Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

Int. J. Modn. Res. Revs.
Volume 3, Issue 1, pp 569-574, January, 2015

ORIGINAL ARTICLE

ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF QUININE DETERMINED BY USING DIFFERENT *IN VITRO* MODELS

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Article History: *Received 2nd November,2014, Accepted December 31st 2014, Published 1st January,2015*

ABSTRACT

Objective: The use of antioxidants in treatment of oxidative stress-related pathologies is a possible therapeutical strategy for the future. Natural product with antioxidant properties could trigger this goal. The aim of this *in vitro* study was to assess antioxidant and free radical scavenging activities of Quinine by using *in-vitro* scavenging assays. **Methods:** 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS, hydroxyl, superoxide, hydrogen peroxide, nitric oxide radicals and reducing power scavenging assays. **Results:** The results of the study were showed that Quinine exhibited substantial inhibition of free radical which was calculated as IC_{50} value, compared with standard ascorbic acid. IC_{50} values of Quinine and ascorbic acid were found to be 45.33µg/mL and 37.76µg/mL in DPPH radical, 42.15µg/mL and 35.97µg/mL in $ABTS^{\bullet+}$, 47.52µg/mL and 34.24µg/mL in hydroxyl radical, 46.55µg/mL and 34.91µg/mL in superoxide anion radical, 43.63µg/mL and 37.00µg/mL in hydrogen peroxide radical, 40.03µg/mL and 34.60µg/mL in nitric oxide radical, and the reducing power of Quinine is 46.16µg/mL We compared to ascorbic acid 40.45 µg/mL. **Conclusion:** We conclude that above results showed Quinine has more potent antioxidant and free radical scavenging properties.

Keywords: Quinine, Ascorbic acid, DPPH, $ABTS^{\bullet+}$, Hydroxyl radical, Reducing power, Antioxidants.

1.INDRODUCTION

Oxidative stress is related to inflammation, playing together an important role in the pathogenesis of cancer (Maeda & Omata 2008). It is an imbalance between production of reactive oxygen species and antioxidant defences (Betteridge 2000). Redox stress accelerates the activation of immune cells which release proinflammatory cytokines, reactive oxygen and nitrogen species causing damage to biological molecules and inducing imbalances in physiological and pathological pathways (Lonkar and Dedon, 2011). *In vivo* studies have provided evidence that dietary intake of antioxidant and anti-inflammatory compounds is a key approach for health endorsement by lowering oxidative stress and inflammation (Watz, 2008). Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) are widely used in the food industry. Mammalian cells have intracellular antioxidant defense mechanism such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) vitamin-E, C and β-carotene in order to protect the cells and tissues from oxidative damage (Ostrovidov *et al*. 2000). Quite a lot of studies reported that phytochemicals plays an important role in scavenging free radicals both *in-vitro* and *in-vivo* (Evan *et al* 2010). Various plants derived phytochemicals have been extensively used in the herbal medicine for the treatment of number of human diseases (Palbag *et al* 2014).

Quinine is a natural alkaloid derived from the dehydrated bulk of *Cinchona* spp. known as *Cinchonae* cortex. It was the first effective treatment for malaria caused by Plasmodiumspp. as early as in the 17th century. Therapeutic role of quinine products is generally related to their obstruction with the protozoan protoplasm and DNA (Van Vugt et al., 2011). *Cinchonae* derivatives as natural extracts are allowed for use in all food producing species (Commission Regulation (EU), No. 37, 2010). Quinine still remains a drug of option for the treatment of severe and complex malaria in most parts of the world and is in fact widely used in most African countries because of its high efficiency and moderately low cost (Barennes et al., 2006). There is no clear report about Quinine in cancer and the present study is mainly focused to evaluate the free radical scavenging properties of Quinine on DPPH, $ABTS^{\bullet+}$, hydroxyl, superoxide, hydrogen peroxide, nitric oxide radicals and reducing power assays.

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2.MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA), Phenozine methosulphate (PMS), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), nitro blue tetrazolium (NBT), 5,5-dithiobis 2-nitrobenzoic acid (DTNB), potassium ferricyanide, ferric chloride, nicodinamide adenine dinucleotide (NADH), glacial acetic acid, hydrogen peroxide (H_2O_2), ethanol, phosphate buffered saline (PBS), and ascorbic acid were purchased from Sigma-Aldrich, and HiMedia Pvt, India. Quinine (CAS No. 130-95-0) was also purchased from Sigma-Aldrich, Pvt, India.

DPPH radical scavenging assay

The effects of Quinine and positive control (ascorbic acid) on 1, 1-diphenyl-2-picrylhydrazyl radicals (DPPH) were estimated according to the method of Lee et al. The sample solution was prepared in absolute ethanol and the resulting concentration was 10 to 50µg/mL. Different concentrations of (10-50µg/ml) Quinine were added to 2mL of methanolic DPPH solution. The mixture was shaken vigorously with a Vortex Mixer and the absorbance was measured by Spectrophotometrically at 517nm immediately and recorded at 5min intervals until the absorbance reached a stable state. The mixture without the addition of sample served as the control. All the tests were performed in triplicate and the (%) of inhibition was calculated according to the equation compared with standard ascorbic acid.

DPPH radical inhibition (%) = [(Abs control – Abs sample) / Abs control] × 100

$ABTS^{\bullet+}$ radical scavenging assay

This method determine the ability of Quinine to scavenge the 2, 2 -azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical cation ($ABTS^{\bullet+}$) by the method of Arnao et al., The antioxidant activity was measured by reaction mixture (0.5mL of 15µM H_2O_2, 0.5mL of 7mM ABTS and 50mM sodium phosphate buffer, pH 7.5) and different concentrations of Quinine (10-50µg/mL). Set blank contained without Quinine replaced water. The absorbance was read in spectrophotometer at 734nm and compared with standard ascorbic acid. We calculated IC_{50} value is the expected concentration of test sample to inhibit 50% of $ABTS^{\bullet+}$ production followed formula.

% $ABTS^{\bullet+}$ scavenging = [(Abs control – Abs sample) / Abs control] ×100

Hydroxyl radical scavenging activity

The % of hydroxyl radicals scavenging ability of Quinine is measured by the method of Kunchandy and Rao. The 1.0mL of reaction mixture containing 100µL of 2-deoxyribose (28mM in 20mM KH_2PO_4-KOH buffer, pH 7.4), different concentration of the test sample (10-50µg/mL), 200µL EDTA (1.04mM) and 200µM FeCl3 (1:1 v/v), 100µL of H_2O_2 (1.0mM) and 100µL ascorbic acid (1.0mM) which is incubated at 37°C for 1 hr. 1.0mL of thiobarbituric acid (1%) and 1.0mL of trichloroacetic acid (2.8%) are added and incubated at 100°C for 20min. After cooling, absorbance is measured at 532nm, set blank reaction mixture water place in test sample.

% of hydroxyl radical scavenging = [(Abs control – Abs sample) / Abs control] ×100

Superoxide radical scavenging assay

The superoxide anion scavenging activity was measurement by method of Fontana et al. Superoxide radical is generated in phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) systems by oxidation of NADH. It measured by the reduction of nitroblue tetrazolium (NBT) to a purple formazan. The 1mL reaction mixture contained phosphate buffer (20mM, pH 7.4), NADH (73µM, NBT (50µM), PMS (15µM) and various concentrations of test sample solution (10 to 50µg/mL). After incubation for 5min at ambient temperature, the absorbance at 562nm was measured against an appropriate blank to measure the formazan generated. The experiment was repeated thrice. The results were compared with standard ascorbic acid.

% Superoxide radical scavenging = [(Abs control – Abs sample) / Abs control] ×100

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging potential of the Quinine was determined using the method of Jayaprakasha et al. A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Quinine and standard ascorbic acid at different concentrations (10 to 50µg/mL) in ethanol (1mL) was added to 2mL of H_2O_2 solution in PBS. After 10min the absorbance was measured at 230nm against a blank solution that contained hydrogen peroxide solution without any substance. The percentage of H_2O_2 scavenging of the Quinine (IC_{50}) values was calculated by following formula.

% Hydrogen peroxide scavenging = [(Abs control – Abs sample) / Abs control] ×100

Nitric oxide radical scavenging assay

The determine nitric oxide radical scavenging activity of [6]-shogaol to screening various method of Garrat. 2mL of sodium nitroprusside (10mM) prepared in phosphate buffer saline (pH 7.4) was mixed with 0.5mL of Quinine at various concentrations at a ranging from 10 to 50 µg/mL and ascorbic acid at various concentrations depend manner from 10 to 50µg/mL. The mixtures were incubated at 25°C for 150 min. After incubation withdrawn 0.5mL solution and mixed with 0.5mL of Griess reagent [1.0mL sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5min with 1mL of naphthylethylene diamine dihydrochloride (0.1% w/v)]. The mixtures were incubated at room temperature for 30min, followed by the measurement of absorbance at 540nm using spectrophotometer. The results were calculated IC_{50} values following the formula.

% Nitric oxide radical scavenging = [(Abs control – Abs sample) / Abs control] ×100

Reducing power assay

The measured Quinine reducing power was determined by the method of Yen and Duh. Sample dissolved in ethanol at a different concentration (10-50µg/mL) were mixed with 2.5mL of phosphate buffer (200mM, pH 6.6) and 2.5mL of 1 % potassium ferricyanide. The mixtures were incubated at 50°C for 20min. After incubation was added 2.5mL of 10% trichloroacetic acid (TBA) these mixture followed by centrifugation at 3000g for 10min. The collect upper layer of the supernatant (5mL) was mixed with 5mL of distilled water (1:1) and added 1mL of 0.1% ferric chloride and the resolved absorbance of the mixed solution were measured at 700nm. Ascorbic acid was used as the standard.

3.RESULTS

The present study demonstrates the free radical scavenging activity of Quinine was evaluated by different scavenging assays compared with standard ascorbic acid. Quinine as well as standard ascorbic acid IC_{50} values were calculated and summarized in Table.1 and graphically presented in figure 1-7. The free radical scavenging consequence activities were increased with the increasing concentrations of Quinine.

DPPH radical scavenging assay

Figure 1 shows the % of DPPH radical scavenging inhibition of Quinine. The IC_{50} values of Quinine (45.33µg/mL) were nearby standard ascorbic acid IC_{50} value (37.76µg/mL). DPPH is unstable nitrogen centered free radical, react with suitable reducing substrate of Quinine that can donate a hydrogen atom become paired off forming the consequent hydrazine with the loss of violet color. In the present study support, Quinine has good antioxidant and scavenges DPPH radicals.

$ABTS^{\bullet+}$ radical scavenging assay

$ABTS^{\bullet+}$ radical scavenging measured based on the reduction of blue/green $ABTS^{\bullet+}$ chromophore generated from the reaction between $ABTS^{\bullet+}$ and potassium persulphate by an electron donating antioxidant. The $ABTS^{\bullet+}$ radical scavenging ability of Quinine showed as IC_{50} values were presented in Figure 2. Quinine significantly inhibit at dose dependent manner inhibition of $ABTS^{\bullet+}$ radical activity with IC_{50} values of 42.15µg/mL. The IC_{50} value of Quinine was compared with the standard antioxidant ascorbic acid (35.97µg/mL).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of Quinine was shows in fig. 3. Hydroxyl radical is singlet electron that scavenging by test sample of Quinine donating free electron to form stable molecules. Our result shows that Quinine potent scavenging hydroxyl radical on IC_{50} value (47.52µg/mL) to compare with ascorbic acid IC_{50} value (34.24µg/mL). The result obtained emphasized the strong antioxidant capacity Quinine.

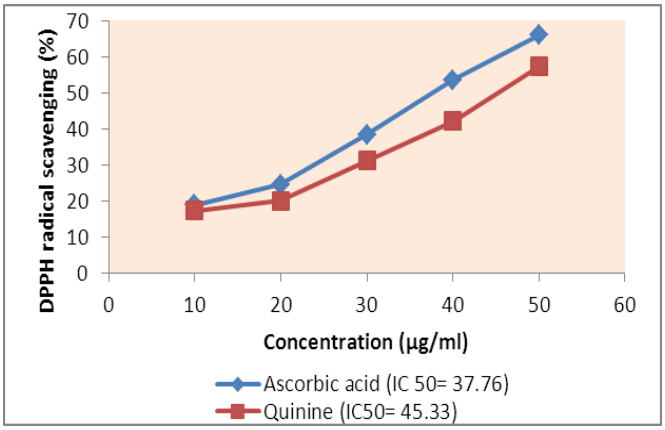

Figure 1: DPPH radical scavenging effect of Quinine compared to that of standard Ascorbic acid

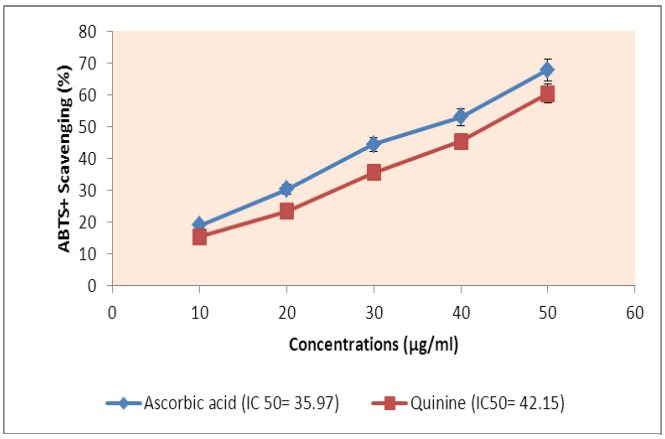

Figure 2: ABTS+ radical scavenging effect of Quinine compared to that of standard Ascorbic acid

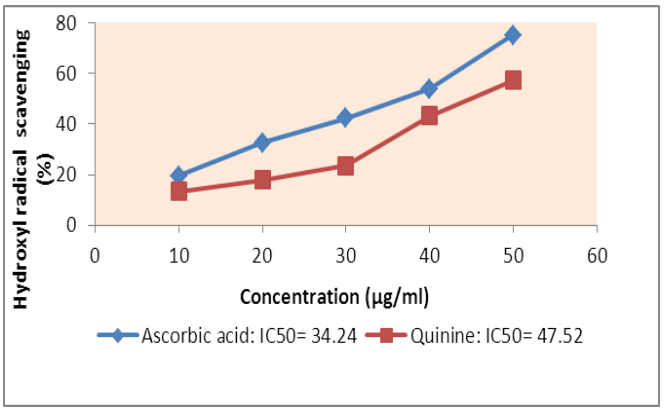

Figure 2: ABTS+ radical scavenging effect of Quinine compared to that of standard Ascorbic acid

Super oxide free radical scavenging activity

Figure 4: Shows the % of super oxide radical scavenging of Quinine. Super oxide anions are generated in the formation PMS-NADH system by the oxidation of NADH and determine by the reduction of NBT in resulting the formation of blue colored formazan that can be measured at 560nm. From results, super oxide radicals scavenged by Quinine in a dose depend manner, IC_{50} value of Quinine (46.55µg/mL) showed potent free radical scavenging activity compared to the standard ascorbic acid IC_{50} value (34.91µg/mL).

Hydrogen peroxide scavenging activity

Free radical scavenging activity of Quinine was evaluated by hydrogen peroxide (H_2O_2) scavenging method and the results were shown in Figure 5. From the results, IC_{50} value of [6]-shogaol showed concentration dependent activity and standard ascorbic acid. The H_2O_2 scavenging effect of Quinine was found in 43.63µg/mL. This was compared to standard ascorbic acid at a concentration of 37.00µg/mL

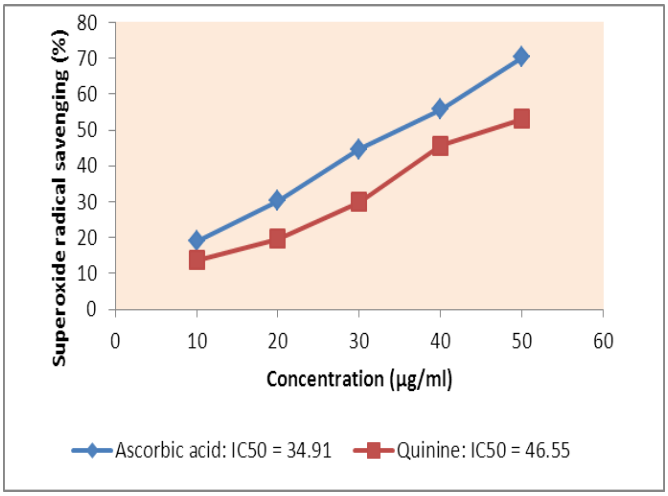

Figure 4: Superoxide radical scavenging effect of Quinine compared to that of standard ascorbic acid

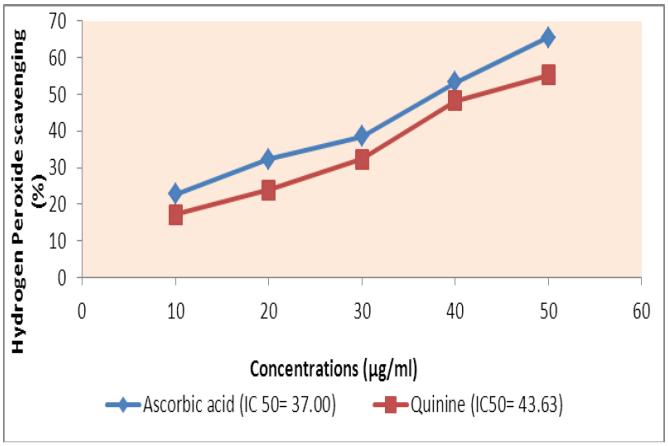

Figure 5: Hydrogen peroxide radical scavenging effect of Quininecompared to that of standard Ascorbic acid

Nitric oxide scavenging activity

The % of Nitric oxide scavenging ability was performed with Quinine compared with standard ascorbic acid were shown in figure 6. From the results, Quinine exhibited most effective dose dependent inhibition of nitric oxide radicals with the IC_{50} values of 40.03µg/mL. It was significantly similar to the scavenging effect of ascorbic acid IC_{50} value at a concentration of 34.60µg/mL.

Reducing power

The reducing power of Quinine was shown in figure 7. In this study, strong antioxidant compounds of Quinine donating electron to convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). From the result reducing power activity of Quinine effectively inhibition of oxidation form of free radical at the concentration Quinine 46.16µg/mL as compared to standard ascorbic acid 40.45µg/mL.

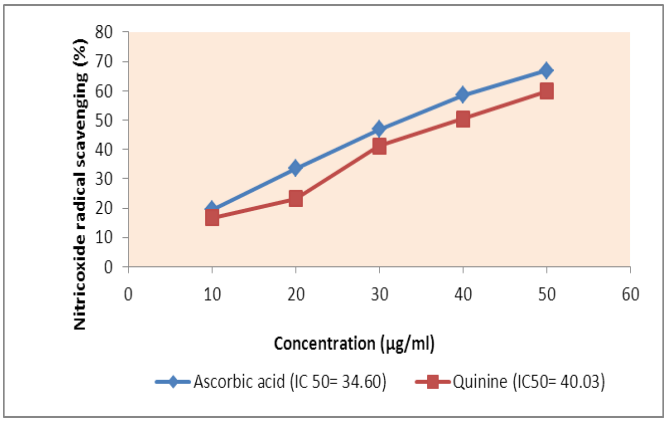

Figure 6: Nitric oxide radical scavenging effect of Quinine compared to that of standard ascorbic acid

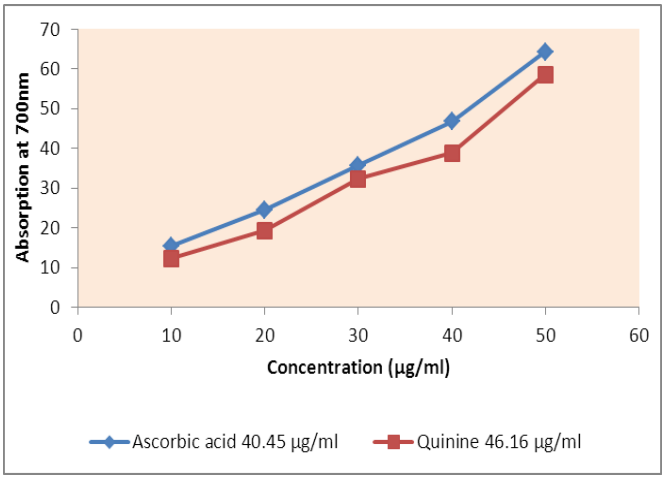

Figure 7: Reducing power inhibition effect of Quinine compared to that of standard Ascorbic acid

4.DISCUSSION

Present study investigated the antioxidant and free radical scavenching potential of Quinine determined by various *in vitro* scavenging assays. Free radicals are atoms or groups of atoms with an unpaired number of electrons, which are highly reactive substances that can result in chain reactions. That chain reactions forming a free radical that triggers the next step (Halliwell, 2007). Free radical includes reactive oxygen species (ROS), reactive nitrogen species (NOS), carbon-centered radicals, and sulfur-centered radicals (Miller et al., 1990). Nitric oxide is a free radical by its unpaired electron, which can also produce hydroxyl radicals and nitrogen dioxide radicals. ROS represent the most important class generated in living systems. The generation of ROS is derived from the contradictory roles that oxygen plays in metabolism. Primary ROS generated in humans are hydrogen peroxide (H_2O_2), superoxide radical (O2•−), and hydroxyl radical (OH•). The superoxide radical is generated in the processes involving oxidases such as NADPH oxidase and xanthine oxidase, the auto-oxidation of hemoglobin, and

photolysis. Superoxide is not particularly reactive by itself, but can be catalytically converted by superoxidase dismutases (SOD) to hydrogen peroxide, which decomposes to yield the highly reactive hydroxyl radical in the presence of iron. The alkaloid substance of Quinine tree (*Rauvolfia caffra*) shows antagonist effect and recognized as powerful antioxidant and also potent scavengers of DPPH. Extracts from stem bark samples of *R. caffra* had a free radical Inhibition of 79.65% ± 1.86, while the leaves showed 70.55% ±1.26 DPPH radicals react with antioxidant substance of Quinine as donate electrons become paired off forming the corresponding hydrazine. In the present study Quinine shows its strong DPPH radical scavenging activity in a dose dependant manner.

Total antioxidant effect of Quinine was determined by inhibition of $ABTS^{\bullet+}$ when compared to standard ascorbic acid. Over production hydroxyl radical leads to increase oxidative stress that cause number of degenerative disease, including precancerous and neoplastic lesions of the oral cavity (Kasote 2013 & Giuca et al 2010). Hydrogen peroxide, a weak oxidizing agent, it can cross rapidly inside the cell membranes and damage intracellular system (Nayana et al 2012). Hydroxyl radicals, it has prominent role in the initiation staging of cancer; removal and neutralization of H_2O_2 is very important for the protection of living systems. In our results, Quinine significantly scavenged the hydroxyradical. Hydroxyl radicals (·OH) generated in the Fenton reaction (Fe^{3+}+ ascorbic acid + H2O2) reacted with 2-deoxyribose to give degradation products that were determined by absorbance as TBA-reaction products. In this system, β-carbolines and melatonin inhibited the degradation of 2-deoxyribose by ·OH (Tomas and Juan 2014). Tomas and Juan work shows that β-carbolines (alkaloid family) has the potential scavenging activity which comes to exceed in concert with our findings.

Mammalian cells can synthesis nitric oxide free radical; it has multiple roles the regulation of many physiological processes (Hagerman et al 1998). $NO^{\bullet}$ radical react with O^{-}_2 radical to form peroxynitrite radicals (ONOO-) to generate toxicity or damage the biomolecules such as proteins, lipids and nucleic acids (Kulbacka et al 2009). Previous study reported that Coumarin strongly inhibits $NO^{\bullet}$ to generate nitrite and peroxy nitrite anions [Patel & Patel 2011]. Above study supports our findings that is Quinine significantly inhibits generation of $NO^{\bullet}$ radicals in a dose-dependent manner.

During the pathophysiological process, generate superoxide radicals (O^{-}_2) in which NADPH oxidase play crucial role induction of degenerative disease. (Droge 2002). Previous study reported that phytochemicals significantly scavenge O^{-}_2 radical (Tupe et al 2013). Above confirmation further implies the importance of Quinine preventing physiological harmful caused by superoxide (O^{-}_2) radical. Primaquine administration increased the activities of some antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase), whereas chloroquine increased the activity of only superoxide dismutase while decreasing that of glutathione peroxidase and catalase. (Naik & J. A.Hasler).

Reducing power are generally associated with the presence of free radical scavenger (Chang et al 2007), which has been shown to exert antioxidant activity by scavenging the free radical chain reaction by donating a hydrogen atom (Ferreira et al 2007). Antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radicals scavenging (Bennett et al 2012). According to above mentioned results Quinine might contain a considerable amount of reductants (–OH, and α,β-carbonyl moiety) which may react with the free radicals to stabilize and terminate from free radical chain reaction. Strychnine (Quinine Family) a plant alkaloid in *in vitro* shows antiangiogenesis, apoptosis and antioxidant potential in MCF-7 cancer cells (Saraswati et al 2010). And another Quinine family (Brucine) shows anticarcinogenic effect in diethylnitrosamine initiated and phenobarbital-promoted hepatocarcinogenesis in rats (Saraswati et al 2013) which supports and denotes Quinine as a well suited compound for free radical scavenging.

5.CONCLUSION

In vitro antioxidant activity of Quinine was carried by different methods such as DPPH, $ABTS^{\bullet+}$, hydroxyl, superoxide, hydrogen peroxide, nitric oxide radicals and reducing power scavenging assays. Our result shows that the free radicals scavenging effect of Quinine have displayed strong antioxidant activity compared to that of ascorbic acid. The overall findings of this study concluded that Quinine have vital antioxidant as therapeutic agents in suppressing the progress of oxidative stress mediated degenerative diseases. The present investigation has shown that, though quinine may be one of the most successful and widely prescribed drugs for the management of malaria. It is therefore suggested that the Quinine be prescribed with Cancer studies too.

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