Antioxidant profiling of C3 quercetin glycosides: Quercitrin, Quercetin 3-β-D-glucoside and Quercetin 3-O-(6”-O-malonyl)-β-D-glucoside in cell free environment

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ABSTRACT

Introduction: Flavonoid Quercetin is a major constituent of fruits and vegetables which naturally exists in the form of its glycosides, foremost as C3 glycosides. Three Quercetin glycosides namely quercitrin, quercetin 3-β-D-glucoside and quercetin 3-O-(6”-O-malonyl)-β-D-glucoside, differing at their C3 glycosylation were explored for their antioxidant properties and were compared to the parent quercetin. **Methods:** Free radical scavenging ability of quercetin glycosides was assessed by UV-VIS spectrophotometry, pulse radiolysis and cyclic voltammetry. Their protective effects in Fenton radical induced DNA strand breaks and mitigating oxidative stress in subcellular organelles such as mitochondria were also examined. **Results:** Unlike earlier reports we found that C3 glycosides of quercetin exhibited better free radical scavenging in cell free environment. These glycosides effectively protected pBR322 DNA against Fenton radical induced-DNA strand breaks and this could be attributed to their interaction with 2 deoxy guanosine base transient. However, amongst these glycosides, Quercetin 3-O-(6”-O-malonyl)-β-D-glucoside which exhibited excellent antioxidant ability in cell-free environment, could not effectively protect mitochondria from lipid peroxidation but conferred protection against protein sulphydryl depletion at lower concentrations. **Conclusion:** All quercetin glycosides exhibited excellent antioxidant activities in cell free environment but did not exert comparable protective effects in biological systems. Varied responses of these derivatives can be attributed to their C3 glycosylation and the derivatizations at C3 sugars.

Key words: C3 quercetin glycosides, DNA protection, Free radical scavenging, Mitochondrial lipid peroxidation and protein sulphydryl depletion, Pulse radiolysis.

INTRODUCTION

Flavonoids, a large group of plant polyphenols have been studied extensively for their chemical, biological and pharmacological properties. Most of their beneficial effects are attributed to their antioxidant and chelating abilities.[1] Flavonoid quercetin is one of the vital constituent of fruits and vegetables. Its biochemical activity is well documented and is reported to be the most potent antioxidants amongst polyphenols.[2] Biological and pharmacological activities attributed to quercetin include anti-inflammatory, antiviral, antibacterial and anticarcinogenic activities.[3] In addition it possesses hypotensive, and anti-glycation effects.[4] The most extensively studied chemical property which augments the health promoting effects of dietary flavonoids is it’s potential to act as antioxidant molecule[2] which is highly dependent on the C-3’ or 4’ OH on the ‘B’ ring while the free C3-OH on ‘C’ ring is found to enhance the reducing ability by almost ten folds.[1] Frequent glycosylation sites in the naturally available quercetin involve C3 and C7-OH either individually or at both the positions. If this glycosylation is at C-3’ or 4’ OH sites on the ‘B’ ring, the reducing ability of antioxidant molecule is highly compromised.[2] The C3 and C7-OH glycosylated sugars most commonly include

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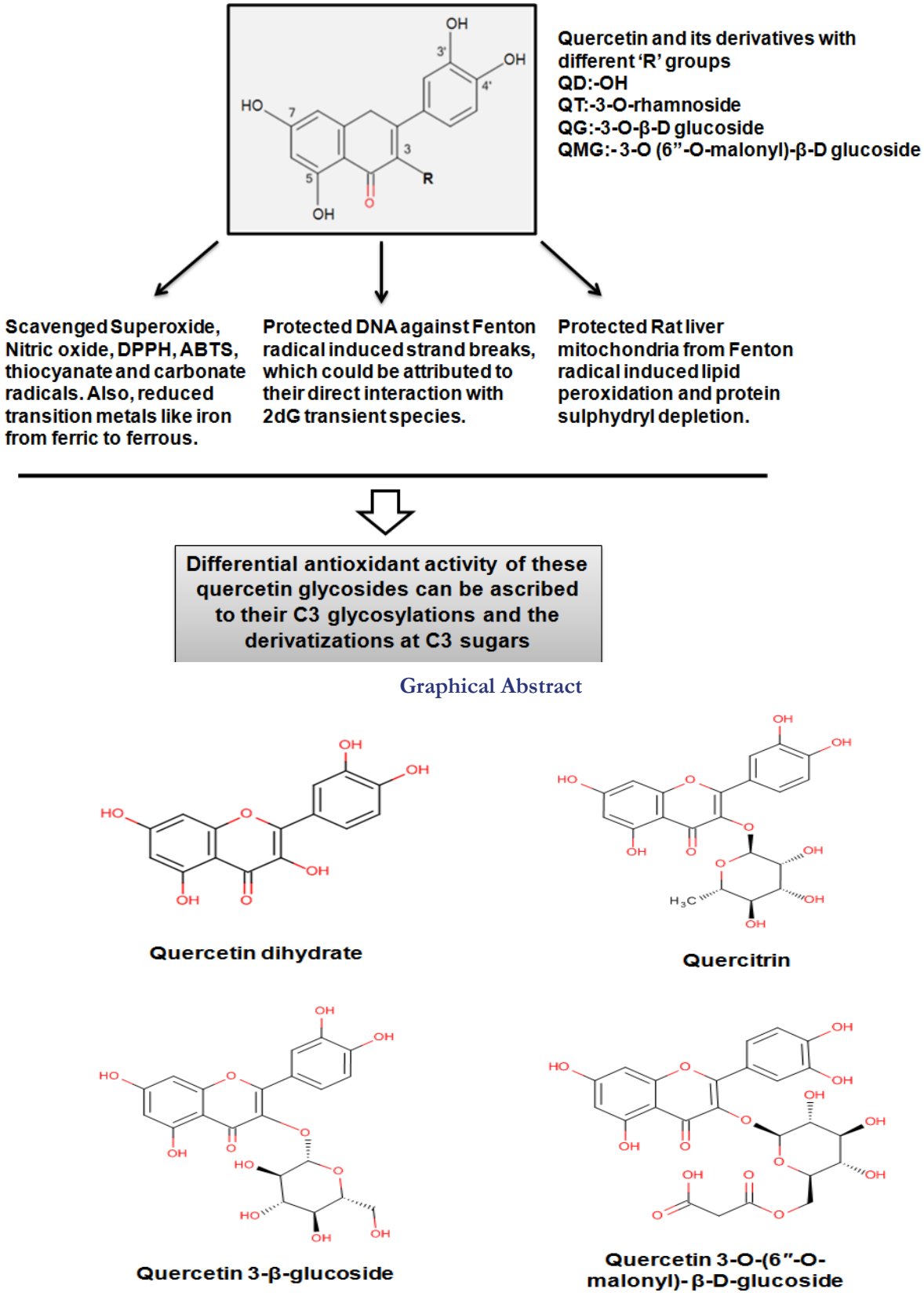
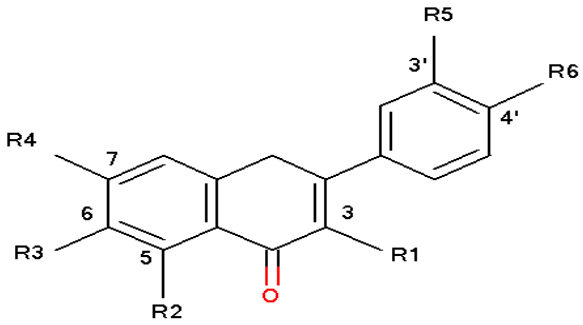


Graphical Abstract

Figure 1: C3 glycosides of quercetin

glucose, galactose, xylose or rhamnose. Amongst the glycosides, the glucosides of quercetin are more efficiently absorbed than the quercetin and therefore are more bioavailable.[5] It was also observed that 3-O-glucosylation of quercetin improves the net absorption of aglycone.[6] To investigate the health promoting effects of the antioxidants,

Table 1: Structural specifications of quercetin glycosides



No.	Molecule	R1	R2	R3	R4	R5	R6
1	Quercetin	OH	OH	H	OH	OH	OH
2	Quercitrin	O-Rha	OH	H	OH	OH	H
3	Quercetin 3-O-β-D glucoside	O-Glc	OH	H	OH	OH	H
4	Quercitin 3-O-(6"-O-malonyl)-β-D-glucoside	-O-Malo-Glc	OH	H	OH	OH	H

Rha- Rhamnose, Glc- Glucose, Malo-Glc- Malonyl glucoside

their net absorption as well as structural pattern are highly important elements. We have assessed different quercetin derivatives with differential glycosylation on C3 of C ring namely, quercitrin, quercitin 3-β-D-glucoside and quercitin 3-O-(6"-O-malonyl)-β-D-glucoside [Figure 1 and Table 1] for their antioxidant behavior and compared them with a parent quercetin molecule using various biochemical assays and pulse radiolysis. Their ability to protect DNA and subcellular organelles such as mitochondria against Fenton radical induced oxidative stress was also investigated. We found that amongst the three glycosides, quercitin 3-O-(6"-O-malonyl)-β-D-glucoside exhibited better free radical scavenging ability in all biochemical assays and pulse radiolysis compared to the parent quercetin molecule. This suggests that differential C3 glycosylation plays important role influencing antioxidant property of the parent quercetin.

MATERIALS AND METHODS

Chemicals

Quercetin dihydrate (QD), Quercitrin (QT), Quercetin 3-β-D-glucoside (QG), Quercetin 3-O-(6"-O-malonyl)-β-D-glucoside (QMG), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1, 1'- diphenyl picrylhydrazyl (DPPH), Na_2-EDTA, thiobarbituric acid (TBA), ammonium persulfate, nicotinamide adenine dinucleotide reduced (NADH), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), 2 deoxy guanosine were obtained from Sigma-Aldrich Chemicals Inc. (St. Louis, MO, USA). All other chemicals used were of analytical grade and procured locally.

2, 2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) (ABTS) radical scavenging

The assay was carried out according to the protocol previously described.[7,8] 2.45 mM (final concentration) aqueous solution of ammonium persulphate was incubated with 7 mM ABTS for 16 h in dark at room temperature to generate the cationic $ABTS^{\bullet+}$ radicals. ABTS and persulfate react with the stoichiometry of l: 0.5 leading to an incomplete oxidation of ABTS to generate $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ solution was diluted by using Phosphate buffered saline (PBS) to an absorbance of 0.7, λ_{max}; 734 nm. 10 µl each of different concentrations of quercetin derivatives were added to 1 ml $ABTS^{\bullet+}$ solution. Change in the absorbance was recorded 10 min after the initial mixing. A control experiment was set using 1XPBS.

1, 1'- diphenyl picrylhydrazyl (DPPH) radical scavenging

The DPPH scavenging assay tests the ability of compound to donate hydrogen atom or electron in order to scavenge free radicals. The assay was carried out as previously described,[9] with minor changes. The reaction mixture (1 ml) comprised of freshly made 100 µM 1, 1-diphenyl-2-picryl-hydazyl (DPPH) in methanol and different concentrations (10, 100 and 1000 µM) of test compounds. $DPPH^{\bullet}$ has an unpaired electron, which imparts purple color to the

solution and when this electron is balanced, the color diminishes. The reaction mixture was kept for 20 min at room temperature in dark and then absorption was measured at 517 nm.

Nitric oxide radical scavenging

Nitric oxide radical scavenging assay was performed following the standard procedure.[10] Briefly, reaction mixture (500 µl) containing sodium nitro prusside (50 mM) in 1XPBS with different concentration of quercetin derivatives (10, 100 and 1000 µM) were incubated for 150 min at room temperature. A control experiment without test compound but with the equivalent amount of vehicles was conducted in an identical manner. After incubation, Griess reagent (1% sulphanilamide and 0.1% N-naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4) was added and absorbance was measured at 550 nm.

Superoxide radical scavenging

Superoxide radical scavenging was carried out as per the procedure previously described,[10] with minor modifications. The mixture contained, NADH (166 µM), NBT (50 µM), different concentrations of quercetin derivatives (10,100 and 1000 µM) and PMS (2.7 µM), in a final volume of 1 ml. The components were dissolved in 20 mM phosphate buffer, pH 7.4. The reaction was initiated by the addition of PMS and conducted at room temperature for 10 min after which the absorbance was recorded at 540 nm. NBT interacts with superoxide molecule and the resulting formazon absorbs at 540 nm.

Ferric reducing antioxidant power (FRAP) assay

Reducing power of quercetin derivatives was measured.[11] FRAP reagent was prepared by mixing 10 mM TPTZ (2, 4, 6 tripyridyl-s-triazine) and ferric chloride (20 mM, $FeCl_3.6H_2O$) in 1:1 proportions in 0.3 M acetate buffer (pH 3.6). These two produce FeIII–TPTZ complex, which is reduced to FeII-TPTZ by an antioxidant. In brief, the reaction mixture of different concentrations of quercetin derivatives in 10 µl volume were incubated with 1 ml FRAP reagent for 30 min at 37°C. The change in the optical density was measured spectrophotometrically at 595 nm. The values of reducing power were expressed in mM of AEAC using ascorbic acid as a standard antioxidant.

Pulse Radiolysis

The Pune University Linear Accelerator Facility (PULAF) at the 'National Center for Free Radical Research' was utilized for this study. Aqueous samples were irradiated with a high energy electron beam of 7 MeV with 100 ns pulse width. Aqueous samples presaturated with nitrous oxide (N_2O) were used to remove dissolved oxygen and to quantitatively convert the hydrated electrons (ea_q^-) to $^{\bullet}OH$. Dosimetry was performed with aerated 0.01 mol dm^{-3} KSCN solution with a G ε of 2.6×10^{-4} m^2 J^{-1} per 100 eV at 480 nm[12,13] and the dose rate per pulse was determined to be 17 Gy. Quartz cells with optical pathlength of 1 cm were used for these measurements. Samples were changed after every pulse to reduce losses due to decomposition. Interaction of the quercetin derivatives, QD, QT, QG and QMG were studied with different oxidizing radicals such as thiocyanate $(SCN)2^{\bullet-}$, 2, 2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) ($ABTS^{\bullet-}$), and a reducing carbonate ($CO3^{\bullet-}$) radical using pulse radiolysis. $(SCN)_2^{\bullet-}$ radicals were generated by radiolysis of 10 mM aqueous potassium thiocyanate,[14] $ABTS^{\cdot-}$ radical was produced by the reaction of radiolytically generated azide radical ($N_3^{\bullet}$) with $ABTS^{2-}$ by using aqueous 0.05 M NaN_3 and 2 mM ABTS.[15] $CO_3^{\cdot-}$ radicals were generated using reaction mixture containing 0.05 M $NaHCO_3$ and 0.05 M Na_2CO_3.[16] The decay traces of $(SCN)_2^{\bullet-}$, $ABTS^{\cdot-}$ and $CO_3^{\cdot-}$ radicals were recorded at the corresponding λ_{max} 480, 420 and 600 nm respectively.

Estimation of Single electron oxidation potentials

Cyclic voltammetry is a well known technique used for characterization of antioxidant power of compounds in a solution. Essentially two parameters viz. oxidation peak positions and area under the peak were used for this purpose.[17] The oxidation peak indicates specific reducing power of compounds in solution. A good antioxidant molecule donates its electrons at lower potentials.[18]

CV measurements were performed by using Metrohm potentiostat/Galvanostat (model Autolab PGSTAT 100). 3 mm commercial gold-disk-electrode, Ag/AgCl, (saturated KCl) and Pt wire were used as working, reference and counter electrode, respectively. All the voltammetric experiments were carried out in phosphate buffer (pH 7.4) in the potential range of-0.1 to +0.8 V using the scan rate 20 mVs^{-1}. Prior to use, the working electrode was polished on 0.4µm Alumina, rinsed with Millipore water and further cleaned electrochemically in 0.5 M H_2SO_4.

Protection to pBR322 DNA against oxidative damage

Oxidative damage to plasmid DNA induced by hydroxyl radicals in Fenton reaction was measured in terms of conversion of super coiled to nicked circular form of

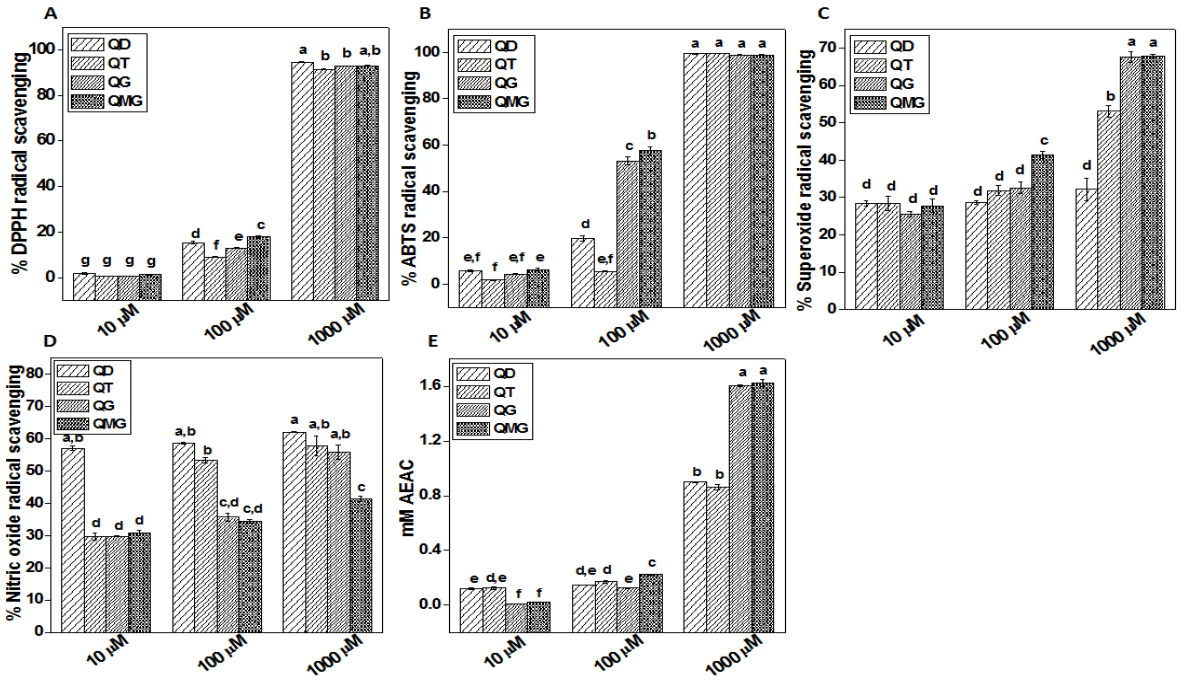


Figure 2: Radical scavenging by quercetin glycosides

(A) DPPH (B) ABTS (C) Superoxide and (D) Nitric oxide radical scavenging and (E) Iron reducing ability of quercetin glycosides. Values were expressed as mean ± SE of three independent experiments. Data was analysed using one way ANOVA, tukey's test for multiple comparisons. Different alphabets indicate significant difference at P ≤ 0.05.

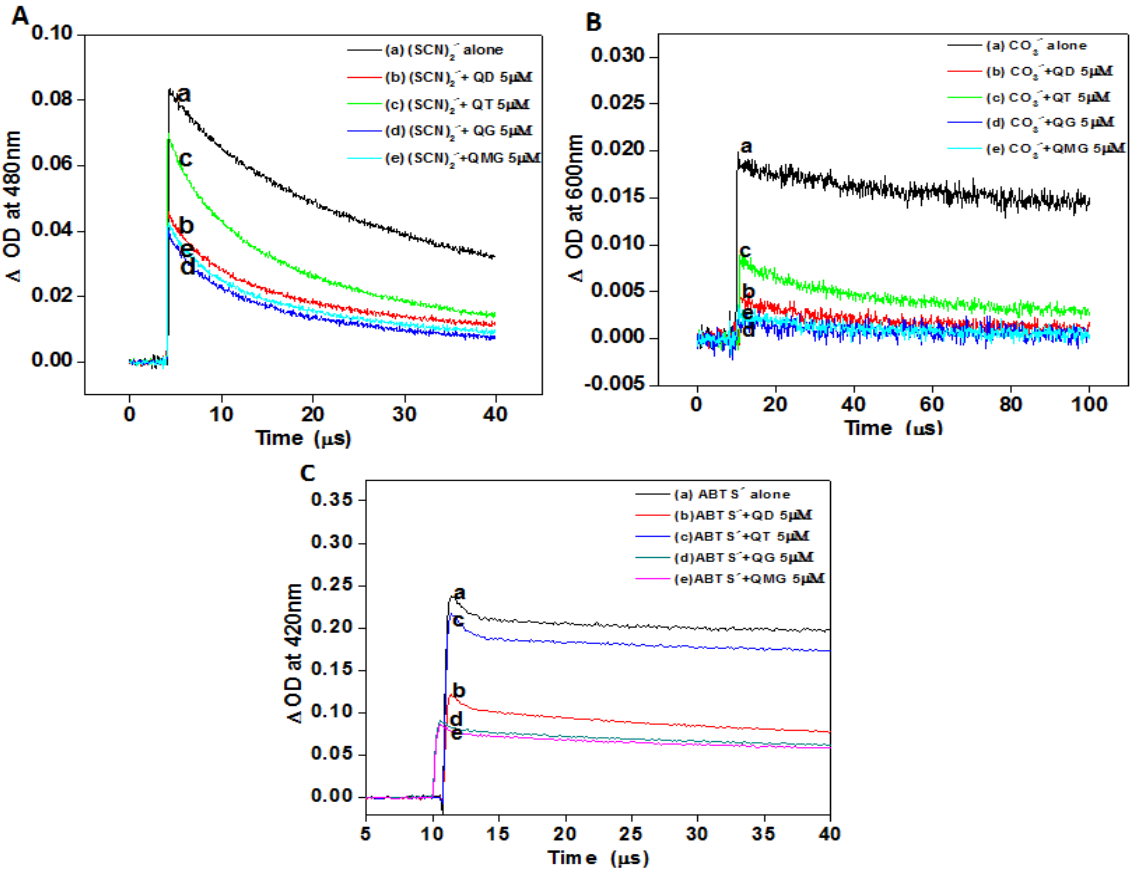


Figure 3: Pulse radiolysis

Decay curves of $(SCN)_2^{\bullet-}$ (A), $CO_3^{\bullet-}$ (B) and $ABTS^{\bullet-}$ (C) radicals in presence or absence of 5 µM QD, QT, QG and QMG.

the pBR322 DNA.[19] Protection conferred by quercetin derivatives was calculated by quantifying the amount of DNA in both nicked circular and supercoiled forms. The reaction mixture contained 1 μM H_2O_2, 500 μM $FeSO_4$, 125 ng pBR322 DNA in a final volume of 20 μl and was incubated at room temperature for 15 min with different concentrations of quercetin derivatives. The samples were resolved in 0.8% agarose gel and after the electrophoresis DNA was visualized by exposing the gel to UV using *Alpha imager* ™ (USA) 3400 gel documentation system and the DNA were quantified by spot densitometry.

Interaction of quercetin glycosydes with 2 deoxy guanosine (2-dG) base

The time resolved transient absorption spectra for the reaction of •OH with 2-deoxy guanosine base were recorded by pulse radiolysis using a dose rate of 7 Gy / pulse. 2 ml of reaction mixture was used for this study. Stabilization of this 2-deoxy guanosine base transient species by quercetin derivatives was monitored using 10 μM concentration of each derivative.

Isolation of Rat Liver Mitochondria

The liver from adult male Wistar rat (weighing 250 ± 25 g) was homogenized in sucrose-EDTA buffer on ice. The homogenate was centrifuged at 2500 rpm for 10 min at 4°C to remove cell debris and nuclear fraction. The pellet was discarded and supernatant was centrifuged at 10000 rpm for 10 min at 4°C to sediment mitochondria. This pellet was washed three times with 50 mM phosphate buffer (pH 7.4), to remove excess sucrose. All procedures were carried out at 4°C, as per described previously in the literature.[20] The pellet was re suspended in 50 mM phosphate buffer and protein concentration was estimated using Bradford's method.[21] The aliquots were prepared in micro-centrifuge tubes and stored at -80°C.

Estimation of Fenton radical induced lipid peroxidation and protein sulphydryl depletion in Rat liver mitochondria

Rat liver mitochondria were exposed to oxidative stress induced by Fenton reaction using Ascorbate-Fe^{2+} in acidic conditions, as per the procedure described,[22] with a few modifications. In brief, the reaction mixture containing 50 mM phosphate buffered medium with 250 μg protein equivalent mitochondria, different concentrations (1, 10 and 100 μM) of quercetin derivatives, 50 μM $FeSO_4$ in 0.1 N HCl and 450 μM ascorbic acid (dissolved in 0.15 M

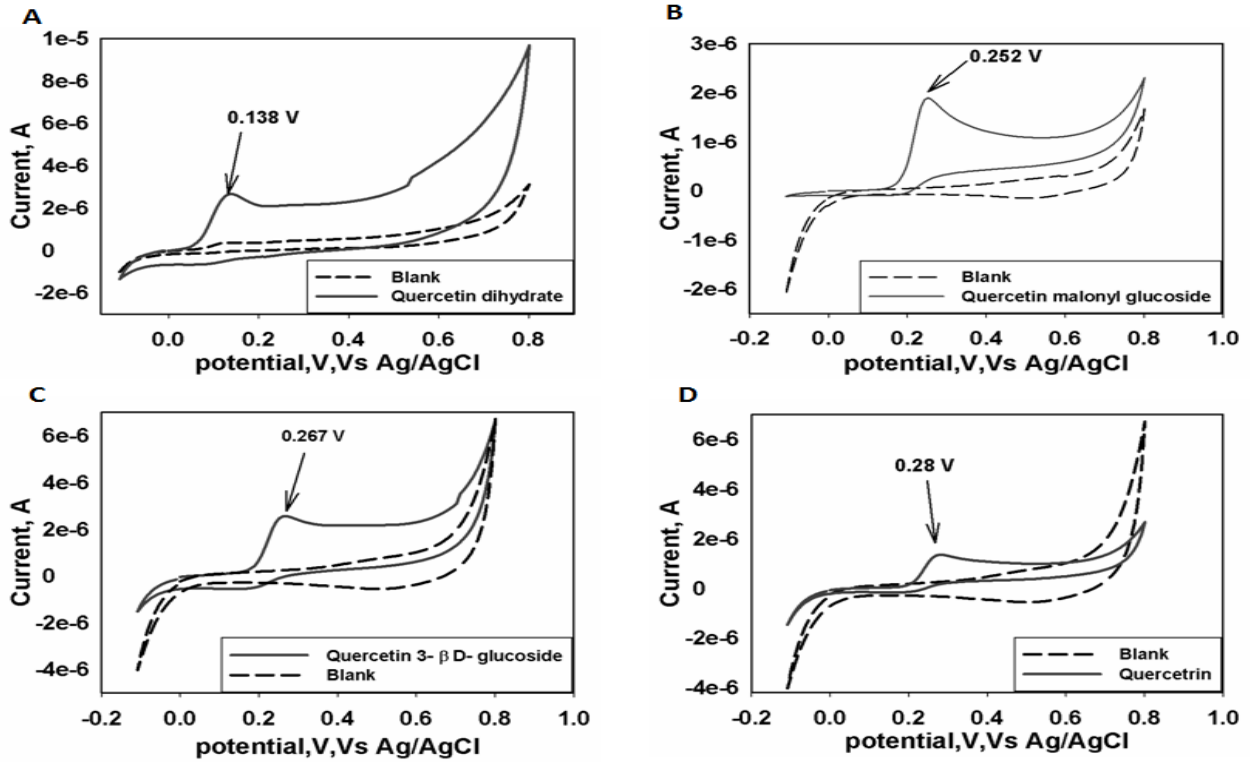


Figure 4: Cyclic voltammetry

Cyclic voltammograms were recorded using gold-disk (d=3mm) as working electrode, Pt wire and Ag/AgCl (saturated KCl) as counter and reference electrodes respectively. Potential window was -100 mV to -800 mV with the scan rate 20 mV/sec. For the measurements 100 μM samples were drop-casted on working electrode and air dried. (A) depicts the cyclic voltammogram of QD with the oxidation potential 0.138V. (B), (C) and (D) show voltammograms for QMG, QG and QT with their respective oxidation potentials at 0.252 V, 0.267 V and 0.28 V.

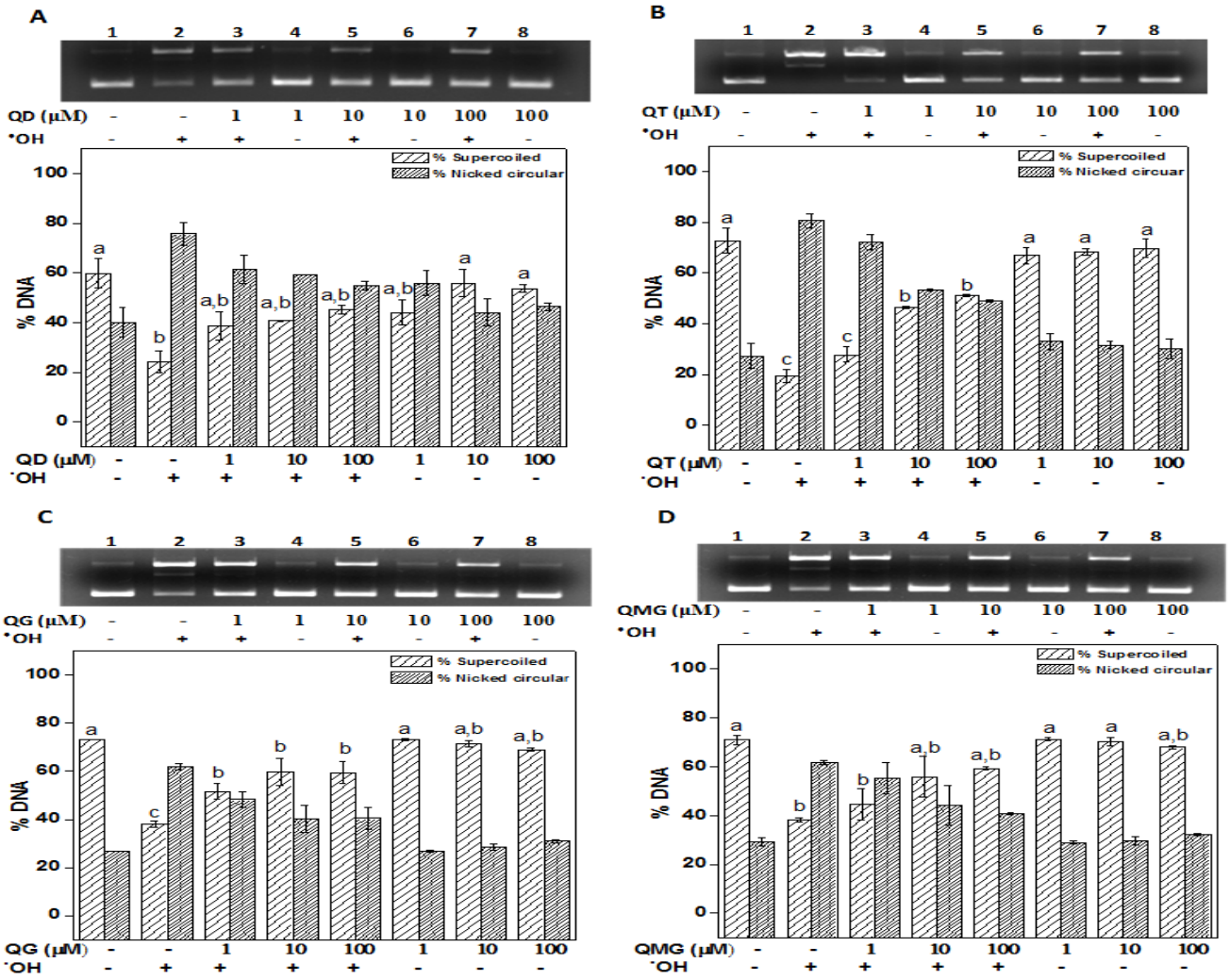


Figure 5: Protection conferred by quercetin QD (A) and its glycosides QT (B), QG (C), QMG (D) to the DNA

Lane 1 is control DNA, Lane-2 oxidatively damaged DNA and Lanes 3, 5 and 7 are pBR322 DNA exposed to •OH in presence of different concentrations (1, 10 and 100 μM) of quercetin glycosides (QD, QT, QG and QMG) and Lanes 4, 6 and 8 are pBR322 DNA in presence of QD (A), QT (B), QG (C) and QMG (D) alone. For quantification of supercoiled and nicked circular forms of DNA, densitometry was performed. Values were expressed as mean ± SE. The data was analyzed using one way ANOVA, tukey's test for multiple comparisons. Different alphabets indicate significant difference at P≤0.05.

Tris-Hcl buffer containing1mM KH_2PO_4, pH 7.4) were incubated at 37°c for 45 minutes. 500 μl of TBA reagent (0.375% TBA, 0.25 M HCl, 15% trichloroacetic acid (TCA) and 6 mM Na_2 EDTA) was added after the incubation. The reaction mixtures were kept in boiling water bath for 20 min at 100^0C, cooled and centrifuged at 14000 rpm for 5 min at 4^0C. Oxidative damage to the lipids was quantitated by recording absorbance of thiobarbituric acid reactive substances (TBARS) at 535 nm.[23] Protein sulphydryls were estimated using Ellman's reagent (DTNB), 5, 5-dithiobis-(2-nitrobenzoic acid).[24] DTNB quantitatively reacts with thiol groups to release 2- nitro- 5-mercaptobenzoic acid (TNB) imparting bright yellow color. After 45 min of incubation with the stressor, mitochondrial mixtures were pelleted down at 10000 rpm. The pellet was suspended in 0.5 ml distilled water and 0.3 ml 14% perchloric acid and was centrifuged at 5000 rpm for 5 min. The supernatant was discarded and the pellet was re suspended in 100 μl of 7% perchloric acid diluted with 0.9 ml water and centrifuged at 4500 rpm for 5 min. Supernatant was discarded and the pellet was dissolved in solution containing 0.2 ml 10% Triton X 100, 0.2 ml phosphate buffer (pH 7.6) and 0.6 ml milli-Q water. 110 μl of 2 mM DTNB was finally added to the mixture and the absorbance was recorded at 412 nm, 5 min after the addition of DTNB. The lipid peroxidation values were expressed as nanomoles of malonaldehyde equivalents per mg protein using 1, 1', 3, 3'-tetra methoxy propane (TMP) standard, while protein sulphydryls were expressed as nanomoles of protein sulphydryls per mg of proteins using GSH standard.

Statistical analysis

Statistical analysis was performed using SPSS 19 software

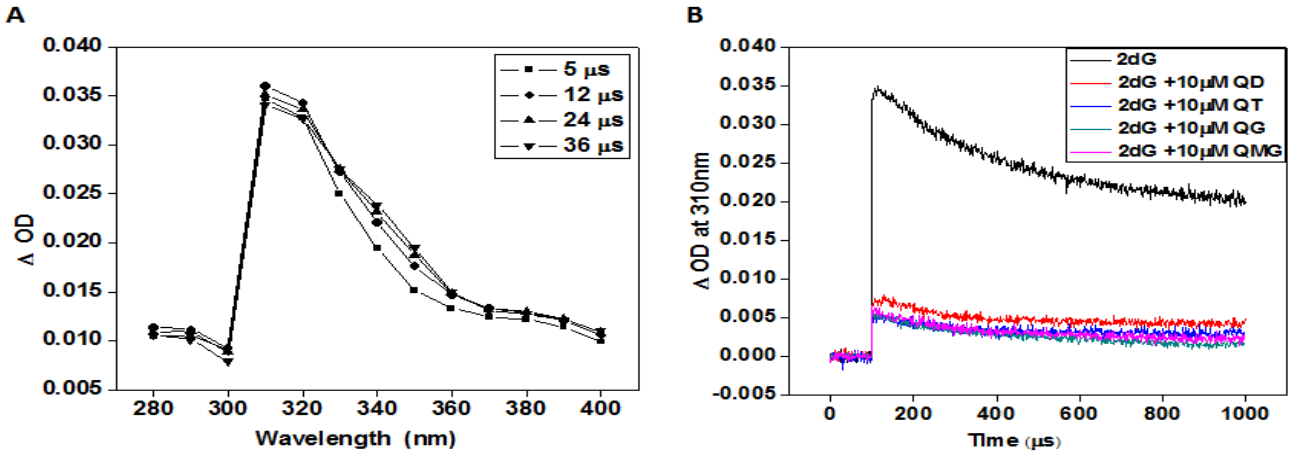


Figure 6: Interaction of quercetin glycosides with 2-deoxy guanosine (2dG) base transient

Figure 6A shows an absorption spectrum (λ_{max}, 310 nm) of 2dG transient species recorded at four different time windows. Figure 6B shows decay of this transient species in presence or absence of 10 µM quercetin glycosides (QD, QT, QG and QMG).

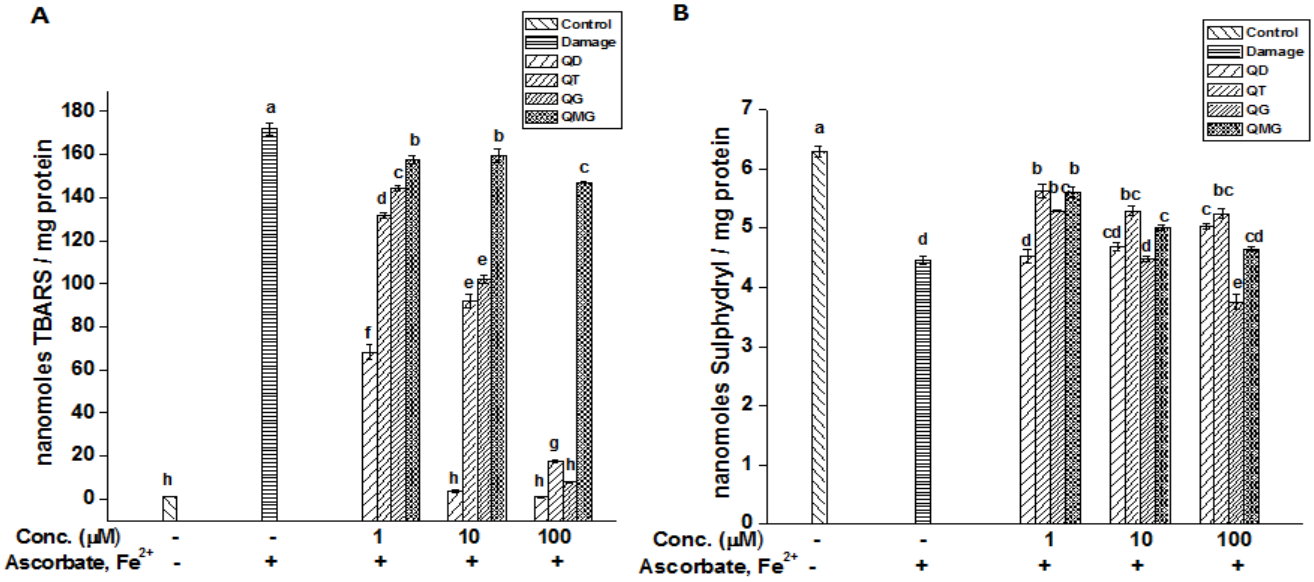


Figure 7: Protection conferred by quercetin glycosides against mitochondrial lipid peroxidation (A) and protein sulphydryl depletion (B)

Oxidative damage to the lipids was estimated by measuring levels of thiobarbituric acid reactive substances (TBARS) using an absorption maxima at 535 nm (Figure 7A) by UV- VIS spectrophotometer. Protein sulphydryls were estimated using Ellman's reagent by recording absorbance at 412 nm (Figure 7B). Values were expressed as mean ± SE of three independent experiments. Data was analyzed using one way ANOVA, tukey's test followed by post hoc. Different alphabets indicate significant difference at P≤0.05.

licensed to Department of Zoology, Savitribai Phule Pune University (SPPU). One way ANOVA- tukey's test paired with posthoc test was performed. The statistical significance of difference at P≤0.05 was indicated using different alphabets (a,b,c,d,e,etc).

RESULTS

Free radical scavenging by quercetin glycosides

DPPH and ABTS both are commercially available stable free radicals. Amongst the quercetin glycosides QMG despite of having bulky structure showed significant (P≤0.05) scavenging of both of these radicals [Figure 2 A and B]. It was equally effective in scavenging superoxide and reducing ferric ion even at low concentrations [Figure 1 A and B, C and E]. QG seems to be a second active scavenger of almost all the radicals including superoxide and nitric oxide.

Pulse radiolysis was used to explore the reactivity of quercetin glycosides with free radicals. Figure 3 A, B and C

show decay traces of thiocyanate $(SCN)_2^{\bullet-}$, carbonate $(CO_3^{\bullet-})$ and 2, 2'-azinobis 3-ethylbenzothiazoline-6- sulfonic acid ($ABTS^{\bullet-}$) radicals respectively alone and in presence of 5 μM of QD, QT, QG and QMG at their respective λ_{max}; 480, 600 and 420 nm. Both, QG and QMG exhibited better scavenging properties of these radicals than rest of the glycosides used in this study. Absorbance of these radicals was the lowest in presence of QG and QMG, implying a potential role of these compounds in affecting the formation of these radicals.

Cyclic voltammograms for detection of oxidation potentials of quercetin glycosides

CV results confirmed the maximum reducing activity of quercetin than rest of the glycosides used in this study. The reducing activity was in the order of Quercetin >Quercetin 3-β-glucoside>Quercetin malonyl glucoside >Quercetrin. Our results clearly indicate that antioxidant properties of quercetin and its derivatives are most affected by the free hydroxyl groups on quercetin molecule. The substitution of the hydroxyl group on C3 ring results in loss of co-planarity and subsequent reduction in antioxidant activity.[18]

Figure 4 A-D depicted a typical cyclic voltammograms recorded on quercetin derivatives in 0.1 M phosphate buffer solutions (pH 7.4), between the potential window -100 mV to 800 mV at 20 mVs-1. All the CVs show quasi-reversible nature with distinct antioxidant peaks. The oxidation potentials were in the order of QD (0.138 V) < QMG (0.252 V) < QG (0.267 V) < QT (0.28 V).

Protective effects of quercetin glycosides against Fenton radical induced DNA (pBR322) damage and their possible interaction with 2-deoxy guanosine base transient

Plasmid pBR322 DNA was exposed to the $^{\bullet}OH$ generated by Fenton reaction. Lane 1 in Figure 5 A, B, C, D is a control pBR322 DNA not exposed to $^{\bullet}OH$, lane 2 is an oxidatively damaged pBR322 DNA on exposure to $^{\bullet}OH$. Lanes 3, 5 and 7 are pBR322 DNA exposed to $^{\bullet}OH$ in presence of different concentrations of QD, QT, QG and QMG (1, 10 to 100 μM) and lanes 4, 6 and 8 are pBR322 DNA in presence of QD, QT, QG and QMG (Figure 5 A, B, C, D) alone respectively. All glycosides alone did not induce any SSBs (single strand breaks) in pBR322 DNA and significantly ($P \leq 0.05$) protected DNA from oxidative damage even at 1 μM concentration.

2-dG which is the most favorable site for DNA damage was exposed to the 7Gy dose of radiation with 50 ns Pulse width using PULAF (Pune University Linear Accelerator Facility) The time resolved transient absorption spectrum for the reaction of $^{\bullet}OH$ with 2-dG was recorded, it showed an absorption maxima at 310 nm (Figure 6 A). The spectral features were similar to those reported from the transient species of 2-dG.[13] The interaction of the dG transient species with quercetin derivatives (10 μM) was monitored at 310 nm (Figure 6 B). All quercetin derivatives strongly affected the formation of dG transient species.

Protective effects of quercetin glycosides in isolated Rat liver mitochondria against ascorbate-Fe^{2+} induced oxidative stress

Rat liver mitochondria were exposed to an oxidative stress induced by Ascorbate- Fe^{2+}. Protection conferred to the lipids by quercetin derivatives was assessed by quantitating lipid peroxidation and was expressed in terms of nanomoles of TBARS (thiobarituric acid reactive substances) formed [Figure 7 A]. In case of proteins oxidative damage was monitored by estimating protein sulphydryls and was expressed in terms of nanomoles of sulphydryls per milligram of protein [Figure 7 B]. All quercetin derivatives except QMG, protected the mitochondria from lipid peroxidation in a concentration dependent manner. QMG, even at higher concentrations was unable to attenuate mitochondrial lipid peroxidation as effectively as other glycosides. In case of protein sulphydryls, lower concentrations of QT, QG and QMG effectively restored the mitochondrial sulphydryls while at higher concentrations they failed to do so.

DISCUSSION

Three glycosides of quercetin used in this study differ in their structure by differential C3 glycosylation. Earlier work has reported different biological activities of these three glycosides.[2,25-28] Querecetin dihydrate (QD), a hydrated form quercetin is reported to decrease the absorption of dietary cholesterol and also the levels of plasma and hepatic cholesterol.[25] Quercitrin is a 3-O-rhamnosyl derivative of quercetin, having O-rhamnoside group at C (3) position. The previous studies on quercitrin demonstrated its anti-inflammatory effect, protective effects in colon by stabilizing fluid transport in rats,[2] preventing diarrhea and has shown a strong antileishamnial activity.[26] It also improved glucose homeostasis in diabetic rats.[27] Quercetin 3-β-D-glucoside is the O-glucoside of quercetin at C3 position. It has low cytotoxicity and has shown antiproliferative effects in cancer cells.[28] Quercetin 3-O-(6"-O-malonyl)-β-D-

glucoside has a 6-O-malonyl glucose at the 3-O-position of quercetin.[29] We have assessed antioxidant potentials of these three glycosides in detail using several biochemical assays, pulse radiolysis and compared them with their parent quercetin molecule.

We investigated the interaction of these quercetin derivatives with stable free radicals such as $ABTS^{\bullet+}$ & $DPPH^{\bullet}$ and biologically significant radicals such as superoxide ($O2^{\bullet-}$) and nitric oxide ($NO^{\bullet}$) in cell free environment. Both model radicals, $DPPH^{\bullet}$ & $ABTS^{\bullet+}$ are nitrogen centered and assess primary radical scavenging ability of an antioxidant through donation of electrons or hydrogen atoms. All glycosides could effectively scavenge both the radicals very rapidly in concentration dependent manner, but QMG and QG, though bulkier than the parent quercetin surprisingly scavenged both the radicals more efficiently than the parent quercetin. We observed similar results in case of superoxide radicals and in ferric reduction property. Ability of these compounds to reduce ferric ion is indicative of their potential to protect biological system under stress of Fenton or Haber weiss reactions. With nitric oxide these glycosides exhibited less reactivity.

The interactions of quercetin glycosides with the free radicals at a microsecond time scale were monitored using pulse radiolysis. The decay traces (Figure 3) of $(SCN)_2^{\bullet-}$, $ABTS^{\bullet-}$ and $CO_3^{\bullet-}$ in the presence of quercetin glycosides suggest that amongst all, QT is least reactive to these radicals while QG and QMG are more reactive, as they scavenged these radicals faster. In addition, quercetin glycosides also affected the radical formations, as evident from the reduction in the absorbance of these radicals.

Cyclic voltammograms carried out at physiological pH (Figure 4) strengthened our observations made from steady state reactions. QT, exhibiting a higher oxidation potential (0.28 V) than the remaining quercetin glycosides, was the least reactive amongst them. These results resemble previous observations by Matereska *et al*. (2008) regarding the activity of quercitrin amongst C3 glycosides. Electro-activity of quercetin glycosides observed from our cyclic voltammetric studies was comparable to their antioxidant behavior in cell free environment, with only exception of the basic quercetin dihydrate, which showed oxidation peak at 0.138 V and was not observed to be as potent antioxidant as its glycosides in the cell free environment studies. All other quercetin glycosides exhibited antioxidant property as expected from their voltammetric analysis. The sub cellular environment with differential pH and also the bioavailability of theses glycosides could be the contributing factors to their antioxidant behavior in the cells.

All glycosides QD, QT, QG and QMG conferred significant ($P \leq 0.05$) protection to the DNA under Fenton radical induced stress in cell free environment while none of them induced strand breaks even at higher concentrations (Figure 5).

In order to understand their mechanism to protect DNA damage, 2-dG base which is the favored site for DNA strand cleavage was subjected to pulse radiolysis studies in presence or absence of quercetin glycosides. The decay of dG transient species was monitored at 310 nm and was found to be stabilized by 10 μM quercetin glycosides. The quercetin glycosides showed strong interaction with the dG transient species as the optical density at 310 nm is quenched by these compounds (Figure 6 B). These observations are significant as it suggests that the decay of the dG transient species can be altered by these compounds, implying a potential role of the glycoside derivatives of quercetin in protecting DNA against free radical induced oxidative assault.

All of these quercetin glycosides along with the parent quercetin molecule were also tested for their role in mitigating the oxidative damage induced in isolated rat liver mitochondria. Our findings suggest that C3 glycosylation with rhamnosyl glucose in QT and glucose in QG enhanced their antioxidant efficacy in mitochondria. Both QT and QG protected mitochondria from lipid peroxidation and protein sulphydryl depletion even at lower concentrations. QMG exhibited excellent antioxidant activity in all of the radical scavenging assays but it could not confer similar protection against mitochondrial lipid peroxidation compared to that of the protection conferred by other glycosides. It was able to protect proteins from sulphydryl depletion at lower concentration.

CONCLUSION

All quercetin derivatives used in the present study exhibited excellent antioxidant activities in cell free environment but failed to exert the comparable protective effects in biological systems. Varied response of these derivatives could be attributed to their C3 glycosylation and the derivatization at C3 sugars.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

Highlights of Paper

- Glycosides of quercetin scavenged biologically significant and model radicals.
- Quercetin glycosides protected vital cellular molecules such as DNA against Fenton radical induced DNA damage in cell free environment, possibly by interacting with 2-deoxy guanosine base transient.
- These glycosides also protected mitochondria from lipid peroxidation and protein sulphydryl depletion against Fenton radical induced damage.
- Differential C3 glycosylations and derivatizations at C3 sugars were observed to be playing important role in influencing the antioxidant property of quercetin glycosides.

About Authors

Saroj Ghaskadbi, *is a Professor at Department of Zoology, Savitribai Phule Pune University (SPPU), Pune. She has been instrumental in the research pertaining to the area of free radical biology. On one hand her research work deals with understanding how cells cope up with oxidative stress. For this different cell systems including islets, liver cells, muscle cells and adipose cells are used. On the other hand she looks at functioning of different antioxidants purified from natural products and their characterizations using in-vitro and in vivo models to test their potential use in varied stress related disorders like diabetes. She has been also involved in development of mathematical models for profiling cellular antioxidant status in diabetics, in association with Dr. Pranay Goel from IISER, Pune.*

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