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Original Article
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Protective Effects of Auraptene against Free Radical-Induced Erythrocytes Damage

Khadijeh Jamialahmadi[1,2], Amir Hossein Amiri[3], Fatemeh Zahedipour[2,4], Fahimeh Faraji[5], Gholamreza Karimi[6,7]*

[1]Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Cancer Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[4]Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[5]School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[6]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[7]Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

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***Corresponding Author**
Gholamreza Karimi
Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad 9177948954, Iran
Tel: +98-513-180-1191
E-mail: karimig@mums.ac.ir

Objectives: Auraptene is the most abundant natural prenyloxycoumarin. Recent studies have shown that it has multiple biological and therapeutic properties, including antioxidant properties. Erythrocytes are constantly subjected to oxidative damage that can affect proteins and lipids within the erythrocyte membrane and lead to some hemoglobinopathies. Due to the lack of sufficient information about the antioxidant effects of auraptene on erythrocytes, this study intended to evaluate the potential of this compound in protecting radical-induced erythrocytes damages.
Methods: The antioxidant activity of auraptene was measured based on DPPH and FRAP assays. Notably, oxidative hemolysis of human erythrocytes was used as a model to study the ability of auraptene to protect biological membranes from free radical-induced damage. Also, the effects of auraptene in different concentrations (25-400 μM) on AAPH-induced lipid/protein peroxidation, glutathione (GSH) content and morphological changes of erythrocytes were determined.
Results: Oxidative hemolysis and lipid/protein peroxidation of erythrocytes were significantly suppressed by auraptene in a time and concentration-dependent manner. Auraptene prevented the depletion of the cytosolic antioxidant GSH in erythrocytes. Furthermore, it inhibited lipid and protein peroxidation in a time and concentration-dependent manner. Likewise, FESEM results demonstrated that auraptene reduced AAPH-induced morphological changes in erythrocytes.
Conclusion: Auraptene efficiently protects human erythrocytes against free radicals. Therefore, it can be a potent candidate for treating oxidative stress-related diseases.

Keywords: auraptene, hemolysis, aaph, peroxidation, oxidative stress

INTRODUCTION

Reactive oxygen species (ROS) have significant roles in the pathogenesis of some disorders, such as atherosclerosis, arthritis, cancer, and neurological disorders. Notably, it is hypothesized that free radicals induce tissue and cellular damage through the oxidation of unsaturated fatty acids in the cell membranes [1].

Erythrocytes are especially sensitive to oxidative damage because of their continuous proximity to oxygen. An enhanced level of plasma-free hemoglobin (pfHb) is an important indicator of hemolysis. Additionally, one of the most important reasons for the lysis of red blood cells (RBCs) is oxidative stress. Regularly in RBCs, oxyhemoglobin is transformed into methe-

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moglobin. Consequently, this conversion produces superoxide radicals. Furthermore, under abnormal conditions, these radicals react with polyunsaturated fatty acid on the erythrocytes membranes, and the process of hemolysis is proceeded [2].

Antioxidants play an important role in protecting human cells against oxidative damage by direct radical scavenging and/or augmenting cellular antioxidant defense [3]. Therefore, it is imperative to search for natural antioxidants and their protective mechanism for limiting the adverse effects of oxidative stress in different diseases. Auraptene is a well-known natural compound belonging to coumarins [4]. Auraptene has been found to have valuable properties, such as anti-inflammatory, anti-tumor, anti-microbial, anti-coagulant, anti-genotoxic, neuroprotective, and antidiabetic properties [5]. Furthermore, it has been found to have various antioxidant activities, such as its protective effect on mitochondrial oxidative damage and scavenging against ROS [6, 7].

Although the antioxidant activity of auraptene has been previously reported, there is little evidence for the antioxidant activity of this compound using cell-based assays. Therefore, we investigated the protective effects of auraptene on human erythrocytes under oxidative stress conditions using AAPH as a peroxyl radical generator [1].

MATERIALS AND METHODS

1. Chemicals

Auraptene was purchased from Golexir Pars Company (Mashhad, Iran). 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), Glutathione (GSH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), malondialdehyde tetrabutylammonium salt (MDA), 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), and guanidine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Additionally, 2-thiobarbituric acid (TBA), 2,4-dinitrophenylhydrazine (DNPH), and trichloroacetic acid were purchased from Merck (Germany).

2. Determination of antioxidant activity

1) Ferric reducing antioxidant power (FRAP)

FRAP assay is an oxidoreductive reaction to evaluate the components' antioxidant capacity described by Benzie and Strain [8]. Briefly, 3 mL of FRAP reagent was added to 100 μL of various concentrations of auraptene solution (25, 50, 100, 200, and 400 μM). The reaction mixture was incubated in the dark at 37℃ for 6 min, and then the absorbance was measured at 593 nm using a UV-Vis spectrophotometer. Ascorbic acid (Vit C, 100 μg/mL) was used as the positive control, and the experiment was conducted in triplicates. Furthermore, the FRAP value was reported using the following equation:

$$\text{FRAP value (\%)} = (1 - \frac{Ac - Ae}{Ac}) \times 100$$

Where Ac is the absorbance of the control, Ae is the absorbance of the sample.

2) DPPH free radical scavenging activity

The ability of auraptene to scavenge the DPPH radical was estimated using the method described by Braca et al. [9] with slight modifications. In brief, fresh DPPH (250 μL, 0.1 mM in methanol) solution was added to 250 μL of auraptene at different concentrations (25, 50, 100, 200, and 400 μM). The reaction mixtures were then shaken immediately and incubated for 30 min at room temperature in the dark, and the absorbance was measured at 517 nm against a blank control. Notably, the blank solution was prepared using the same amount of methanol and DPPH. Additionally, ascorbic acid (100 μg/mL) was used as the positive control, and all tests were run in triplicate. Inhibition of DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Inhibition of DPPH radicals (\%)} = (\frac{A0 - AT}{A0}) \times 100$$

Where A0 is the absorbance of negative control and AT is the absorbance of the sample.

3) Preparation of erythrocyte suspension

Fresh blood (5-10 mL) from healthy, non-smoking volunteers who were 20-30 years old and not taking any medication was used after providing their informed consent. Blood samples were collected in heparinized tubes and immediately used to prepare erythrocyte suspensions. The erythrocytes were separated by centrifugation at 3,000 rpm for 10 min at 4℃ in a refrigerated centrifuge. Phosphate-buffered saline (PBS, pH 7.4) was used to wash the erythrocyte pellets three times. Finally, erythrocytes were resuspended in the same buffer to give the 10% hematocrit level and stored at 4℃ [10].

The protocol of this study was approved by the Medical Ethics Committee of the Mashhad University of Medical Science

(Mashhad, Iran).

4) Preparation of erythrocyte ghost membranes

Fresh human blood samples were collected in heparinized tubes and centrifuged at 2,500 rpm for 20 min at 4℃. The supernatant was carefully removed, and the pellet was washed twice using PBS under the same conditions. For RBC ghost preparation, the isolated washed erythrocytes were hemolyzed by adding 40 mL of cold hypotonic PBS to 1 mL of cells and incubated overnight at room temperature. The following day, the samples were centrifuged at 12,000 rpm for 20 min at 4℃, and the supernatant was discarded. To remove the hemoglobin, the pellet was washed 2-3 times with PBS at the same condition until its red color was removed entirely. The RBC ghosts were then resuspended in PBS, and the amount of protein in the sample was measured by the Bradford method. The erythrocyte solutions were kept at −20℃ until further assessments [11].

5) Hemolysis assay

To induce oxidative hemolysis of erythrocytes, proxy radicals generated by the thermal decomposition of AAPH in oxygen were used [12]. RBC suspensions (10% hematocrit) were incubated with different concentrations of auraptene (25, 50, 100, 200, and 400 μM) at 37℃ for 10 min. Subsequently, the reaction mixtures were treated with and without 25 mM AAPH in PBS (pH 7.4) and incubated in a shaker incubator at 37℃ for 6 h. At the indicated time points, 100 μL of the reaction mixture was removed, diluted with 900 μL of PBS, and centrifuged at 3,000 rpm for 5 min. The absorbance of the supernatant was determined at 540 nm. Vitamin C (100 μg/mL) was used as the positive control. Additionally, reference values were determined using the same volume of RBC suspension in a hypotonic phosphate buffer (pH 7.4: complete hemolysis). Furthermore, the hemolysis percentage was calculated as the sample/absorbance of the reference value × 100.

6) Measurement of lipid peroxidation of erythrocytes

Malondialdehyde (MDA), an end product of lipid peroxidation, was determined in erythrocyte membranes induced by 25 mM AAPH using a modified TBA method [13]. The reaction mixture (0.5 mL) containing 10% RBC suspension was added to 1 mL of TBA reagent containing (0.375% (w/v) TBA, 15% (w/v) TCA, 0.25 M HCl) and incubated at 95℃ for 1 h. the absorbance was read at 532 nm following cooling and centrifugation at 1,000 g for 10 min. A calibration curve of MDA (5-50 μM nmol/mL) was applied to quantify the extent of MDA in erythrocyte membranes and expressed as nmol/mg hemoglobin (Hb). This method used vitamin C (Vit C) as the positive control. Notably, the colorimetric method was used to determine the hemoglobin content of the lysate. Furthermore, the concentration of total Hb was determined using Drabkin and Austin's [14] method.

7) Determination of glutathione content in erythrocytes

The amount of GSH in erythrocytes was measured using DTNB according to the Yang et al. [15] method. A 10% suspension of RBCs was prepared and added to the tubes. To prepare the simultaneous reactions, various concentrations of auraptene with AAPH were added to them. To perform the delayed reactions, various concentrations of auraptene were added 2 h after the initiation of the reaction. At the end of each hour, 0.5 mL of samples were mixed with cold hypotonic PBS to lyses the RBCs. To precipitate the proteins, 0.5 mL metaphosphoric acid solution (1.67 g metaphosphoric acid, 0.2 g EDTA, and 30 g NaCl in 100 mL water) was added to 0.5 mL lysate and vortexed. After 5 min, it was centrifuged at 18,000 g for 10 min at 4℃ to separate the precipitated protein. Then, 0.45 mL of supernatant was mixed with Na_2HPO_4 (300 mM), and the samples' absorbance was measured at 412 nm versus a blank (0.45 mL solution plus 0.45 mL water). 100 μL of DTNB was added to all samples and the blank, and the absorbance of samples was measured against a blank at 412 nm. The GSH values were reported as μmol/gHb.

A standard curve was prepared using 20-100 μM of the GSH solution to measure the volume of GSH. Then, 0.45 mL of Na_2HPO_4 (300 mM) was mixed with 0.45 mL of GSH solution, and the absorbance was measured at 412 nm. Subsequently, 100 μL of DTNB solution was applied to the sample and blank, and the absorbance of the sample was read at 412 nm.

8) Determination of carbonyl protein extent in RBC ghost

The amount of GSH in erythrocytes was measured using DTNB, according to Levine et al. [16] and Wang et al. [17]. First, RBC ghosts were prepared. According to previously mentioned methods to prepare the simultaneous and delayed reactions, AAPH and various concentrations of auraptene were added to the RBC ghosts. After 2 h, 100 μL of samples were added to the test and the control tube. Then, 800 μL of HCl (2.5N) was added to the control tube, and 800 μL of DNPH (10 mM) prepared in 2.5N HCl was added to the test tube. Tubes were incubated at darkness and room temperature for 1 h every 10 min and intermittently shaken. Then, 1 mL of TCA (20% w/v) solution was

added to both tubes. After vortexing and placing on ice for 10 min, the tubes were centrifuged at 10,000 g for 10 min to obtain the protein pellets. The supernatant was removed, and 1 mL TCA (10% w/v) was added to the pellet and centrifuged with the same condition. Following the added centrifugation, the supernatant was removed, and the pellets were washed three times with ethanol: ethyl acetate (1:1, v/v) solution to remove unreacted DNPH and lipid remnants. Finally, 500 μL guanidium hydrochloride solution (6M) was added to the pellet after vortexing and incubated at 37℃ for 10-30 min to dissolve completely. Then it was centrifuged at 10,000 g for 10 min at 4℃ to remove insoluble materials. The absorbance of the supernatant was read at 370 nm versus a blank (tube of control). After ghost preparation, 100 μL of the sample was added to the cell quartz and thoroughly mixed with 900 μL guanidium hydrochloride to measure the total protein of RBCs. The absorbance of the sample was read at 280 nm. Bovine serum albumin (BSA) in 6M guanidine hydrochloride was used as a standard. The carbonyl content was calculated using an absorption coefficient (e) of 22000 M^{-1} cm^{-1}, and the data were described in nmol/mg protein.

9) Morphology of RBCs affected by auraptene and AAPH

First, to evaluate the morphology of RBCs, 10% suspension of RBC was prepared in PBS and incubated gently at 37℃ for 10 min. Then, auraptene (25-400 μM) and AAPH (25 mM) simultaneously were added to them and incubated for 6 h. After drying the sample on an aluminous sheath, the samples were coated with gold particles and were finally seen via FESEM electronic microscopy. Vit C and PBS were used as the positive and negative control, respectively.

10) Statistical analysis

Instat software, ANOVA test, and Tukey-Kramer were applied to compare results, and Prism software was used to draw the plots. All tests were done 3 times independently, and the results were reported as Mean ± SD. Significant differences between results were considered at $p < 0.05$.

RESULTS

1. Antioxidant capacity of auraptene in FRAP

According to Fig. 1 and the extent of reduced Fe^{2+} of various concentrations of auraptene compared with reduced Fe^{2+} of Vit C (100 μM), it was concluded that auraptene has a low antioxidant effect, and there was a significant difference between them ($p < 0.001$). Therefore, auraptene in different concentrations is unable to reduce Fe^{+3}.

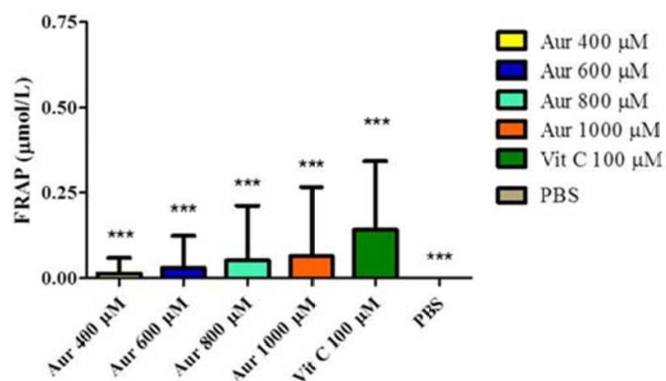

Figure 1. Results of auraptene antioxidant effect via FRAP reagent for 30 min incubation. Columns are showed Mean ± SD. The samples were compared with highest concentration of positive control (Vit C). n = 3, statistically significant difference is shown as follows: ***p < 0.001. Aur, auraptene; FRAP, ferric reducing ability of plasma; PBS, phosphate buffered saline; SD, standard deviation; Vit C, vitamin C.

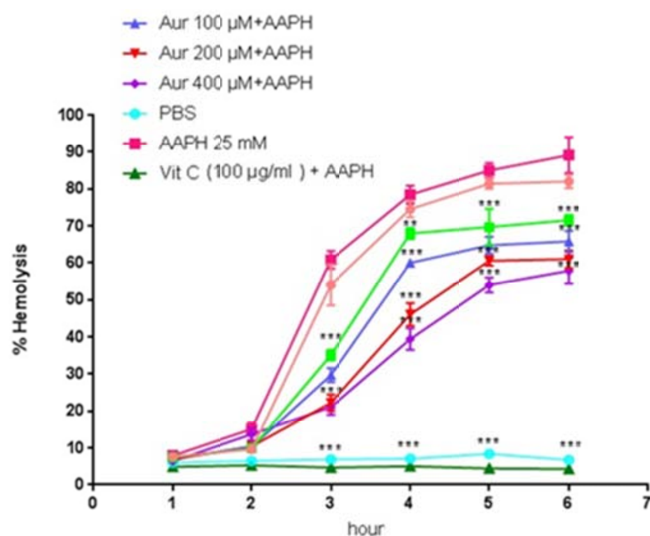

Figure 2. Effects of various concentrations of auraptene on AAPH 25 mM induced hemolysis simultaneously. The samples were compared with AAPH 25 mM. The dots demonstrate Mean ± SD. n = 3, statistically significant differences are shown as follows: **p < 0.1, ***p < 0.001. Aur, auraptene; AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; PBS, phosphate buffered saline; SD, standard deviation; Vit C, vitamin C.

2. Radical scavenging activity of auraptene in DPPH assay

After the addition of DPPH with various concentrations of auraptene and positive and negative controls and incubation, the color of the auraptene samples became lavender. However, the color of the Vit C solution became pale yellow. Positive control inhibited DPPH radicals up to 89%. In contrast, increasing the concentration of auraptene did not affect its radical scavenging. Therefore, it is concluded that auraptene did not exhibit considerable radical scavenging activity toward DPPH, even with an increase in its concentration.

3. Effects of auraptene on AAPH-induced hemolysis in erythrocytes

Erythrocytes were suspended in PBS and incubated at 37℃ in the absence of AAPH to show autohemolysis of the negative control. Notably, there was slight hemolysis within 6 h. After the addition of AAPH (50 mM) to the erythrocyte's suspension, time-dependent hemolysis occurred ($p < 0.001$). After adding various concentrations of auraptene and AAPH 25 mM simultaneously to the suspension of RBCs, the amount of hemolysis significantly decreased in a concentration and time-dependent manner ($p < 0.001$; Fig. 2).

The lysis of RBCs began after the 2 h incubation with 25 mM AAPH, as seen in Fig. 2. Anti-hemolysis effects of auraptene increased with its concentration, and auraptene 400 μM showed better results. As the positive control, Vit C (100 μM) showed anti-oxidant and anti-hemolysis activities.

4. Effects of auraptene on lipid peroxidation in erythrocytes

The addition of AAPH 25 mM to RBC suspension induced lipid peroxidation and increased MDA level in a time-dependent manner compared to the PBS sample. After incubation of the samples with different concentrations of auraptene, the AAPH-induced MDA generation was significantly inhibited in a concentration-dependent manner ($p < 0.001$) (Fig. 3). Incubation of RBCs with the concentrations of auraptene in the absence of AAPH 25 mM formed MDA levels similar to PBS.

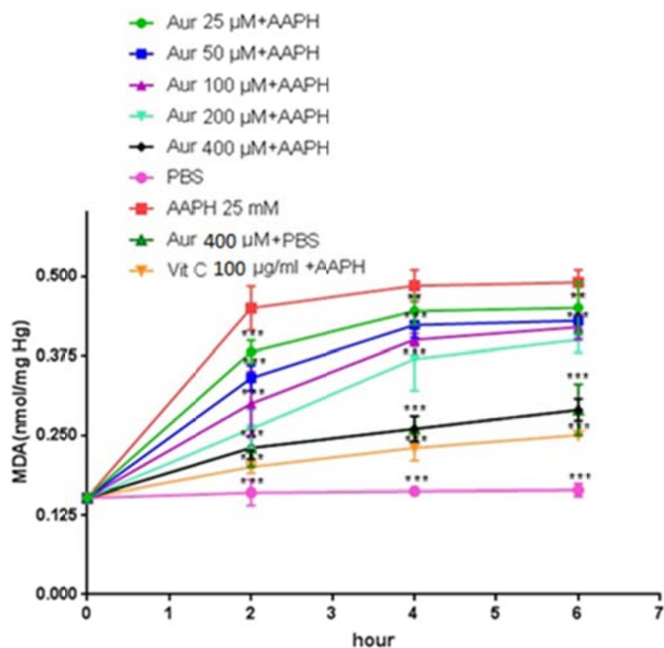

Figure 3. Results of simultaneous addition of auraptene and AAPH 25 mM in inhibition of MDA formation from lipid peroxidation. The samples were compared with AAPH 25 mM. The dots demonstrate Mean ± SD. n = 3, statistically significant differences are shown as follows: $^{**}p < 0.1$, $^{***}p < 0.001$. Aur, auraptene; AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; MDA, malondialdehyde; PBS, phosphate buffered saline; SD, standard deviation; Vit C, vitamin C.

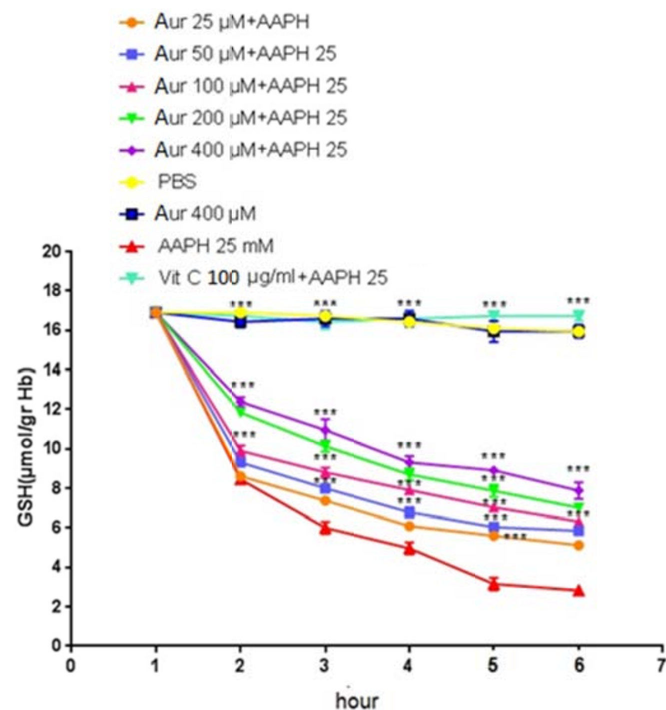

Figure 4. Results of simultaneous addition of auraptene and AAPH 25 mM on the extent of reduced glutathione. The samples were compared with AAPH 25 mM. The dots demonstrate Mean ± SD. n = 3, statistically significant difference is shown as follows: $^{***}p < 0.001$. Aur, auraptene; AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; PBS, phosphate buffered saline; SD, standard deviation; Vit C, vitamin C.

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However, higher concentrations of auraptene after 4 and 6 h incubation showed significantly reduced MDA levels compared to AAPH 25 mM ($p < 0.001$).

5. Effects of auraptene on GSH levels of erythrocytes

RBC suspension incubated with PBS as a negative control group for 6 h at 37℃ showed no significant changes in the cytosolic GSH content of erythrocytes. Incubation of erythrocytes with AAPH 25 mM only showed a time-dependent decrease in the amounts of cytosolic GSH. After the addition of AAPH along with different concentrations of auraptene to RBC suspension, the content of intracellular GSH was remarkably decreased in a concentration-dependent manner ($p < 0.001$) (Fig. 4). In contrast, the addition of 400 μM auraptene to RBC suspension without AAPH for 6 h did not change the GSH content of the erythrocytes. Notably, the higher concentrations of auraptene after 4, 5, and 6 h incubation had more effects on inhibiting the cytosolic GSH content.

6. Effects of auraptene on protein carbonyl content of erythrocytes

Protein oxidation in erythrocytes induced by AAPH represents protein carbonyl formation. AAPH 25 mM was added to RBC ghost, which time-dependently oxidized the proteins and raised protein carbonyl content. Additionally, the level of carbonyl protein affected by AAPH in RBCs had a significant difference compared with RBCs incubated with PBS ($p < 0.001$). After adding auraptene, the amount of carbonyl protein was remarkably reduced in a concentration-dependent manner ($p < 0.001$) (Fig. 5). In contrast, incubation of erythrocytes with auraptene in the absence of AAPH did not change the level of carbonyl proteins like that control group (PBS sample). Also, the effect of the delayed addition of auraptene to RBC Ghost was investigated (data are not shown). Furthermore, different concentrations of auraptene significantly reduced the amount of carbonyl protein ($p < 0.001$).

7. Effects of auraptene and AAPH on the morphology of erythrocytes

As shown in Fig. 6, AAPH (25 mM) induced hemolysis in RBCs (F). After adding auraptene with different concentrations, the number of lysed RBCs via AAPH was reduced, indicating the protective effect of auraptene on AAPH-induced hemolysis in RBCs. In this figure, the RBCs with particles illustrate the hemolysis, and the RBCs with a round border show intact cells.

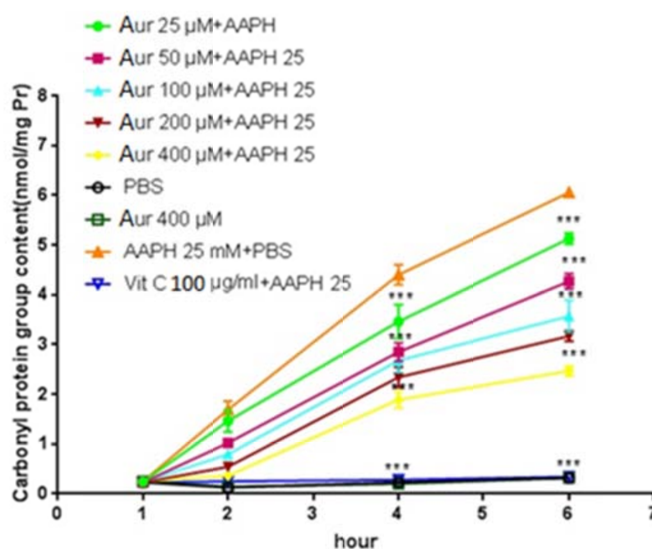

Figure 5. Results of simultaneous addition of auraptene and AAPH 25 mM on carbonyl protein formation. The samples were compared with AAPH 25 mM. The dots demonstrate Mean ± SD. n = 3, statistically significant difference is shown as follows: ***$p < 0.001$. Aur, auraptene; AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; PBS, phosphate buffered saline; SD, standard deviation; Vit C, vitamin C.

DISCUSSION

Some hemoglobinopathies are associated with oxidative damage to proteins and lipids as a pre-hemolytic event [18]. Therefore, developing models to test erythrocyte hemolysis inhibition is critical to study cell membrane oxidative damage caused by free radicals. In the current study, the maximum auraptene concentration (400 μM) would decrease by 40% of erythrocyte hemolysis. However, this was less than the Vit C positive control.

FRAP assay is a simple and valid method applied to measure antioxidant capacity. Auraptene had no significant antioxidant effect, according to the FRAP assay. This lack of effect may be due to its chemical structure, as it cannot transfer hydrogen or electron like an antioxidant or free radical scavenging and catalysis of hydrogen peroxide. The number of conjugated double bond (CDB) systems primarily affects the ferric-reducing activity [19]. Thus, auraptene with a few numbers of CDBs does not have the potential to reduce Fe^{3+} to Fe^{2+}. Due to the result of this test not depending on the concentration of antioxidants,

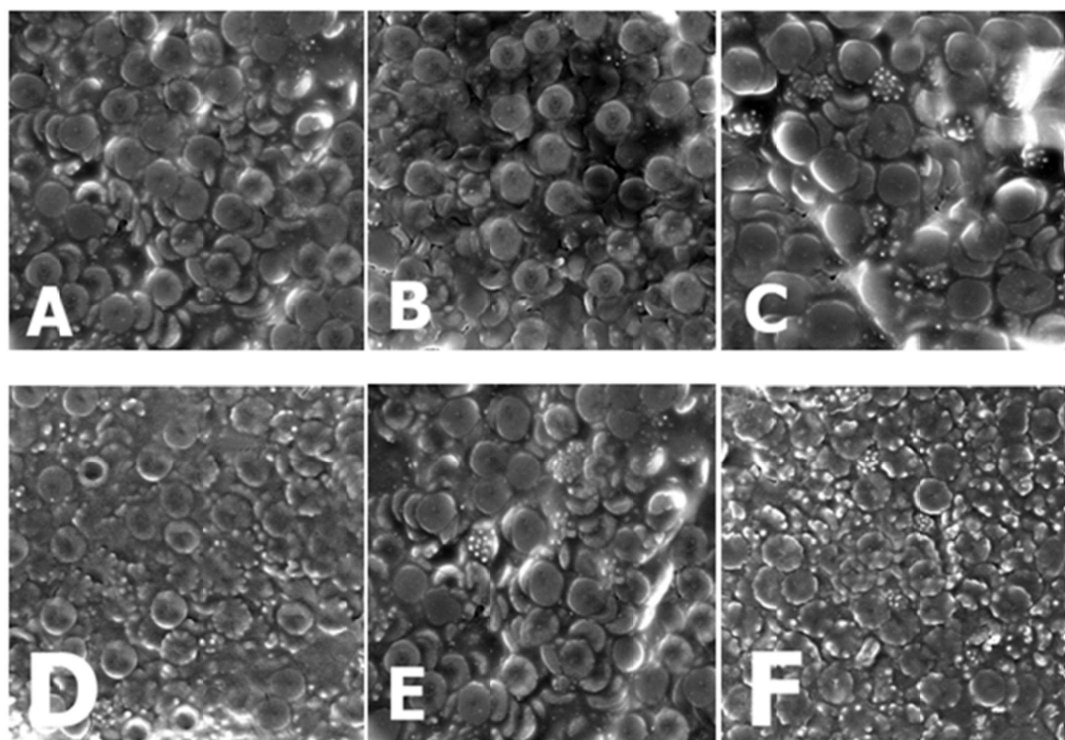

Figure 6. Erythrocyte in PBS (A), erythrocyte + AAPH 25 mM treated with Vit C (B), erythrocyte + PBS treated with auraptene 400 μM (C), erythrocyte + AAPH 25 mM treated with auraptene 25 μM (D), erythrocyte + AAPH 25 mM treated with auraptene 400 μM (E), erythrocyte + AAPH 25 mM (F). AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; PBS, phosphate buffered saline; Vit C, vitamin C.

increasing auraptene concentrations did not make a difference in its observed antioxidant property.

DPPH is a stable free radical usually applied as a substrate to estimate antioxidant activity. Our results confirmed that different concentrations of auraptene did not inhibit DPPH radicals. It has been reported that umbelliprenin, as a prenylated coumarin with a close structure to auraptene, does not have scavenging activity concordant with our observations [20]. It has been shown that the extra hydroxyl group in the structure of antioxidants increases the ability of reduction and DPPH scavenging [21]. Also, the presence of esters with gallic acid in the antioxidant structure raised the ability of DPPH scavenging [22]. In addition, the inhibitory effects of some antioxidants on oxidation systems have been attributed to chelating ions, such as Fe^{2+} and Cu^{2+} [23]. However, the chemical structure of auraptene is unable to transfer hydrogen to DPPH radical and produce $DPPH_2$ [21]. The action between free radical scavenger and DPPH is reversible in that this usually makes the antioxidant capacity of many compounds less of a prospect.

It has been shown that auraptene is unable to scavenge free radicals. However, it can inhibit oxidative stress by inhibiting free radical species formation in activated leukocytes [24]. Using auraptene in higher concentrations significantly reduced hemolysis. Comparing Vit C and auraptene demonstrates that auraptene has fewer antioxidant effects. Ascorbic acid protects the cells and inhibits cell lysis, with the property of peroxyl radicals scavenging. However, auraptene does not scavenge free radicals and carries out its antioxidant effects by inhibiting free radical formation. This suggests that the lesser impact of auraptene is because of its disability in reducing already formed radicals.

Auraptene probably decreases lipid peroxidation through the inhibition of alkoxide radical formation. Thus it protects the membrane of RBC ghosts and decreases hemolysis. It has been reported that auraptene protects lymphocytes against H_2O_2-induced oxidative DNA damage, probably through the improvement in the activities of intracellular enzymes involved in the antioxidant mechanism and the geranyl group's activity in this compound's structure [25]. Furthermore, it has been indicated that rosemary essential oil and silymarin inhibited AAPH-induced hemolysis in a concentration-dependent manner. This may be because the mentioned compounds delayed

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the loss of glutathione in erythrocytes and its ability to scavenge peroxyl radicals, prevent oxidation damage to membrane proteins and lipids, and increase the ATP content of erythrocytes [26]. The delayed addition of auraptene severely reduced its efficacy. It has been previously shown that auraptene, in oral usage, reduced the production of aldehydic lipid peroxidation and increased the activity of metabolizing enzymes such as glutathione S transferase and kinone reductase in the colon and liver [27].

Incubation of RBCs with AAPH decreased the amount of glutathione by 12%, demonstrating the susceptibility of GSH to oxidative damage induced by AAPH. Auraptene could raise the GSH content of the cell, and this effect of auraptene might be due to the inhibition of alkoxyl radical formation induced by AAPH. Notably, some studies have supported that auraptene increased the activities of GSH in several tissues [28, 29]. Furthermore, the delayed addition of auraptene only at special conditions inhibited the effect of AAPH on intercellular GSH content. The inhibitory effect of auraptene against GSH reduction increased as its concentration rose.

Protein carbonylation is a kind of protein oxidation that ROS might produce. This phenomenon in RBCs changes membrane transportations, enzyme activity, membrane permeability, damages the membrane integrity, and exposes RBCs to lysis [30]. The simultaneous and delayed addition of auraptene to RBCs generally decreased protein carbonylation induced by the oxidation of proteins. This effect is likely because of the inhibition of free radical formation. However, the delayed addition of auraptene was less effective because free radicals had already formed.

According to our findings from FESEM microscopy, it was concluded that auraptene in 400 μM drastically inhibited the lysis of RBCs and the number of engrailed RBCs. Consequently, our results indicated that auraptene had protective effects against free radical-induced RBC damage.

To our knowledge, this is the first to suggest that auraptene has protective effects against free radical-induced erythrocyte damage by decreasing lipid peroxidation, preserving intracellular GSH levels, and inhibiting the production of carbonyl protein in red blood cells. These results are similar to our previous work on osthole [3]. However, further studies need to investigate the exact mechanisms of action by which auraptene induces antioxidant activity in cells.

To complement the results of this study, it is suggested to evaluate the antioxidant effects of auraptene with enzymatic tests such as superoxide dismutase and catalase, to perform *in vivo* experiments, and to investigate the effects of auraptene on other body tissues.

CONCLUSION

In our study, auraptene decreased lipid peroxidation, preserved the extent of intracellular GSH, and inhibited carbonyl protein production-induced oxidation. Ultimately, auraptene kept the normal form of RBCs in the highest concentration. Overall, our study provides evidence for the antioxidant activity of auraptene on erythrocytes using cell-based assays that could bring forth significant information to better understand this compound's potential therapeutic role.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ORCID

Khadijeh Jamialahmadi, https://orcid.org/0000-0003-2173-003X
Amir Hossein Amiri, https://orcid.org/0000-0003-0132-5715
Fatemeh Zahedipour, https://orcid.org/0000-0002-8486-2964
Fahimeh Faraji, https://orcid.org/0000-0002-7647-6211
Gholamreza Karimi, https://orcid.org/0000-0002-1273-5448

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