Indian Journal of Natural Products and Resources
Vol. 14(1), March 2023, pp. 50-54
DOI: 10.56042/ijnpr.v14i1.1127

Curcumin ameliorates oxidative stress in red blood cells during ageing

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Received 10 November 2022; revised received 10 February 2023; accepted 17 February 2023

An essential dietary flavonoid known as curcumin has positive health effects and inhibits the synthesis of reactive oxygen species (ROS). The aim of this study is to look at signs of oxidative stress in red blood cells (RBCs) treated with curcumin as a function of age. A total of 116 healthy volunteers ranging in age from 20 to 81 years provided clinically pertinent blood samples for the investigation. Three groups of subjects were created: young (20 to 35 years), middle (36 to 60 years), and old (>60 years). Oxidative stress was induced *in vitro* by incubating RBCs with 10^{-5}M *tert*-butyl hydroperoxide (*t*-BHP). Malondialdehyde and reduced glutathione were detected by co-incubating RBCs with curcumin (10^{-8} M to 10^{-5} M final concentration) and *t*-BHP to assess the effect of curcumin. After being incubated with *t*-BHP, the results revealed higher MDA levels (P <0.001) in comparison to their respective controls and the GSH level significantly (P <0.001) decreased during ageing. By raising GSH and lowering MDA levels, curcumin treatment *in vitro* considerably (P <0.01) mitigated the harmful effect of oxidative stress in RBCs from all age groups. The results of this study showed the potential role of curcumin in the ageing process and it will facilitate the quick screening of novel chemical compounds that may protect RBCs from oxidative stress.

Keywords: Ageing, Curcumin, Glutathione, Malondialdehyde, Oxidative stress

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 36/906, A61P 39/00

Introduction

Turmeric is a member of the ginger family (Zingiberaceae) and is widely used in both traditional Indian and Chinese medicine to treat a variety of diseases[1]. Turmeric formulations are used to treat fresh wounds and bruises, as well as to soothe insect stings. It is used to treat chronic urinary tract infections, hepatobiliary diseases, Alzheimer's disease, Parkinson's disease, diabetes, pulmonary, and cardiovascular disease[2,3]. Turmeric is also widely used as a spice in curries. Because of the presence of its oleoresins and essential oil, it is typically responsible for its characteristic colour and flavour[4]. The turmeric's yellow pigment, curcumin (Fig. 1), has demonstrated a variety of bioactivities, particularly anti-tumour properties in both *in vitro* and *in vivo* studies[5]. Curcumin, also known as diferuloylmethane[6], shows keto-enol tautomerism depending on the pH of the solution: the keto-form is predominant at less than pH 7, while the enol form is predominant at pH greater than 7[(Ref.7)]. *Curcuma aromatica* (0.1 µg/g), *Curcuma longa* (1-2 µg/g), and *curcuma zedoaria* (>100 µg/g) are notable for their curcuminoid level in the roots[8] (Fig. 1).

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Human erythrocytes or red blood cells (RBCs), spend 120 days in the circulatory system, transporting oxygen from the lungs and nutrients from the gastrointestinal tract to all other body tissues and carbon dioxide back to the lungs, and waste from tissues to the excretory system. Hematopoietic stem cells differentiate to create nucleated RBCs, and these RBCs are produced and mature in the bone marrow through a differentiation process. Reticulocytes arise in circulation once the endoplasmic reticulum and nuclei are degraded. RBCs are constantly exposed to high oxygen tension as oxygen carriers. During human ageing, RBCs are more susceptible to oxidative damage than other cells[9]. The irreversible damage caused by oxidative stress, which lowers antioxidant capacity, causes erythrocytes to be damaged by hemolysis and removed from circulation. RBCs cannot repair damaged components as they lack cell organelles[10].

Several sorts of reactive oxygen species (ROS) have piqued attention in biomedical research. The foundation for a free radical theory of ageing was laid by early investigation of free radical processes. According to certain theories, ROS plays a significant role in unregulated processes involving several kinds of macromolecules. A range of ageing mechanisms, such as non-enzymatic alteration via Schiff base

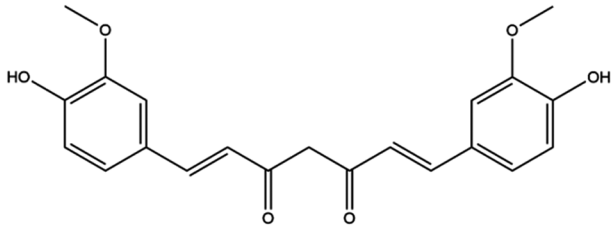

Fig. 1 — Structure of curcumin.

creation or Michael addition, can be attributed to cumulative macromolecular damage, over time[11]. Excessive ROS causes ageing and age-related disorders by damaging nucleic acid (RNA and DNA), proteins, carbohydrates, and lipids[12]. Aerobic organisms, on the other hand, have an antioxidant defence system that protects them against oxidative stress. Enzymatic mechanisms including glutathione peroxidase, superoxide dismutase, catalase, and glutathione -s-transferase are part of this defence system[13]. When the body's antioxidant defence is overloaded, ROS causes oxidative damage. Some oxidative damage happens even under normal conditions, but as we age, our ability to repair damage and use antioxidants effectively, declines, increasing the pace of this damage[14]. Spectroscopic analysis and an *in-vitro* model were utilized to detect GSH and MDA to study the impact of curcumin (Fig. 2).

Materials and Methods

Selection of subjects

The study was completed on normal healthy males and females (n=116) who were classified into three age groups: young (n= 40), middle (n=42), and old (n=34). The samples were collected from Mahendergarh, Haryana region. The selection criteria were based on previously published reports data[15]. The participants were checked for major diseases like asthma, tuberculosis (TB), diabetes, and other severe illnesses. None of the participants was on any medications not even taking any dietary supplements. Informed consent was obtained from all individual participants included in the study.

Ethics approval

This study was performed in line with the principles of the declaration of the Institutional Human Ethics Committee (CUH/2020/IHEC/04).

Reagents

Curcumin was procured from Sigma and all other analytical chemicals like 5, 5'-dithiobis, 2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were procured from Himedia India.

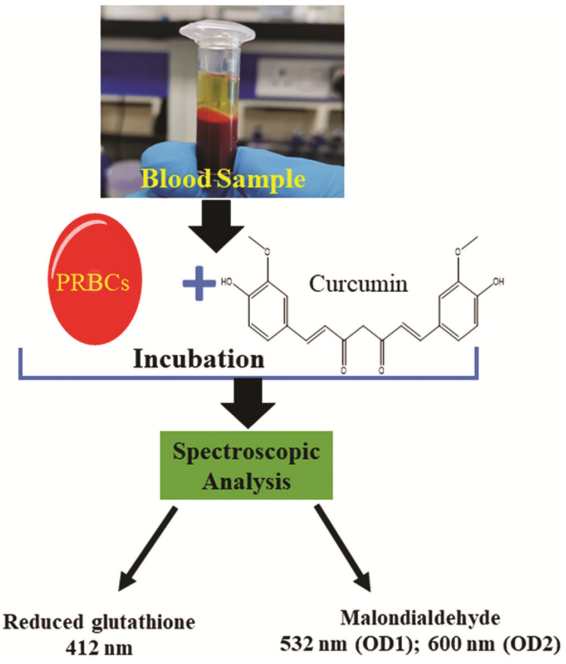

Fig. 2 — Illustrates the methods used to examine the oxidative stress biomarker in a blood sample.

Determination of reduced glutathione (GSH) and malondialdehyde (MDA) content in red blood cells

Reduced glutathione in red blood cells was determined using Butler's method[16]. The method is based on the sulfhydryl group's ability to decrease 5,5-dithiobis, 2-nitrobenzoic acid (DTNB), which results in a yellow-coloured anionic product that can be detected spectrophotometrically at 412 nm, was used to measure the GSH content in mg/mL of packed red blood cells (PRBCs). The malondialdehyde content was determined with slight modifications in Esterbauer and Cheeseman's method[17]. The erythrocytes (0.2 mL) were placed in Krebs'-Ringer phosphate buffer with pH 7.4. The lysate was mixed with ten per cent TCA and centrifuged at 3000 rpm for 5 min. The supernatant was mixed with an equal volume of 0.67 per cent TBA in 0.05 mol/L NaOH and heated for 30 min at a temperature >90ºC. The absorbance was measured at OD1 at 532 nm and OD2 at 600 nm. After subtracting OD2 from OD1, the net optical density (OD) was calculated. A standard plot was measured to determine the amount of MDA in erythrocytes. MDA concentration is measured in nmol/mL of PRBCs.

In vitro treatment with curcumin

To ascertain the impact of curcumin on RBCs, blood was washed 2 to 3 times with Krebs Ringer

phosphate buffer (KRP) containing 5 mmol/L glucose (KRP-G) of pH 7.4. The erythrocytes were placed in four volumes of KRP-G. *In vitro,* oxidative stress was induced by incubating washed erythrocytes for 1 h at 37ºC with 10^{-5}M *tert*-butyl hydroperoxide (*t*-BHP) (final concentration). The amount of *t*-BHP utilized in this investigation to cause erythrocyte oxidative stress was comparable to those employed in other reports[18]. *In vitro* effect of curcumin was tested by incubating erythrocytes with different curcumin concentrations: $10^{-8}$to 10^{-5}M and *t*-BHP 10^{-5}M for 1 h at 37ºC in the dark. Following a previous study which showed particular concentrations of polyphenols in RBCs, we chose similar curcumin concentrations for the current study[18]. RBCs were then washed 2 to 3 times with KRP, pH 7.4 before being replaced with PRBCs for the experiment. Similarly, for control, blood was incubated without curcumin.

Statistical analyses

Statistical Package for Social Science (IBM, SPSS, version 20.0) was used to do the statistical analysis. With the help of multivariate ANOVA (MANOVA), the impact of curcumin was examined. Post hoc analysis for comparisons between the young, middle-aged, and old groups [control, *t*-BHP treatment (zero curcumin), and *t*-BHP + curcumin]. A probability (P) value < 0.05 was used to define statistical significance.

Results and Discussion

Human RBCs GSH content decreased significantly (P <0.001) below baseline in all age groups after being exposed to increased oxidative stress by being incubated with *t*-BHP. *In vitro*, RBCs incubated with curcumin protect from oxidative stress induced by *t*-BHP, as increased GSH levels in all age groups. In comparison to *t*-BHP, treated (zero curcumin), RBCs in the young age group 10^{-8} M does not show any significant change (p = 0.5150), 10^{-7} M showed significant change (P <0.01), while the most significant change was observed at 10^{-6}M and 10^{-5}M (P <0.001), in the middle age group 10^{-5}M (P <0.001) showed most significant change and 10^{-6} M and 10^{-7}M (P <0.01) while 10^{-8}M showed insignificant change (p = 0.7132). Old age group 10^{-8}M also showed significant results (P <0.01), and 10^{-7} to 10^{-5}M showed the most significant results (Fig. 3). Glutathione is a key antioxidant found in RBCs that protects them from Reactive oxygen species and reactive nitrogen species (ROS/RNS). The findings revealed that GSH content in RBCs decreases with age, as seen by the drop in

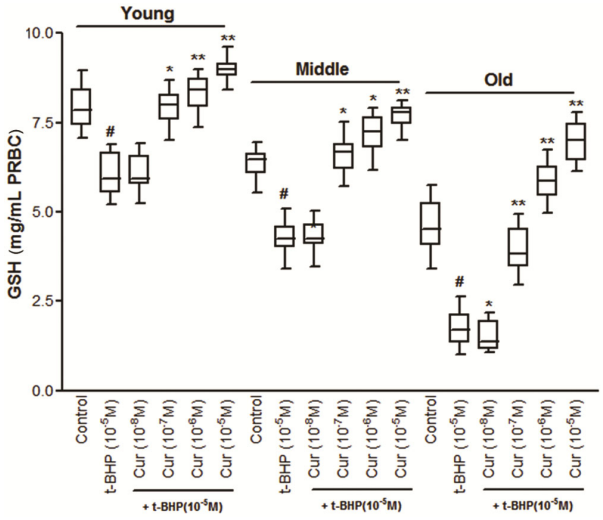

Fig. 3 — The effect of curcumin treatment (10^{-8}M to 10^{-5}M final concentration)on red blood cells. Young (n = 40), middle (36-60 years; n = 42), and old (> 60 years; n = 34) groups had lower GSH levels in oxidatively damaged red blood cells. GSH levels were measured in mg/mL of PRBC. Data are expressed as mean SD. #, P<0.001 compared to the relevant control and *, P<0.01, ** P<0.001 compared to the respective oxidative stressed induced group. GSH stands for reduced glutathione; PRBC is for packed red blood cells. *t*-BHP stands for *tert*-butyl hydroperoxide, while M stands for molar.

GSH level in the heterogenous population of RBCs throughout human ageing. Curcumin therapy *in vitro* protects all age groups against oxidative stress-induced reduction in GSH levels.

Our data demonstrated (Fig. 4) that lipid peroxidation in erythrocytes increases with age. Lipid peroxidation significantly increased (P <0.001) in all age groups when erythrocytes were exposed to a known amount of oxidative stress by being incubated with *t*-BHP in comparison to their respective controls. At 10^{-8} M and 10^{-7}M, curcumin caused a substantial (P <0.001) decline in MDA content when compared to zero curcumin (*t*-BHP treated only) in all three age groups of RBCs.

In all three age groups, the result was more significant (P <0.0001) at 10^{-6} M and 10^{-5}M (Fig. 4). Under oxidative stress, the RBC membrane is susceptible to lipid peroxidation, which involves the cleavage of double bonds present in polyunsaturated fatty acids, resulting in the generation of MDA[19]. Considering the antioxidant property of curcumin and its link to ageing, curcumin acts as a mediator in defence against ageing and has been studied. The present findings indicate that curcumin has potent antioxidant properties consequently influencing the ageing process.

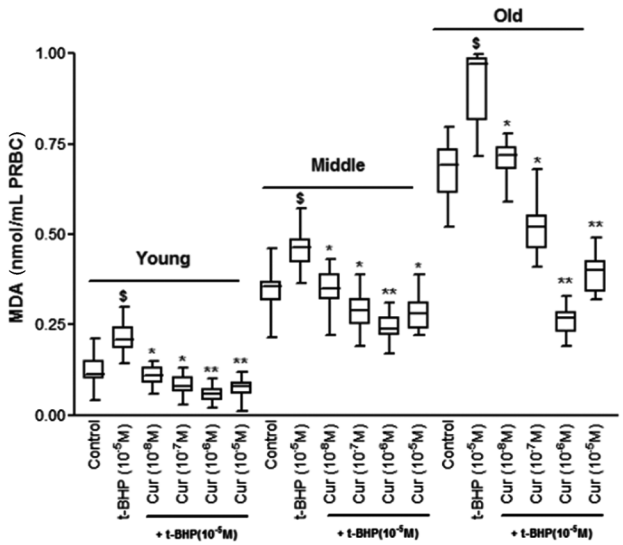

Fig. 4 — The effect of curcumin treatment (10^{-8}M to 10^{-5}M final concentration) on malondialdehyde content in oxidative stressed red blood cells in young (n = 40), middle (36-60 years; n = 42), and old (> 60 years; n = 34) people. MDA concentrations were measured in nmol mL^{-1} PRBC. Data are expressed in mean SD. $, P<0.001 compared to the respective control, and *, P<0.001, ** P<0.0001 compared to the respective oxidative stressed induced group. MDA stands for malondialdehyde; PRBC stands for packed red blood cells; Cur stands for curcumin; *t*-BHP stands for *tert*-butyl hydroperoxide; M stands for molar.

Curcumin significantly inhibits oxidative stress in a dose-dependent manner in RBCs by donating hydrogen and scavenging free radicals. Flavonoids can also chelate metal ions such as iron, and excess iron ions cause lipid peroxidation in cells by increasing hydroxyl radical[20].

Oxidative stress has been demonstrated to impair the RBC membrane and affect its deformability in a previously published study[21]. The utmost crucial antioxidant found in RBCs is GSH. GSH is biosynthesized by glutamyl cysteine synthetase and glutathione synthetase and then transformed to oxidized glutathione (GSSG) by glutathione peroxidase to prevent peroxidation of macromolecules in RBCs and finally reduced to GSH by glutathione reductase[22]. Curcumin pre-treatment dramatically reduced ROS production and prevented GSH depletion[23]. *In vitro* model of Alzheimer's disease showed that treatment of curcumin in $A\beta_{1-42}$ cells significantly increased the GSH level[24].

MDA is the most well-studied lipid peroxidation product. According to Kalpravidh, *et al.,* hydrogen peroxide (H_2O_2) induced levels of MDA in RBC were considerably greater in beta-thalassemia/HB E patients than in control, although they decreased significantly after treatment for up to 6 months[25]. Curcuminoid dosages of 600 mg/day decreased the MDA level, this effect was more significant when curcuminoids were co-incubated with piperine[26]. Curcumin may have an antioxidant effect by lowering MDA levels in the blood and raising GSH activity. All three active sites in curcumin can be oxidized by hydrogen abstraction and electron transfer. Extensive research by several research groups has proven that the phenol-OH group in curcumin is the one from which hydrogen can be readily abstracted during the free radical reaction, producing phenoxyl radicals that resonance stabilized the structure of the keto-enol[27].

Limitations and future prospectus

The present results should be understood from the perspective of some limitations. Because of their high reactivity aldehyde, such as their ability to react with proteins via the Michael addition reaction or with DNA to form adducts, measuring their free concentrations as valid oxidative damage levels is difficult[28]. Temperature and diet may also regulate the oxidative status of the RBCs.The human body has a highly complicated and efficient antioxidant system made up of many interconnected antioxidant compounds and enzymes. The mechanism is thought to be involved in the increased oxidative stress as a function of human age including changes in the tissue/plasma content as well as the activity of the antioxidant defence system. These findings may have implications for developing strategies for the use of curcumin in the treatment of ageing and age-associated diseases. Curcumin is a potent oxidative species scavenger and our finding may have wide implications for ageing and age-associated diseases. Additional studies are needed to assess the full therapeutic potential of curcumin on ageing.

Conclusion

Healthy ageing, longevity, and the effect of various flavonoids have been studied in several models. The direct measurement of MDA and GSH in curcumin-treated RBCs will provide valuable data for anti-ageing studies. Curcumin's application in treating oxidative stress is expected to bypass medication resistance and achieve treatment selectivity in a variety of clinical disorders. The influence of curcumin on ageing and age-related disorders should be studied further to discover the molecular mechanism behind it. Finally, we infer that curcumin's antioxidant activity is linked to its possible function in human ageing. These findings will help to establish

the reference value of polyphenols for oxidative stress biomarkers in different age populations.

Conflict of interest

The authors have no conflict of interest. The authors have no relevant financial or non-financial interests to disclose.

Acknowledgement

This study was supported by a Fellowship (Senior Research Fellow) from the Council of Scientific and Industrial Research (CSIR), Government of India to Mr. Akshay Kumar (09/1152(0016)/2019-EMR-I).

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