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# Protective effect of ferulic acid on erythrocytes against experimental oxidative stress models

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## Abstract

Various pathological conditions in thebodyare observed as a result of cellular damage. Damage to erythrocytes makes them less capable of carrying oxygen. Some therapeutic agents are known to cause lysis of erythrocytes. In the present study, ferulic acid was used to assess protective effects on erythrocytes against various hemolytic insults. Experimental models viz. hemoglobin- induced linoleic acid peroxidation, H2O2 induced hemolysis and CuOOH-induced hemolysis were used to study theprotective effect of ferulic acid. Ferulic acid demonstrated concentration-dependent inhibition in the experimental models. In all the three cases, maximum inhibition of hemolysis was observed at 250 µg/ml of ferulic acid. The study establishes ferulic acid as apotential antihemolytic agent.

**Keywords:** erythrocytes, oxidative stress, antioxidant, ferulic acid

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## 1. Introduction

Cellular damage is thekey origin for numerous 'physiological' and 'pathological' conditions. [1]Red blood corpuscles are often used as a suitable model for such studies. Oxidative stress in erythrocytes alters in particular 'membrane', 'hemoglobin' and 'cellular structure'. [2] Hemoglobin seems to be the 'key spot' and 'easy target' to be deteriorated by drugs. [3]

Oxidative changes in hemoglobin bring about disruption in its structure leading to its denaturation. [4]This process commences with increment in the accumulation of 'oxidative agents in erythrocytes. Mass of 'denatured hemoglobin' as a result of oxidative stress produces 'Heinz bodies' [5, 6]. Development of 'Heinz bodies' is closely related to prognosis of 'hemolytic anemia'. [7-9]Some chemicals and pharmaceuticals are known to cause theformation of Heinz bodies leading to thedestruction of erythrocytes and hemolytic anemia. [10-13]

Polyphenols are amongst an important class ofplant-derived chemicals extensively found in plants. Maize is the richest source of it. [14-16]Attendance of phenolic nucleus together with conjugation of side chain leads to the development of 'stabilized phenoxy radical' which leads to 'antioxidant activity'. It contributes to the perpetuation of the crucial biological function of the cell.[17] By the virtue of antioxidant activity, ferulic acid has demonstrated hepatoprotective, radioprotective, pulmonary protective, neuroprotective, and antiatherogenic effects.[5-9] Apart from this it has also shown anticancer [18], anti-apoptotic [19] and anti-aging[20] effects. However, there is no report about the antihemolytic effect of ferulic acid. The present work was aimed to determine the antihemolytic effect of ferulic acid.

## 2. Materials and methods

### 2.1 Chemicals

Ferulic acid, linoleic acid, ascorbic acid and hydrogen peroxide were purchased from Central Drug House, India. All other chemicals were of analytical grade. Double distilled water was used in the present study.

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### 2.2 Blood collection

Two milliliters of blood from healthy goat were freshly collected into $K_3$ *EDTA tubes*. Blood sample was stored at 4°C for 24 h before use. An aliquot of 1.0 ml of blood transferred into 1.5 ml micro-centrifuge tubes and was centrifuged at 2500 rpm for 5 min, and the supernatant was removed. The cell suspension was washed with asterile saline solution (0.89% w/v NaCl) and centrifuged at 2500 rpm for 5 min. This was repeated three times till the supernatant was clear and colorless, and the packed cell volume (PCV) was measured. The cellular component was reconstituted to a 40% suspension (v/v) with phosphate buffered saline (10 mM, pH 7.4) and was used in the assays.

### 2.3 Experimental

Antioxidant activity in hemoglobin- induced linoleic acid peroxidation test

Antioxidant activity of the ferulic acidwas determined by a reported method. Briefly, reaction mixtures (200 µl) containing 40 µl of ferulic acid (50-250 mg/ml in ethanol), 40 µl of linoleic acid emulsion (1 mmol/l), 70 µl of phosphate buffer (pH 6.5), and 50 µl hemoglobin (0.0016%), were incubated at 37°C for 45 min. After the incubation, 2.5 ml of 0.6% HCl in ethanol was added to stop the peroxidation oflipid. The amount of peroxide in the test solution was measured in triplicate using the thiocyanate method by reading the absorbance at 480 nm after coloring with 100 ml of 0.02 mol/ l of $FeCl_2$ and 50 ml of ammonium thiocyanate (300 mg/ml). [21]Vitamin C was used as a positive control.

### 2.4 Antihemolytic activity of ferulic acid against $H_2O_2$ induced hemolysis

The inhibition of goat erythrocyte hemolysis by extracts was evaluated as previously described. Hemolysis was induced using $H_2O_2$ as free radical initiator. To 100 µl of a 5% (v/v) suspension of washed erythrocytes in phosphate buffered saline (PBS), aliquots of 50 µl containing different amounts of ferulic acid (50-250 µg/ml in PBS pH 7.4), was added. To this, 100 µl of 100 µM $H_2O_2$ (in PBS pH 7.4) was added. The reaction mixtures were shaken gently during incubation at 37°C for three h. The reaction mixtures were diluted with 8 ml of PBS and centrifuged at 2000 × g for 10 min. The absorbance of the resulting supernatant was measured at 540 nm by spectrophotometer to determine the extent of hemolysis. Likewise, the erythrocytes were treated with 100 µM of $H_2O_2$ without plant extract to obtain complete hemolysis. The absorbance of the supernatants was measured under the same conditions. The inhibitory effect of the extract was compared with that of the standard antioxidant vitamin C. To evaluate the hemolysis induced by the extract; erythrocytes were pre- incubated with 50 µl of extract for one h and the hemolysis was determined. [22] Percentage of hemolysis was calculated by taking hemolysis caused by 100 µM $H_2O_2$ as 100%.

### 2.5 Antihemolytic activity of ferulic acid against CuOOH-induced hemolysis

To 1% RBC suspension, aliquots (3.5 ml) were incubated at 37°C for 21 0 min in the presence of 50 µM CuOOH (dissolved in ethanol) and the cellular integrity determined turbidimetrically at 710 nm at 30 min intervals. The samples (dissolved in ethanol; final concentrations 0.5 µg ml-1) were pre-incubatedfor 30 min with RBC before the addition of CuOOH [blanks were RBC added with ethanol, at a final concentration always less than 0.1% (v/v)].[23] Percentages hemolysis was determined to set as a 100% hemolysis the absorbance value determined in RBC suspensions sonicated for 5 seconds (mean values of 3 determinations were used for the calculation).

### 2.6 Statistical analysis

The results are expressed as a mean±standard error of themean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (*$P$<0.05).

## 3. Results

### 3.1 Antioxidant activity in hemoglobin-induced linoleic acid peroxidation test

Unsaturated fatty acids are abundantly found in membrane lipids that are sensitive to oxidation out of whichlinoleic and arachidonic acid are essential.[24] The inhibition of peroxidation of lipids by antioxidants could be due to their ability to scavenge free radicals. Antioxidant activity of ferulic acid was observed in a dose-dependent manner by thiocyanate method, as evident from figure 1 (Group

I 22.30±0.98 %, p<0.05; Group II 32.53±1.12 %, p<0.05; Group III 43.26±1.23 %, p<0.05; Group IV 52.21±1.24 %, p<0.05; Group V 61.52±1.26 %, p<0.05). Ferulic acid displayed a comparable antioxidant activity to the reference standards by protecting theintegrity of red blood corpuscles.

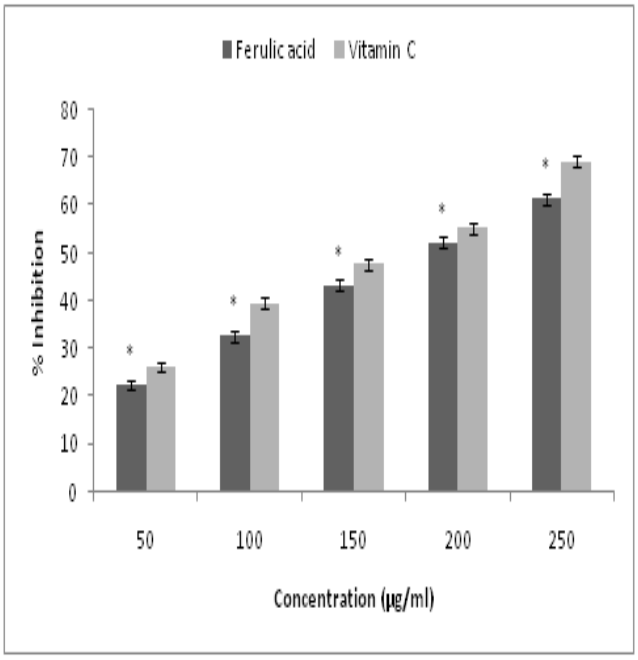



**Figure 1.** Protective effect of ferulic acid on hemoglobin-induced linoleic acid peroxidation test

The results are expressed as mean±standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (*P<0.05).

## 3.2 Antihemolytic activity of theextract against $H_2O_2$ induced hemolysis

Erythrocytes are regarded as achieftarget for free radical attack because of the attendance of high concentration of polyunsaturated fatty acids and the $O_2$ transport allied with redox active hemoglobin molecules, which are powerfuladvocates of ROS.[25, 26]Inhibition of lipid peroxidation by antioxidants could be due to their ability to scavengefree radical. In this study, ferulic acid caused concentration-dependent inhibition of hemolysis as compared to ascorbic acid (Group I 62.31±0.96 %, p<0.05; Group II 55.14±1.23 %, p<0.05; Group III 46.96±1. 43 %, p<0.05; Group IV 32.42±1.31 %, p<0.05; Group V 19.24±1.41 %, p<0.05; Figure 2).

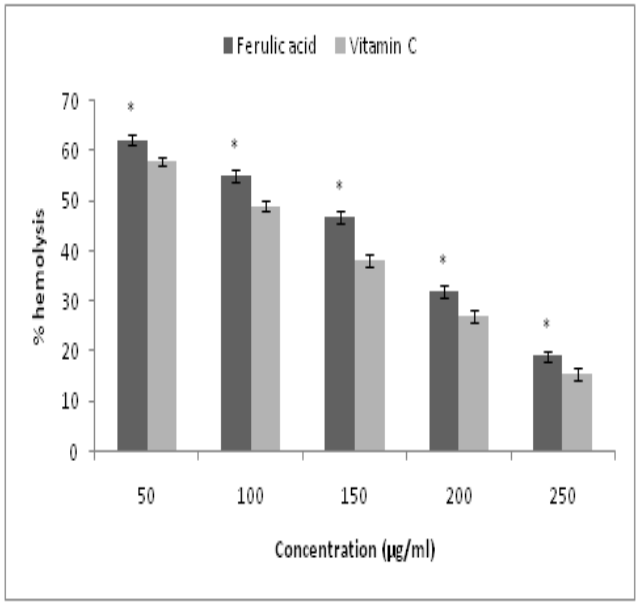



**Figure 2.** Antihemolytic activity of theextract against H2O2 induced hemolysis

The results are expressed as mean±standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (*P<0.05).

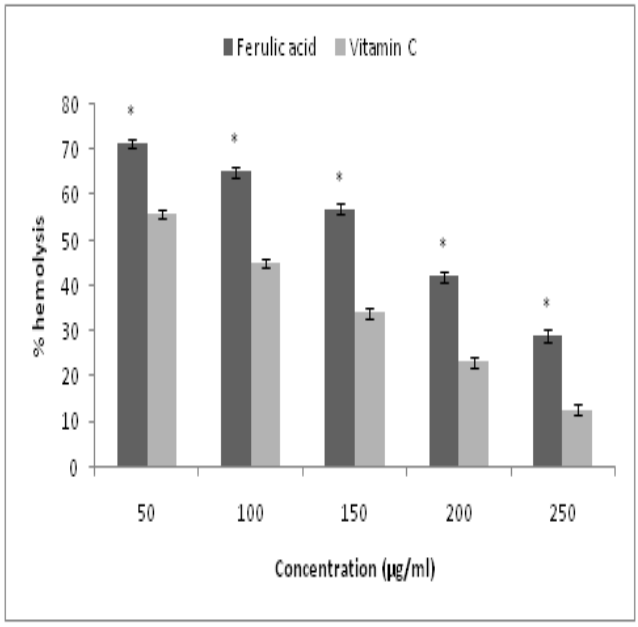



**Figure 3.** Antihemolytic activity of theextract against CuOOH-induced hemolysis

The results are expressed as mean±standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (*P<0.05).

### 3.3 CuOOH-Induced Hemolysis

The antioxidant activity of the ferulicwas established in goat erythrocytes (RBC) exposed to CuOOH.In this study, erythrocyte membrane resistance to free radical-induced hemolysis was determined. Incubation of RBCs with ferulic acid (250 µg/ml) caused asignificant reduction in hemolysis as compared to other concentrations (Group I 71.37±0.87 %, $p<0.05$; Group II 65.23±1.41 %, $p<0.05$; Group III 56.91±1. 55 %, $p<0.05$; Group IV 42.37±1.42 %, $p<0.05$; Group V 29.14±1.55 %, $p<0.05$; Figure 3).

## 4. Discussion

Free radicals are considered to be the 'by-products' generated as a result of various biochemical reactions prevailing in the body. Free radicals are thought to be the key reason for prognosis of different diseases. Various biological macromolecules like DNA, RNA, lipoproteins, proteins, lipids are harm deteriorated due to ill effects of free radicals. They are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseasesare well documented. The ROS easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA.[27]This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also in the ageing process. Based on the growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is supported. Epidemiological studies have found that the intake of antioxidants, such as Vitamin C, reduce the risk of coronary heart disease and cancer.[28] A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases. [29]

Plant-derived polyphenols are one of the important classes of therapeutic agents, which are also known for immuno-modulatory effects. [30-32]Ferulic acid, a phenolic compoundhasnoticeable antioxidant effects. [33]. The present work was undertaken to evaluate thebeneficial effect of ferulic on oxidative stress induced by various agents. Various experimental strategies were followed to extend stress in erythrocytes. In hemoglobin-induced linoleic acid peroxidation test, maximum inhibition (61.52 ± 1.26) was observed at 250 µg/ml. Similar results were seen in H2O2 induced hemolysis andCuOOH-induced hemolysis.

## 5. Conclusion

The hemo-preventive property of ferulic acid might be useful when a drug with hemolytic potential is co-administered. Thus, in brief, it can be concluded that ferulic acid effectively inhibited hemolysis of erythrocytes in variousexperimental models. Further systematic studies in animal models will provide an insight towards themechanism of action.

## 6. Conflict of Interest

None Declared

## 7. References


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