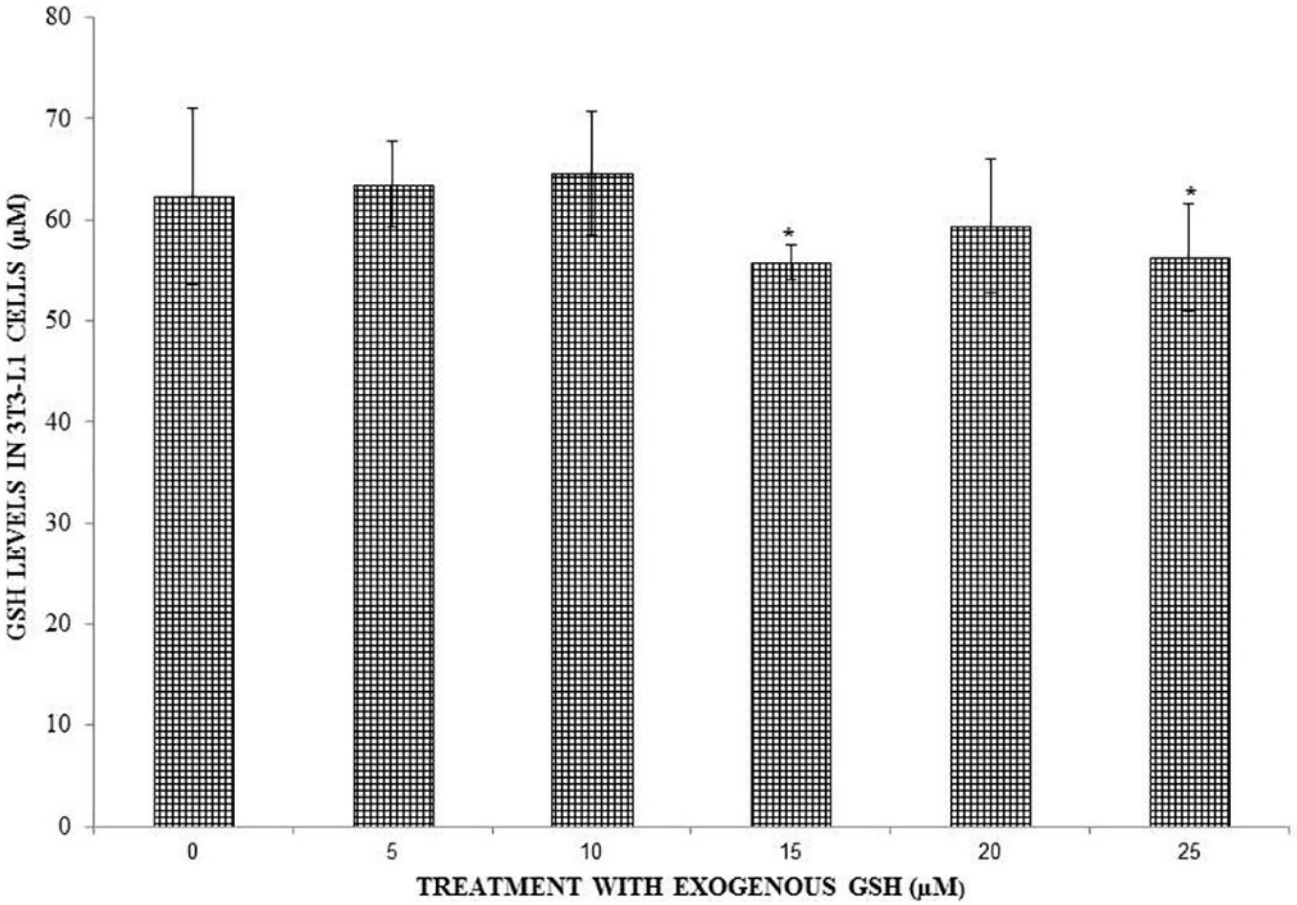

Figure 5.

The effects of several doses of exogenous ⊞GSH (0, 5, 10, 15, 20 and 25 μM) on intracellular GSH levels in Fe^{2+} induced oxidative damage in 3T3-L1 preadipocytes following incubation at 37°C and 5% CO_2 for 24 h. Each bar chart ± standard error in this and other figures in this paper represent mean for 4 different experiments for each dose level of exogenous GSH tested and, which was assayed in triplicates. Statistical significances denoted by asterisk in this Figure are shown as: comparison between the control (i.e., without exogenous GSH) and GSH-3T3-L1 cells treated groups. * denotes $P<0.05$. Vertical bars in this and other figures denote standard deviation. The X-axis labels for Figures 5 are defined as follows: 0 – means control cell samples not treated with exogenous GSH; 5, 10, 15, 20 and 25 μM – means cell samples were each treated with the respective dose of exogenous GSH for 24 h.