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ORIGINAL ARTICLE

6-Gingerol Prevents Free Transition Metal Ion [Fe (Ii)] Induced Free Radicals Mediated Alterations by *In vitro* and Ndv Growth in Chicken Eggs by *In ovo*

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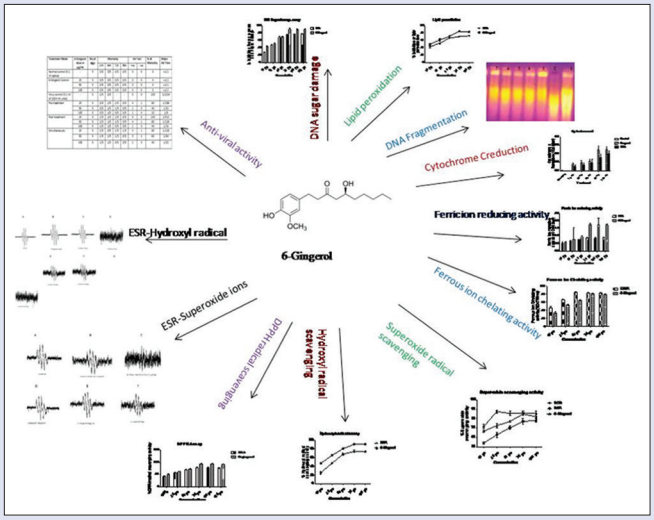
ABSTRACT

Background: The study of free radicals and their reactions is implicated in the pathogenesis of various human diseases. Therefore, antioxidants treatment plays a pivotal role in preventing the free radical-induced diseases. **Objective:** The objectives of this study were to examine the protective role of 6-gingerol from free transition metal ion-induced alterations by *in vitro* and Newcastle disease virus (NDV) in chicken eggs by *in ovo*. **Materials and Methods:** In this study, lipid peroxidation, DNA sugar oxidation, chelation of ferrous ions, DNA fragmentation, Cytochrome c-oxidation, ferric ion reducing activities and DPPH, superoxide and hydroxyl radicals scavenging activity by ESR studies were examined with 6-gingerol by *in vitro*. Furthermore, antiviral activity was screened with 6-gingerol by *in ovo*. **Results:** *In vitro* results showed that 254 µM/ml of 6-gingerol firmly inhibited erythrocyte membrane lipid peroxidation by 85.28% and DNA sugar oxidation by 91.26% when compared with butylated hydroxyanisole 74.42% (554 µM/ml) and 78.30% (824 µM/ml), respectively. Whereas, chelating activity of 6-gingerol was 80.2% which nearly similar to the EDTA activity (50 µg/ml) shown 83.93%. In addition, 6-gingerol (254µM/ml) scavenged the superoxide, hydroxyl, DPPH radicals by 84.96%, 90.45%, and 94.63% respectively and the same was strongly supported with ESR studies. Further, 254 µM/ml of 6-gingerol inhibited the DNA fragmentation, Cytochrome c-oxidation and reducing the ferric ion by in vitro and antiviral activity shown on NDV at 100 µg/ml. **Conclusion:** This study could enlighten that the 6-gingerol has a good metal sequester property and it could prevent the free radicals by *in vitro* and NDV growth in chicken eggs by *in ovo*.

Key words: 6-Gingerol, antioxidants, cytochrome c, DNA fragmentation, lipid peroxidation

SUMMARY

- 6-gingerol prevents free transition metal ion induced alterations
- 6-gingerol protects the DNA from metal ions induced free radicals
- 6-gingerol inhibits the NDV growth in chicken eggs.



Abbreviations used: NDV: Newcastle Disease Virus; ESR: Electron Spin resonance; SOD: Super oxide Dismutase; HA: *Hemagglutination*.

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INTRODUCTION

The study of free radicals and their reactions has been led to increase the interest in the pathogenesis of human diseases. However, free transition metal ions induced free radicals interact the biomolecules and caused the oxidative damage. Thus, they play a major role in the contribution of oxidative stress-related diseases.[1] However, all aerobic animals have antioxidant defense system to counteract the harmful effects induced by free radicals.[2] Sometimes, these protective mechanisms are disrupted by various pathological conditions due to an increase of oxidative stress.[3]

Newcastle disease virus (NDV) causes Newcastle disease in all domestic and wild avian species. Its effect the egg production, hemorrhagic intestinal lesions, nervous disorders and respiratory distress.[4] Consequently, its effect on economic status of food production sectors in all around the world because of high mortality and morbidity was associated with disease.[5] Moreover, viral infections are accompanied with profound changes in cell/tissue metabolism, which lead to generate the reactive oxygen species. In this scenario, antioxidant supplementations were needed to overcome the oxidative stress. Although a number of synthetic

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antioxidants accomplish to be promising, their toxicity and side effects were restricted on their extensive prescription.

The phenolic compounds of plants have gained much attention due to their free radical scavenging and antioxidant activities with potential beneficial implications in human and animals health.[6] When they consumed with food, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life.[7] Many medicinal plants have shown good antimicrobial activity. Several epidemiological studies have also been reported that the consumption of natural antioxidants reduced the risk of a number of chronic diseases.[8,9]

Ginger (*Zingiber officinale*) belongs to *Zingiberaceae* family. Since over 2000 years, ginger rhizome has been used worldwide as a spice and flavoring agent. Ginger has a long history of medicinal use for various diseases including dementia, fever, infectious diseases,[10] arthritis,[11] and diabetes.[12] Ginger rhizome contains biological active constituents including gingerol. Gingerols are found in greater abundance in fresh ginger.[13] Gingerol exhibit anti-inflammatory, antihyperglycemic, and anticancer activity.[14] To the best of our knowledge, there is no report related to ginger volatile compound 6-gingerol effect on free transition metal ion-induced free radical-mediated alterations and antiviral activity against NDV by *in vitro* and *in ovo* conditions. Therefore, we investigated the transition metal ion-induced free radical-mediated alterations and antiviral activity against NDV by 6-gingerol.

MATERIALS AND METHODS

Chemicals

6-Gingerol was purchased from TCI chemicals, China. 5,5'-dimethyl-l-pyrroline-N-oxide (DMPO), xanthine oxidase, cytochrome c, superoxide dismutase (SOD), and calf thymus DNA were purchased from Sigma-Aldrich, USA; remaining all chemicals were analytical grade and purchased from Sigma and HiMedia.

In vitro studies

Erythrocyte membrane lipid peroxidation

Lipid peroxidation was induced in erythrocyte ghost by adopting the method of Dodge *et al*.[15] Lipid peroxidation was measured by the assessment of thiobarbituric acid reactive substances using Dahle *et al*.'s[16] method. Briefly, 6-gingerol was added at various concentrations ranging from 10 to 100 μg/ml to the reaction mixture. Butylated hydroxyanisole (BHA) was used as a positive control. The absorbance was measured calorimetrically at 535 nm. The percent inhibition of lipid peroxidation was calculated by comparing the absorbance of the test samples with negative control.

Fenton reactant-induced DNA sugar damage

Oxidative DNA sugar damage was measured by the following Sultan *et al*.'s[17] method. Briefly, 6-gingerol at various concentrations ranging from 10 to 100 μg/ml was added to the reaction mixture and absorbance was read at 535 nm using a Shimadzu ultraviolet (UV)-1800 Spectrophotometer (Tokyo, Japan). BHA was used as a positive control. The percent inhibition of DNA oxidation was measured by comparing the absorbance of the test sample with a negative control.

Hydroxyl radical-mediated DNA damage

DNA damage was measured on agarose gel by deoxyribose assay.[18] Briefly, 100 μg of calf thymus DNA was added to the 100 μl of reaction mixture and 10 μl of loading buffer was run on 1% agarose with ethidium bromide. The electrophoresis was conducted and DNA was visualized under a UV transilluminator (Bio-Rad, Sydney, Australia). BHA (50 and 100 μg/ml) was used as positive control.

Cytochrome c reduction

The cytochrome c-reducing capacity of 6-gingerol was measured using the method of Suter and Richter.[19] Briefly, 6-gingerol was added (10–100 μg) to the oxidative cytochrome c and absorbance was assessed at 550 nm using a Shimadzu UV-1800 Spectrophotometer. BHA was used as positive control.

Test for ferric ion-reducing capacity (Fe^{3+} to Fe^{2+})

The ferric ion-reducing capacity of 6-gingerol was assessed using the method followed by Wang *et al*.[20] Briefly, 6-gingerol was added at various concentrations ranging from 10 to 100 μg to the reaction mixture. The absorbance was recorded at 700 nm using Shimadzu UV-1800 spectrophotometer. Absorbance increases with an increase in ferric ion-reducing capacity.

Test for ferrous ion-chelating activity

Ferrous ion-chelating activity of 6-gingerol was assessed by the method proposed by Suter and Richter.[19] 6-gingerol at various concentrations ranging from 10 to 100 μg was added to the reaction mixture. The absorbance was measured at 700 nm using Shimadzu UV-1800 Spectrophotometer. Ethylenediaminetetraacetic acid (EDTA) (10–100 μg) was used as positive control. The percent of ferrous ion-chelating activity was measured by comparing the absorbance of the test samples with that of the negative control.

Superoxide-scavenging activity

Superoxide radical (O^{-2})-scavenging activity was measured using the method followed by Lee *et al*.[21] Briefly, 6-gingerol was added at various concentrations ranging from 10 to 100 μg to the reaction mixture. The absorbance was measured calorimetrically at 560 nm. SOD (10–100 μg/3 ml) was served as positive control. The percent superoxide-scavenging activity was determined accordingly by comparing the absorbance of test samples with the negative control.

Hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activity was measured by deoxyribose assay.[18] Briefly, 6-gingerol was added at various concentrations ranging from 10 to 100 μg to the reaction mixture and absorbance was measured at 532 nm. BHA was used as positive control. The percent inhibition of hydroxyl radical-scavenging activity of 6-gingerol was evaluated by comparing with negative control.

1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity was evaluated by the method of Shimada *et al*.[22] Briefly, 6-gingerol was added to the reaction mixture at various concentrations ranging from 10 to 100 μg/ml. The absorbance was read at 517 nm in a Shimadzu UV-1800 Spectrophotometer. BHA at 400 μM was used as positive control. The percent DPPH radical-scavenging activity of 6-gingerol was calculated by decrease in absorbance at 517 nm in comparison with the negative control.

Free radical-scavenging activity of 6-gingerol by electron spin resonance

Free radical-scavenging activity of 6-gingerol was determined by electron spin resonance (ESR) spectrophotometer. In this study, JES-FA200 model of X-band ESR (Japan) was used.

Hydroxyl radical scavenging assay

ESR studies were carried out by the method of Morales *et al*.[23] Briefly, 10 μl of 6-gingerol at various concentrations (10–100 μg/ml) was added to the reaction mixture. Ascorbic acid was used as a reference antioxidant. The ESR spectrum was recorded for every 60 s after the addition of H_2O_2.

Superoxide anion radical scavenging assay

ESR studies were carried out using Morals *et al*.'s[23] method. Briefly, 10 µl of 6-gingerol at various concentrations (10–100 µg/ml) was added to the reaction mixture. SOD was used as a reference antioxidant. The ESR spectrum was recorded for every 120 s after the addition of xanthine oxidase at ambient temperature.

Antiviral activity by *in ovo*

Virus

Komarov mesogenic stain of NDV was obtained from Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India. In our study, 1024 hemagglutination (HA) units of NDV stock was prepared and it was preserved at −40°C until use. Embryonic infective dose (EID_{50}) was evaluated by the method of Reed and Muench[24] and it was found $10^{9.7}$ units/ml in chick embryos.

Embryonated chicken eggs

Embryonated chicken eggs were obtained from the Poultry Department, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India.

Inoculation of eggs

After 9–12 days incubation of embryonated eggs, they were used for NDV infection and this was followed by the treatment with 6-gingerol. Eggs were mainly divided into six groups in which 2nd, 4th, 5th, and 6th groups were again divided each into three subgroups (n = 5) based on the inoculation of 6-gingerol concentrations, 25, 50, and 100 µg/ml, respectively. They were perforated and immediately inoculated by the NDV and 6-gingerol as follows: Group-1 eggs were inoculated with 0.1 ml of phosphate buffered saline, Group-2 eggs were inoculated with 6-gingerol, and Group-3 eggs were treated with virus stock (1024 HA units), Group-4, i.e., pretreated group, where eggs were infected with 0.1 ml of stock virus after 1 h inoculation of 0.1 ml 6-gingerol, Group-5, i.e., posttreatment group, where eggs were treated with 6-gingerol after 1 h inoculation of virus, and Group-6 simultaneously treated group where eggs were treated with 0.1 ml of viral stock along with 6-gingerol. Embryo survival rate was observed daily for 5 days. Allantoic fluid was collected from all the groups for HA test to determine the viral titer in the eggs.

Hemagglutination test

HA test was carried out according to OIE manual (1996) standard diagnostic test. HA test was performed and the observations were recorded. All tests were repeated in triplicates.

Statistical analysis

Data were represented as mean ± standard deviation by Microsoft corporation , Redmond, Washington, United States GraphPad Software, San Diego, California, USA.

RESULTS AND DISCUSSION

Some of the ginger nonvolatile pungent compounds (gingerols, shogaols, and zingerone) have antioxidant, anti-inflammatory, and gastroprotective activity,[25,26] and anticancer activity might be due to it's free radical scavenging activity.[26] The phenolic bioactive compounds could reduce the viral growth may due to their antioxidant activity.[27,28] However, the redox active metals play a major role in the generation of free radicals.[29] In stress condition, excess of superoxide release the iron from iron-containing molecules which generate the highly reactive hydroxyl radicals and make the changes in cell membrane, DNA, and other cellular molecules and which induce the diseases.[30] Therefore, in this study, we investigated the effect of 6-gingerol on free transition metal ion-induced free radical-mediated alterations by *in vitro* and antiviral activity on NDV by *in ovo*.

Inhibition of ghost lipid peroxidation by 6-gingerol

Lipid peroxidation is a complex process occurring in cells and it induces the alterations in the membrane and generates potentially toxic products which are covalently interacting with proteins and DNA[31] bases and damaged. The inhibitory effect of 6-gingerol against ferrous sulfate and ascorbic acid induced ghost lipid peroxidation as shown in Figure 1a. 6-gingerol inhibits the lipid peroxidation at high level about 85.28% at low concentration (75 µg/ml) while BHA showed 73.42% at 100 µg/ml. The levels of lipid peroxidation products may use as biomarkers for the measurement of oxidative stress. Our *in vitro* results suggested that 75 µg/ml concentration of 6-gingerol significantly broke down the chain reaction of lipid peroxidation maybe by donating proton or electrons to the free radicals or chelation of ferrous ions in the Fenton reaction which prevents the generation of free radicals.

Inhibitory effect of 6-gingerol on Fenton reactant-induced DNA sugar damage

The results shown in Figure 1b exhibit a positive relationship between the concentration of 6-gingerol and inhibition of oxidative DNA sugar damage. The maximum inhibition of DNA sugar damage by 91.26% at 75 µg/ml of 6-gingerol was observed when compared with BHA, which showed 78.30% at 125 µg/ml. Our results suggested that 6-gingerol may have the ability to combat against free radical-mediated degradation of DNA sugar moiety by their antioxidant and metal sequester property.[32,33]

Protective effect of 6-gingerol on hydroxyl radical-mediated DNA damage

Increased production of reactive oxygen species concerned to increase the oxidative stress by deterioration of antioxidant mechanism which can lead to proliferate the DNA damage and it has been linked to various pathological disorders.[34] In the present study, calf thymus DNA was subjected to the Fenton reactants for 30 min; as shown in Figure 2a, high mobility of DNA (lane-2) was observed on agarose gel when compared with untreated DNA (lane-1) due to extensive DNA fragmentation by hydroxyl radical oxidation. Inhibition of DNA fragmentation of 6-gingerol was observed at various concentrations ranging from 25 to 100 µg/ml. The maximum inhibition of DNA fragmentation was observed at 75 µg/ml (lane 7) which was higher than the known synthetic antioxidant BHA (100 µg/ml) (lane 4). This effectiveness of the 6-gingerol to prevent oxidative DNA damage was concomitant to its hydroxyl radical-scavenging activity. This suggests that 6-gingerol might have the ability to combat free radical-mediated oxidative damage.

6-Gingerol cytochrome c reduction

In this study, cytochrome c has been used as a model protein. It is an electron transporter in the respiratory chain. 6-gingerol significantly reduced the oxidized cytochrome c in a concentration-dependent manner (25–100 µg/ml). The maximum cytochrome c-reducing capacity of 6-gigenrol was observed at 75 µg/ml which is higher than the known synthetic antioxidant BHA (100 µg/ml) [Figure 2b]. The earlier studies were shown similar results with the ethyl alcohol and water (1:1) extract of curry leaves which contain reducing compounds that reduce ferric-cytochrome c to ferro-cytochrome c.[35] Reduction of cytochrome c by 6-gingerol might also implemented to maintain the cytochrome c redox state which is an important to regulate the apoptosis.

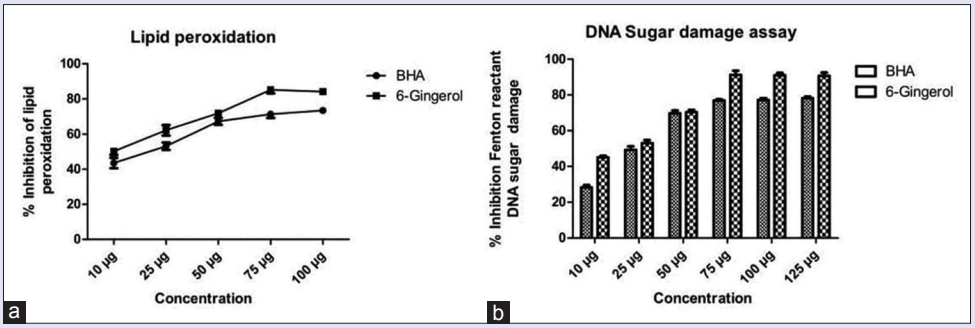

Figure 1: (a) Inhibition of rat red blood cell membrane lipid peroxidation by various concentration of 6-gingerol. (b) Inhibition of DNA sugar damage at various concentrations of 6-gingerol compared with butylated hydroxyanisole. The negative control was without any antioxidant or extract. Results are shown as mean ± standard deviation (*n* = 3)

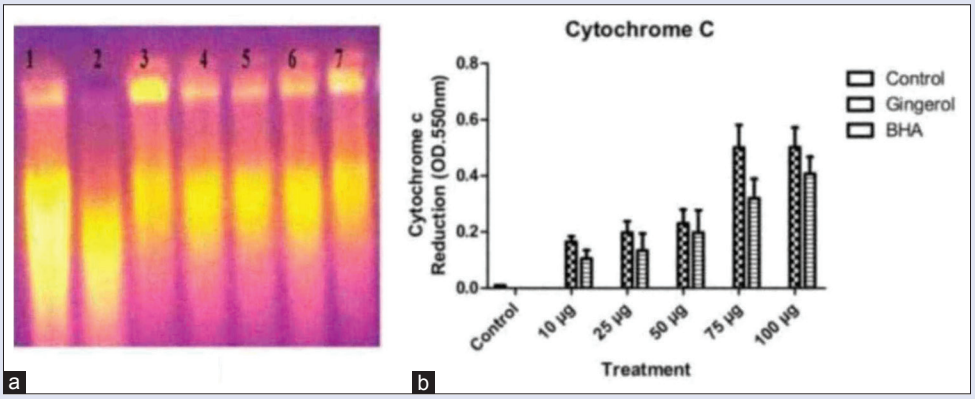

Figure 2: (a) Inhibition of hydroxyl radicals-mediated DNA degradation by 6-gingerol. Lane 1: calf thymus DNA untreated; lane 2: Calf thymus DNA + Fenton reactants lanes 3–7 show the results for calf thymus DNA + Fenton reactants solution + butylated hydroxyanisole (50 µg and 100 µg), 6-gingerol at 25, 50, and 75 µg, respectively. Butylated hydroxyanisole is a standard antioxidant. (b) Cytochrome c reduction capacity of 6-gingerol. The positive control was ascorbic acid. Results are shown as mean ± standard deviation (*n* = 3)

6-Gingerol ferric ion-reducing capacity

It was mainly depended on the substances those have dropping the electrons or hydrogen atoms for reducing the Fe^{+3}/ferricyanide into ferrous form and consequently terminate radical chain reaction. The previous studies have been reported that the extract of spices and herbs showed ferric ion-reducing activity.[35,36] In the present study, as shown in Figure 3a, the maximum reducing capacity of 6-gingerol (1.77 ± 0.10) was observed at lower concentration (75 µg/ml) when compared with BHA at 125 µg/ml concentration (0.912 ± 0.02). The reducing capacity of 6-gingerol might be due to their hydrogen-donating ability.

6-Gingerol ferrous ion-chelating activity

Iron is an essential mineral for normal physiology; when it is excess, it facilitates the formation of free radicals via Fenton reaction. The substances those were chelate the iron and removal of free iron from circulation could have valuable antioxidant capability. This can be a promising approach to prevent oxidative stress-induced diseases. Iron pro-oxidant property was last when it is chelated. Hence, we examined the chelation of Fe^{+2} by 6-gingerol in a competitive way with potassium ferricyanide. Interestingly, as shown in Figure 3b, maximum (80.2%) Fe^{+2} binding capability of 6-gingerol was found at 75 µg/ml as evidenced by the low absorption at 700 nm and its chelating effect is comparable with EDTA (50 µg/ml) which showed a chelating effect of 83.93%.

6-Gingerol superoxide radical-scavenging activity

The aerobic animals have able to suppressed superoxide anions by their antioxidant defense system when they are produced in certain limits. However, in excess, they cause cell damage.[21] In the present *in vitro* study, superoxide anions were generated via nitroblue tetrazolium (NBT) assay system. The results in Figure 4a showed superoxide anion-scavenging activity of 6-gingerol at various concentrations ranging from 10 to 100 µg/ml. The maximum superoxide anion-scavenging activity of 6-gingerol (84.96%) was observed at 75 µg/ml while it was 78.27% at 100 µg/ml of BHA. SOD was used as a positive control that showed 87.23% at 25 µg/ml concentration. The active inhibition of the NBT reduction by 6-gingerol suggests that 6-gingerol is a potential scavenger of superoxide anion. The previous studies have also been supported to our results; the ginger significantly scavenges the superoxide anions and acts as a dietary antioxidant *in vivo*.[37]

6-Gingerol hydroxyl radical-scavenging activity

Hydroxyl radicals are highly reactive substances and are easily to initiate the cell damage *in vivo* when compared with other reduced forms of dioxygen.[38] However, from our results [Figure 4b], 6-gingerol shown maximum hydroxyl radical-scavenging activity by 90.45% at 75 µg/ml which was higher than a well-known synthetic antioxidant BHA (77.25%) at 100 µg/ml. This implies that 6-gingerol may able to reduce the Fe^{+3}–EDTA reactions which was initiated the OH radicals using H_2O_2.

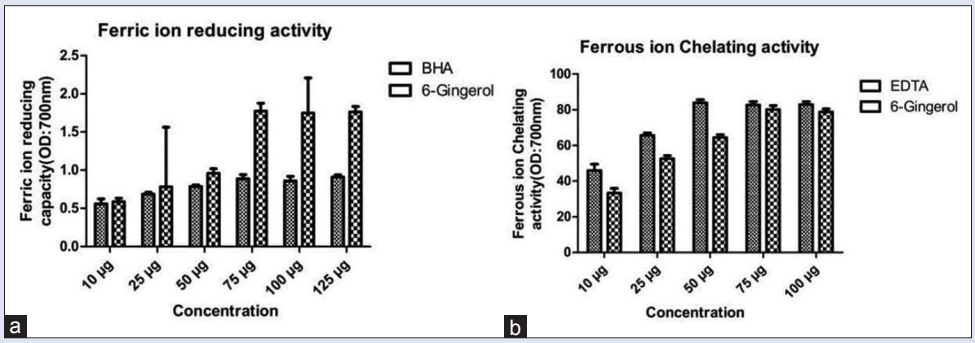

Figure 3: (a) Dose-dependent ferric ion-reducing capacity of 6-gingerol. Results are shown as mean ± standard deviation (n = 3). The positive control was butylated hydroxyanisole. (b) Dose-dependent ferrous iron-chelating activity of 6-gingerol. The negative control was without any antioxidant or extract. Results are shown as mean ± standard deviation (n = 3). Positive control was ethylenediaminetetraacetic acid

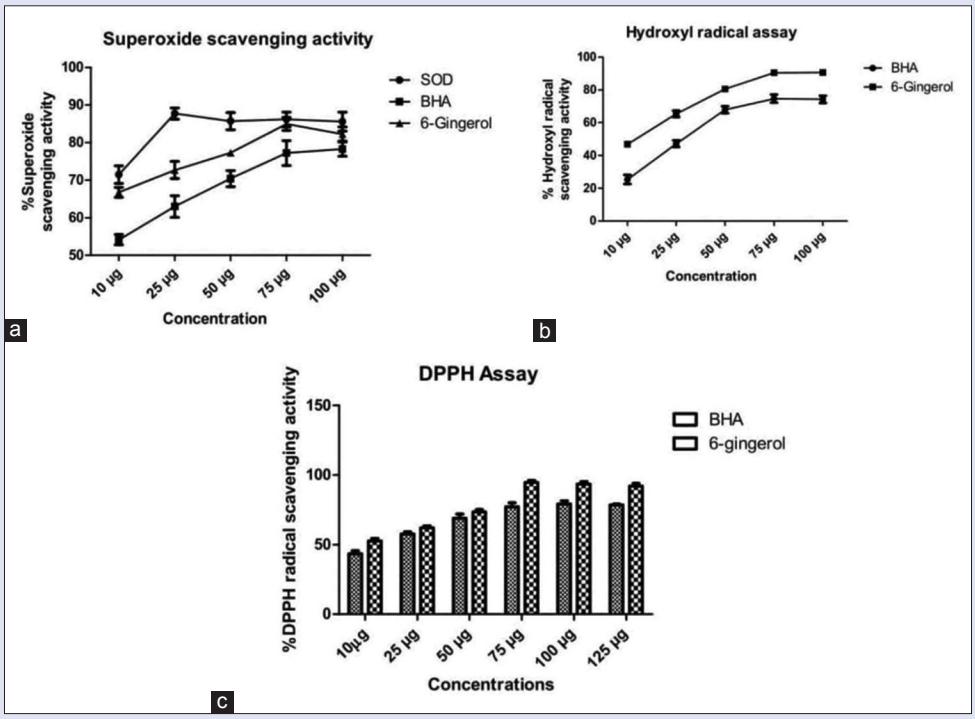

Figure 4: (a) The superoxide-scavenging activity of 6-gingerol. The negative control was without any antioxidant or extract. Positive control superoxide dismutase. (b) Hydroxyl radical-scavenging activity of various concentration of 6-gingerol in comparison with butylated hydroxyanisole. The negative control was without any antioxidant or extract. (c) Inhibition of 1,1-diphenyl-2-picrylhydrazyl radicals at various concentrations of 6-gingerol in comparison with butylated hydroxyanisole. Results are shown as mean ± standard deviation (n = 3)

The earlier studies have demonstrated that phenolic compounds showed high antioxidant activity by donating electrons to the free radicals and suppress their activity.[35]

6-Gingerol 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity

DPPH analysis is one of the tests used to prove the ability of 6-gingerol to act as donor of hydrogen atoms or electrons. The obtained results shown in Figure 4c explain 6-gingerol exhibited a significant effect on inhibition of DPPH radicals in dose-dependent manner. The maximum activity of 6-gingerol (94.66%) was observed at 75 µg/ml and it was higher than the known synthetic antioxidant BHA (79.19%) at 100 µg/ml. The results indicated that 6-gingerol is a powerful free radical scavenger compared with known antioxidant. The hydrogen-donating abilities of the phenolic compounds of the ginger are thought to be responsible for the inhibition of DPPH-free radical.[39]

6-Gingerol hydroxyl radical-scavenging activity by electron spin resonance

The ESR spectrum of DMPO-OH adducts showed a typical 1:2:2:1 quarted line. The ESR signal is proportional to the concentration of spin adducts in the solutions. The ESR signal significantly diminished with increase of 6-gingerol concentrations and it was higher at 75 µg/ml concentration [Figure 5a-g] when compared with other concentrations. Consequently, this activity was similar to the reference antioxidant ascorbic acid (75 µg/ml). This activity shown by gingerol may be due to (a) suppression of hydroxyl radicals formation by 6-gingerol in the Fenton reaction, (b) donation of proton or electron to radical spin

Table 1: Antiviral activity of 6-gingerol at various concentration

Treatment Mode	6-Gingerol dose in µg/ml	No. of eggs	Mortality				HA Test		% of mortality	Mean Ab Titre
			24 h	48 h	72 h	96 h	+ve	-ve		
Normal control (0.1 ml saline)	-	5	0/5	0/5	0/5	0/5	0	5	0	≤1/2
6-Gingerol Control	25	5	0/5	0/5	0/5	0/5	0	5	0	≤1/2
	50	5	0/5	0/5	0/5	0/5	0	5	0	≤1/2
	100	5	0/5	0/5	0/5	0/5	0	5	0	≤1/2
Virus control (0.1 ml of 1024 HA units)	-	5	2/5	3/5	-	-	5	0	100	1/1024
Pre treatment	25	5	0/5	2/5	2/5	0/5	4	1	80	1/256
	50	5	0/5	1/5	1/5	0/5	2	3	40	1/32
	100	5	0/5	1/5	0/5	0/5	1	4	20	1/8
Post treatment	25	5	2/5	1/5	2/5	0/5	5	0	100	1/512
	50	5	2/5	1/5	1/5	0/5	4	1	80	1/128
	100	5	1/5	1/5	0/5	0/5	2	3	40	1/32
Simultaneously	25	5	2/5	0/5	1/5	1/5	4	1	80	1/128
	50	5	1/5	1/5	1/5	0/5	3	2	60	1/64
	100	5	1/5	1/5	0/5	0/5	2	3	40	1/32

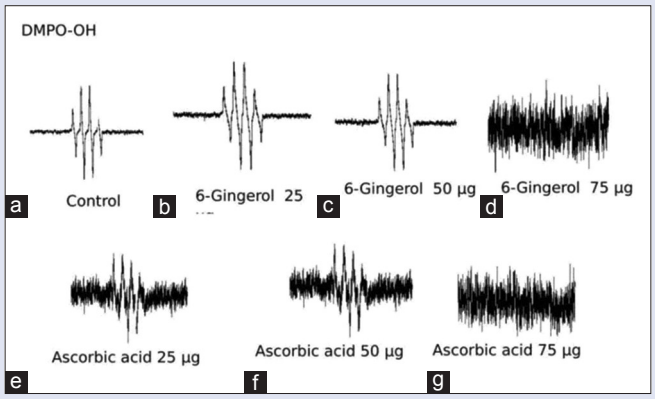

Figure 5: Quenching effect of various concentrations of 6-gingerol using 5,5′-dimethyl-l-pyrroline-N-oxide to trap the OH radical. Examples of electron paramagnetic resonance spectra signal ranging from 326–346 mT: (a) Control; (b-g) Effects of concentrations ranging from 10 to 75 µg/ml. The positive control was ascorbic acid

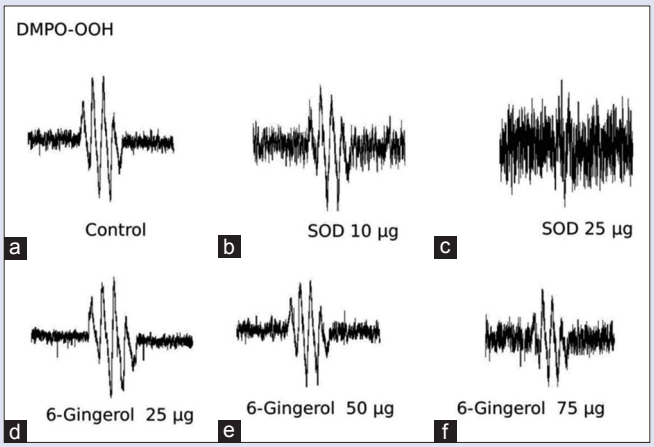

Figure 6: Quenching effect of various concentrations of 6-gingerol using 5,5′-dimethyl-l-pyrroline-N-oxide to trap the O2 radical. Examples of electron paramagnetic resonance spectra signal ranging from 326 to 346 mT: (a) = control; (b-f) Effects of concentrations ranging from 10 to 75 µg/ml. The positive control was superoxide dismutase

adduct, and (c) competition between 6-gingerol and the spin trap agent for the hydroxyl radical.[40,41]

Superoxide anion radical-scavenging activity of 6-gingerol by electron spin resonance

The results obtained are as shown in Figure 6a-f, the intensity of the signals virtually abolished with increasing of 6-gingerol concentrations from 10 to 100 µg/ml, and it was higher at 75 µg/ml of 6-gingerol concentration when compared with other concentrations. However, this activity was similar with the antioxidant enzyme SOD at 25 µg/ml concentration.

In this study, ESR signals of DMPO-OOH were gradually decreased when increased the concentration of 6-gingerol, it may be due to (a) the fact that 6-gingerol inhibits the xanthine oxidase activity and (b) transformation of superoxide anion to oxygen.[42,43]

Inhibition of Newcastle disease virus growth in chicken eggs by 6-gingerol

NDV has a single-stranded negative probing filamentous RNA.[44] At present, there is no specific treatment for NDV, and it was only controlled by the vaccination. Recently, there has been a growing interest in plants as natural sources for the treatment of viral diseases as therapeutics.

The results from our *in ovo* study, as shown in Table 1, showed that normal control and 6-gingerol alone treated embryonic eggs were not shown any agglutination in HA test and mortality whereas untreated NDV-infected eggs have shown agglutination in HA test (1/1024 virus titer) and all infected eggs died within 48 h. Six-gingerol pretreated viral infected eggs shown 80%, 40%, and 20% mortality rate at concentrations of 25, 50, and 100 µg/ml of 6-gingerol, respectively, whereas in HA test, their viral titer values 1/256, 1/32, and 1/8, respectively. Six gingerol posttreated group eggs showed 100%, 80% and 40% mortality rate at various concentrations of 25, 50, and 100 µg/ml 6-gingerol and their viral titer values were 1/512, 1/128, and 1/32, respectively. Virus and 6-gingerol (1:1 v/v) simultaneously treated group showed 80%, 60%, and 40% mortality rate at various concentrations of 25, 50, and 100 µg/ml of 6-gingerol and their titer values were 1/128, 1/64, and 1/32, respectively.

Several studies made a comparative evaluation of the antioxidant and the antiviral activities of plant extracts and naturals substances. The higher amount of phenolic compounds particularly flavonoids and tannins are known to possess good antiviral activities.[27,28] In our *in ovo* study, 6-gingerol pretreatment significantly exerts antiviral activity in a dose-dependent manner which indicates that 6-gingerol inhibited the viral growth. Post and simultaneously 6-gingerol treated NDV-infected

eggs have shown low titer value in HA test and mortality when compared with NDV control. This could be suggested that 6-gingerol has ability to prevent the NDV growth at some extent. The antiviral effect of 6-gingerol on NDV might be due to killing the virus and/or interfering with viral multiplication[45] or may it exhibit protease inhibition, and hence interferes with cleavage of hemagglutinin neuraminidase and fusion protein, which are important to glycoproteins for NDV attachment and multiplication or virucidal effect.[46,47]

CONCLUSION

Our research results clearly demonstrated that 6-gingerol has shown a good metal sequesters property by recuperation of free transition metal ion-induced free radical alterations by *in vitro* and it also inhibits the NDV growth in embryonic chicken eggs by *in vivo*. Thus, this study provides a basis for developing a valuable food additive to protect from the free radical-induced alterations and NDV in chickens

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Conflicts of interest

There are no conflicts of interest.

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