



Liposome-Encapsulated Curcumin Offers Sustained Release of Curcumin to Ameliorates the Oxidative Stress Biomarkers of Red Blood Cells During Aging

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Abstract

In this study, curcumin-encapsulated liposomes were evaluated in the red blood cells (RBCs) of different age groups. We conducted research on a sample of 38 healthy individuals of both sexes. We examined established aging biomarkers in red blood cells and tested the in vitro effect of curcumin liposomes against tert-butyl hydroperoxide (t-BHP), which induces oxidative stress. Our results show that liposome-encapsulated curcumin significantly ($p < 0.05$) decreases the malondialdehyde (MDA) and increases reduced glutathione (GSH) levels during aging. In silico biological target prediction of curcumin revealed that curcumin mainly targets monoamine oxidase A (MAOA). Our findings reveal that the curcumin liposome formulation gives a controlled release of curcumin in comparison to free curcumin in RBCs during the aging process. The finding has great potential in biomedical research for exploring curcumin-loaded liposomes as an anti-aging compound.

Keywords Curcumin liposomes · Aging · Malondialdehyde · Glutathione

1 Introduction

Aging is generally understood to be the gradual loss of physiological function over time, which is linked to a higher risk of mortality and morbidity [1]. Pre-clinical models indicate that aging is, in fact, a treatable and adjustable ailment that can be manipulated through dietary changes, pharmacological treatments, or genetic engineering [2]. The markers of cellular aging fall into several categories: genome-based problems, signaling dysfunctions, organelle compromise, and changes in cell phenotype [3]. By increasing our knowledge of the physiological causes of aging, researchers have designed strategies to counter aging and age-associated

diseases. Some studies on fruit flies indicate that oxidative stress is the primary determinant of the aging process [4]. More researchers are interested in the mechanism of ROS's involvement in disease [5]. The most prominent hypothesis on aging is the oxidative stress theory. Oxidative stress leads to the impairment of macromolecules, such as lipids, nucleic acids, carbohydrates, and proteins [6]. The aging process is marked by the steady buildup of injured cells, a gradual loss in physical activity, and an increasing vulnerability to the development of multiple illnesses [7]. Thus, for normal physiological activities in animals, maintaining and enhancing antioxidant status, especially through herbal remedies, is crucial.

Curcumin is a flavonoid belonging to the Zingiberaceae family, also known as diferuloylmethane [8]. Curcumin is found abundantly in *Curcuma longa*'s rhizome. Curcumin has a rich history in Ayurvedic medicine and conventional Indian curry varieties. The poor water solubility of curcumin limits its application besides its anti-diabetic, anti-cancer, inflammatory, and anti-antioxidant properties [9, 10]. Liposomes are synthetic, microscopic lipid-based spherical vesicles that self-assemble into a bilayer around an interior aqueous compartment [11, 12]. It is used as a delivery agent for various biomolecules like drugs, proteins, and nucleic acids. Additionally, liposomes have been extensively used

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in the food and cosmetics industries [13, 14]. The solubility of curcumin in aqueous environments is improved by encapsulating it with liposomes, which enhances the solubility of conventional curcumin formulations [15]. Curcumin absorbs more effectively in the gastrointestinal system and enters the bloodstream due to its increased solubility. Different factors might lead to the breakdown of curcumin, but liposomal encapsulation protects it from environmental elements like heat, light, and enzymatic destruction [16]. The improvement in cellular absorption helps curcumin to have anti-aging benefits. Age-related alterations in lipid metabolism are closely linked to a disturbed systemic lipid profile, which can accelerate the development of aging phenotypes [17]. Among the most notable and persistent byproducts of oxidative stress is malondialdehyde (MDA). The bloodstream continuously circulates it, and urine excretes it in both conjugated and unbound forms, particularly in conjunction with L-serine and L-lysine. It is reported that the relationship between oxidative stress and disease gets stronger with age [18]. The other commonly assessed biomarker is reduced glutathione (GSH). GSH is an extremely important hydrophilic antioxidant found in erythrocytes. Cells contain this tripeptide molecule, which consists of three amino acids and lacks any proteins. The molecule also contains a sulfhydryl ($-SH$) group [19].

In this study, we made a liposomal form of curcumin and looked at how it affected red blood cell biomarkers of oxidative stress linked to aging. Our goal was to make curcumin safer, more effective as an antioxidant, and easier to control its release and distribution while also increasing its therapeutic potential.

2 Material and Methods

Egg phosphatidylcholine, cholesterol, DPPH, and curcumin were bought from Sigma-Aldrich. Trichloroacetic acid, reduced glutathione, and thiobarbituric acid (TBA) were procured from HI Media Laboratories, India. Other chemicals and solvents used in the study were of analytical grade.

3 Sample Collection

The present investigation was conducted on 38 people in good health, comprising both males and females, from Haryana, India. The samples underwent a thorough examination to detect any significant health problems. The criteria of selection were in line with the previous study [20]. All

subjects provided informed consent for using their blood samples. The study was approved by the Institutional Human Ethics Committee (IHEC) (Ref. No. CUH/2020/IHEC/04) of the University.

A total of 10 mL of intravenous blood was obtained from healthy individuals of varying age groups, specifically “young (18–35 years), middle-aged (36–60 years), and elderly (> 60 years)”, using EDTA vials. The blood was subjected to centrifugation at a temperature of 4 °C and centrifuge at 1800 g for 10 min. The plasma and the buffy coat were separated, resulting in the collection of packed red blood cells (PRBC). The obtained PRBCs were rinsed 2–3 times in Krebs–Ringer phosphate (KRP) buffer with a pH of 7.4. The liquid portion was discarded, and the packed red blood cells (PRBC) were promptly utilized in our investigation.

4 Obtaining Liposomes with Curcumin Liposomes

Curcumin liposomes were synthesized as per the method described earlier by Singh et al. [21] with little changes. Briefly, egg PC (10 mg/mL), curcumin 600 µg/mL (dissolved in ethanol), and cholesterol at a concentration of 1 mg/mL were combined in a test tube. A thin lipid film was formed by purging nitrogen gas onto it. The film was hydrated using PBS (pH 7.4) and heated at 60 °C in a water bath for 30 min with vortex after every 2–3 min and was then sonicated for 30 min. A slight yellowish semi-cloudy suspension was extruded with the use of a mini extruder with a polycarbonate filter having a pore size of 100 nm to yield homogeneous curcumin liposomes. Empty liposomes were also prepared using the above-mentioned protocol without curcumin.

5 Characterization of Liposomes

The morphology and size of the empty and curcumin-loaded nanoliposomes were assessed using a transmission electron microscope (TEM) (JEOL1400 FLASH, JEOL). For TEM analysis, a 10 µL nanoliposome formulation was loaded to a carbon-coated copper grid with a 300-mesh grid structure. To enable TEM observation, the samples were negatively stained with a 5-times diluted solution of uranyl acetate and subsequently air-dried overnight. Drug encapsulation efficiency (%) was measured after the disruption of liposomes with absolute ethanol and was determined by employing a UV–vis spectrophotometer at 425 nm. The equation, absorbance of curcumin liposomes – empty liposomes = final absorbance, was used to calculate the concentration of curcumin encapsulation, using a standard graph of curcumin.

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Drug in Liposomes}}{\text{Total Drug}} \times 100$$

Curcumin release from liposomes was studied using the dialysis method. Dialysis bags having a molecular cut-off weight of 12,400 Da were soaked in hot water for 10 min to remove preservatives. Liposomes were put into the dialysis bag; the bag was closed with the help of clips and checked for leakage. This bag was immersed in the 200 mL beaker containing 100 mL Kreb's Ringer Bicarbonate–Reduced Glutathione (KRB-GSH) buffer (pH 7.4). After that beaker was put on the hot plate cum magnetic stirrer with 400 rpm and 37 °C temperature. Aliquots were withdrawn at various time gaps (0, 0.5, 1, 2, 3, 6, 12, and 24 h). The optical density of collected samples was measured at 425 nm.

$$\text{Drug Release (\%)} = \text{Curcumin Release} / \text{Curcumin Encapsulated} * 100$$

The zeta potential and hydrodynamic size of curcumin liposomes were determined using dynamic light scattering (Anton Paar, Particle Analyzer, Litesizer 500). Curcumin liposomes were diluted with PBS (pH 7.4) in a 1:10 ratio (Curcumin liposomes/PBS), and a minimum of 30 scans for size and 50 scans for zeta potential were taken for dynamic light scattering analysis.

6 Evaluation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

As previously mentioned [22], the ability of curcumin, curcumin liposomes, empty liposomes, and gallic acid (25, 50, 100, 150, and 200 µg/mL) to quench free radicals was assessed. The 0.002% DPPH solution was prepared in methanol. A total of 190 µL of DPPH solution and 10 µL of the test sample were introduced into 96-well plates. The plates were incubated at a temperature of 37 °C for 30 min while being kept in darkness. Afterward, the O.D. was estimated at 515 nm. Gallic acid was employed as an established antioxidant.

7 In-Vitro Effect of Curcumin, Curcumin Liposomes, and Empty Liposomes on Biomarkers of Oxidative Stress

The MDA level in erythrocytes was determined according to a previously published report [23]. 0.1 mL of packed erythrocytes were suspended in 1.5 mL of pH 7.4 KRP buffer. One milliliter of lysate was mixed with 1 mL of trichloroacetic acid (10%), and then the centrifugation was done at 3000 rpm for 5 min, and the supernatant was mixed with an equal volume of 0.67% thiobarbituric acid (TBA) and heated for 30 min at > 90 °C temperature. The absorbance

was measured at 532 nm (OD1) and 600 nm (OD2); after deducting the absorbance at OD2 from that at OD1, the net optical density (OD) was computed. The concentration of MDA in RBCs was calculated as nmol/mL PRBC, using a standard plot of absorbance versus MDA concentrations. For the estimation of GSH in erythrocytes, the Beutler method was utilized [24]. This method is based on the –SH group activity decreases the 5,5'-dithiobis, 2-nitrobenzoic acid (DTNB) and the final yellow colored product gives maximum absorbance at 412 nm. The amount of GSH in RBCs was determined in mg/mL PRBC using a standard plot.

In this study, in vitro experiments were performed; curcumin and curcumin liposomes (3 µg/mL) were added to the assay medium. Afterward, the mixture was placed in an incubator at a temperature of 37 °C for a duration of 60 min before the assay. Control experiments were conducted in parallel, where curcumin and curcumin liposomes were not added to the assay medium. To create oxidative stress in a laboratory setting, packed red blood cells (PRBC) were treated with tert-butyl hydroperoxide (t-BHP) at a concentration of 10^{-5} M.

8 Biological Prediction and Computational Structural Biology

The protein targets of curcumin were predicted using SwissTargetPrediction (<http://swisstargetprediction.ch/>). The computational structure-based active/binding site prediction in Monoamine oxidase A (PDB ID, 2Z5X) was performed using a web tool named COACH (<https://zhanggroup.org/COACH/>). COACH is a method that uses a meta-server technique to predict protein–ligand binding sites. COACH utilizes two comparative methods, TM-SITE and S-SITE, to generate complementary predictions of ligand binding sites based on the given structure of target proteins. These methods identify “ligand-binding templates from the BioLiP protein function database by comparing binding-specific substructures and sequence profiles” [25, 26]. Molecular docking was performed using EADock (<http://www.swissdock.ch/>) using default parameters [27].

9 Statistical Analysis

The results are reported as the standard error of the mean (SEM). The paired *t*-test was utilized to ascertain significant associations between variables. Statistical significance was defined as having *p*-values less than or equal to 0.05 (*p* < 0.05). The data analysis was conducted using GraphPad Prism version 5.00 for Windows, developed by GraphPad Software.

10 Results

In this study, nanoliposomes were fabricated using the lipid-thin hydration method. Curcumin exhibited a distinct peak at 425 nm (Fig. 1, red curve), attributable to its characteristic yellow color. In the case of curcumin liposomes (Fig. 1, black curve), a similar peak was observed, albeit with reduced intensity. This observation indicates the successful encapsulation of curcumin within the liposomes. Notably, empty liposomes did not display any absorbance peak (Fig. 1, green curve) at 425 nm, confirming that the 425 nm peak is solely attributed to the presence of curcumin within the curcumin-loaded nanoliposomes. Further, we conducted measurements of the Zeta potential and hydrodynamic size of the nanoliposome formulation using dynamic light scattering (Table 1). The hydrodynamic diameters of empty liposomes and curcumin-loaded liposomes were 266.73 ± 5.07 nm and 214.53 ± 7.47 nm, respectively. The curcumin-loaded nanoliposomes had smaller hydrodynamic sizes than the empty liposomes. This was probably because the curcumin was added to the lipid bilayer, which can make the liposome structure smaller.

Egg phosphatidylcholine is a neutral lipid; thus, the zeta potential of the empty and curcumin-loaded nanoliposome was -2.30 ± 0.66 mV and -3.53 ± 0.29 , respectively. The size and shape of the empty and curcumin-loaded nanoliposomes were characterized using TEM (Fig. 2). The empty nanoliposome showed (Fig. 2A and B) and curcumin-loaded nanoliposomes (Fig. 2C and D) were quasi-spherical in shape and ~ 100 nm and 90 nm in size, respectively. The thick outer layer of the curcumin-loaded nanoparticles showed that the drug had been successfully inserted into the lipid bilayer, even though it does not like water. The preparation of TEM samples necessitates thorough drying of the specimens. As a result, it is anticipated that the nanostructures within liposomes may experience a notable reduction in size compared to their respective hydrodynamic diameters. Consequently, in our experimental setup, there is a considerable variance in the average liposome diameter when assessed through both DLS and TEM due to this drying process.

To assess the effectiveness of encapsulation, we determined the encapsulation efficiency by examining various ratios of egg phosphatidylcholine/cholesterol/curcumin. The encapsulation efficiency for the ratios 2.5:0.25:0.600, 5:0.5:0.600, and 10:1:0.600 was found to be 16.39%, 33.55%, and 57.20%,

Fig. 1 UV–visible spectra of free curcumin (red curve), curcumin-loaded liposomes (black curve), and empty liposomes (green curve)

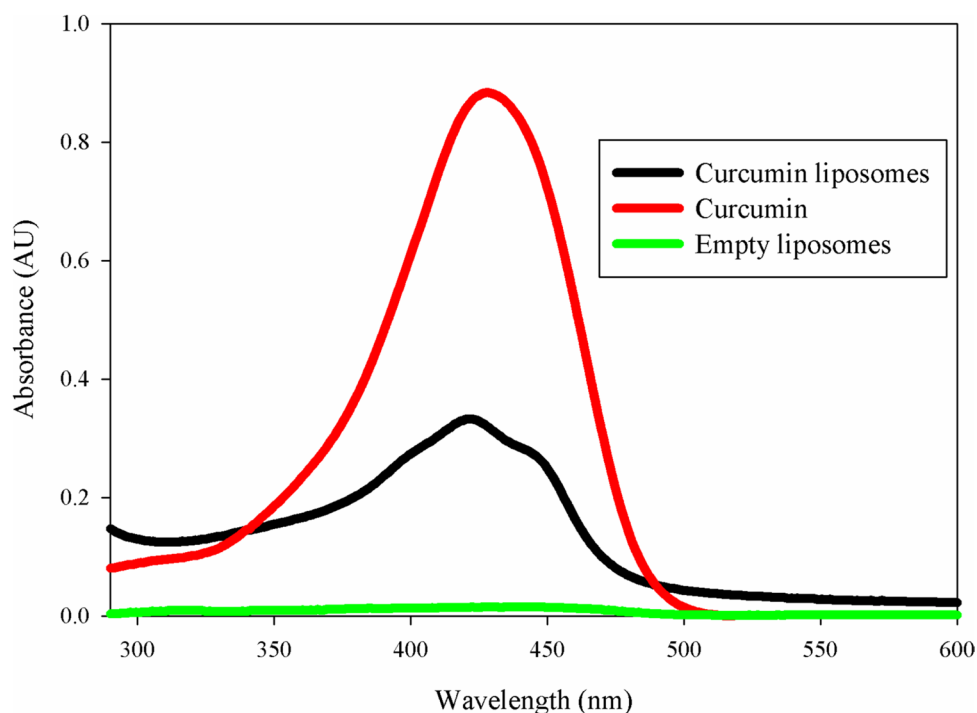


Table 1 Hydrodynamic size, polydispersity index, and zeta potential of empty and curcumin liposomes

Liposomes	Hydrodynamic size	Polydispersity index (%)	Zeta potential (mV)
Empty liposomes	266.73 ± 5.07	9.1 ± 0.45	-2.30 ± 0.66
Curcumin-loaded liposomes	214.53 ± 7.47	11.76 ± 0.76	-3.53 ± 0.29

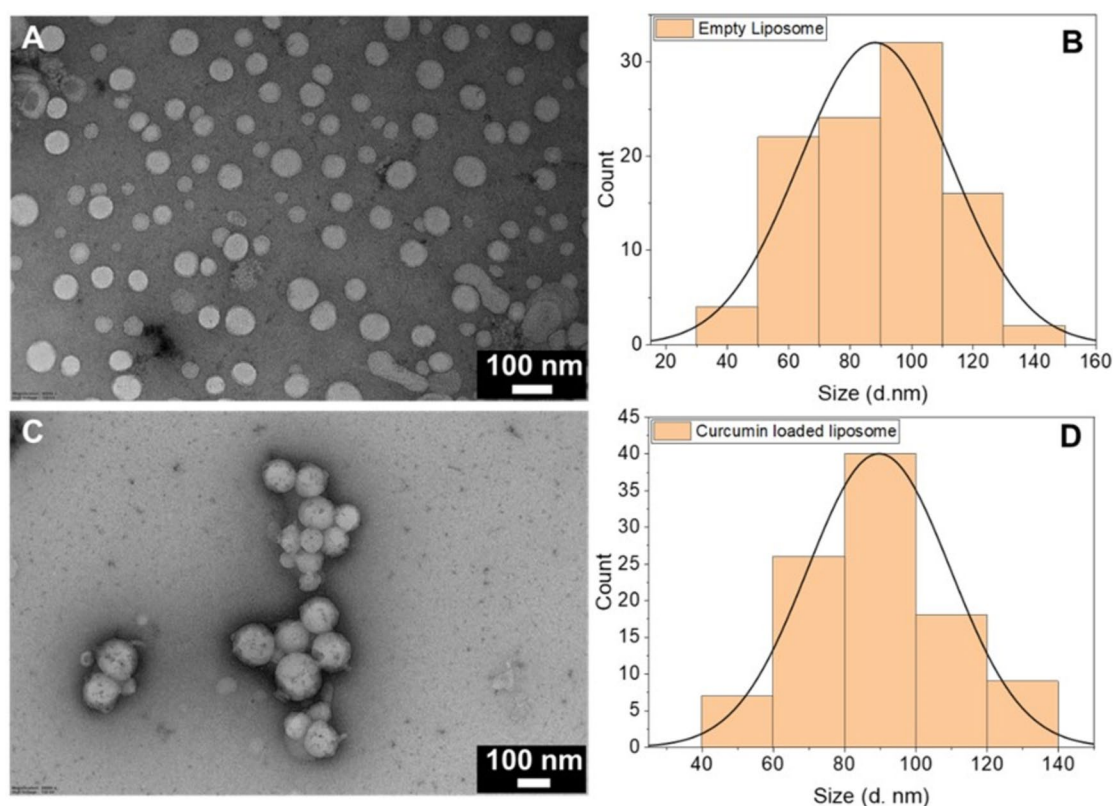


Fig. 2 **A** Transmission electron micrographs depicting empty liposomes. **B** Bar graph illustrating the particle size distribution of empty liposomes. **C** Transmission electron micrographs depicting curcumin-loaded nanoliposomes. **D** Bar graph illustrating the parti-

cle size distribution of curcumin-loaded nanoliposomes. The particle size distribution data were obtained by measuring 100 nanoliposomes from various TEM images using ImageJ software

Table 2 Percent drug encapsulation efficiency of liposomes at various ratio of egg phosphatidyl choline, cholesterol, and curcumin

Egg PC/cholesterol/curcumin (mg/ml)	Avg. % drug EE ($n=3$)	$\pm$ SEM
2.5:0.25:0.6	16.39	0.464124
05:0.5:0.6	33.55	0.520651
10:01:0.6	57.20	3.068557

respectively (Table 2). The quantity of curcumin in liposomes was found using the standard curve of curcumin. Further, we examined the effect of curcumin liposomes on red blood cells by analyzing the drug release profile in Kreb's Ringer bicarbonate buffer in the presence of glutathione at pH 7.4 under in situ conditions. To simulate conditions resembling red blood cells in vitro, we utilized Kreb's Ringer bicarbonate. Drug release was assessed at different time intervals (0, 0.5, 1, 2, 3, 6, 12, and 24 h) as presented in Fig. 3.

The antioxidant activity of curcumin and curcumin liposomes was evaluated using 2,2-diphenyl-1-picrylhydrazyl as shown in (Fig. 4 and Table 3). DPPH percent radical scavenging activity increased with an increase in the concentration

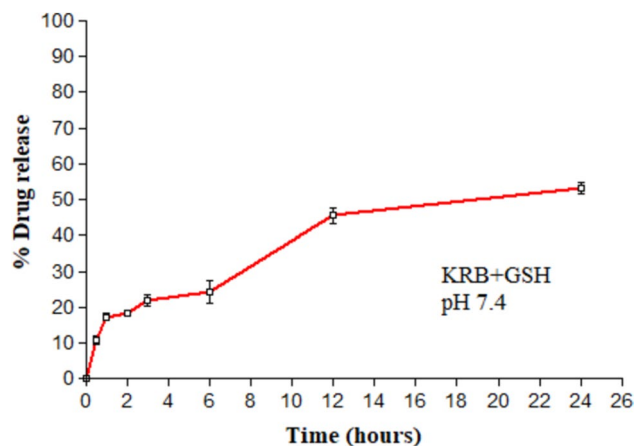


Fig. 3 In situ release of curcumin encapsulated in liposomes (KRB + GSH buffer, pH 7.4). After 24 h 53.22 (± 1.54) % curcumin was released from the liposomes. Data are expressed as $\pm$ SEM; $n=3$. KRB + GSH stands for Krebs ringer bicarbonate buffer with reduced glutathione

(25, 50, 100, 150, and 200 μ g/ml) of curcumin in both curcumin liposomes and curcumin. Empty liposomes show very little radical scavenging activity which means the radical

Fig. 4 Percent DPPH radical scavenging activity of curcumin, curcumin liposomes, empty liposomes, and gallic acid. Data are expressed as $\pm$ SEM; $n = 3$

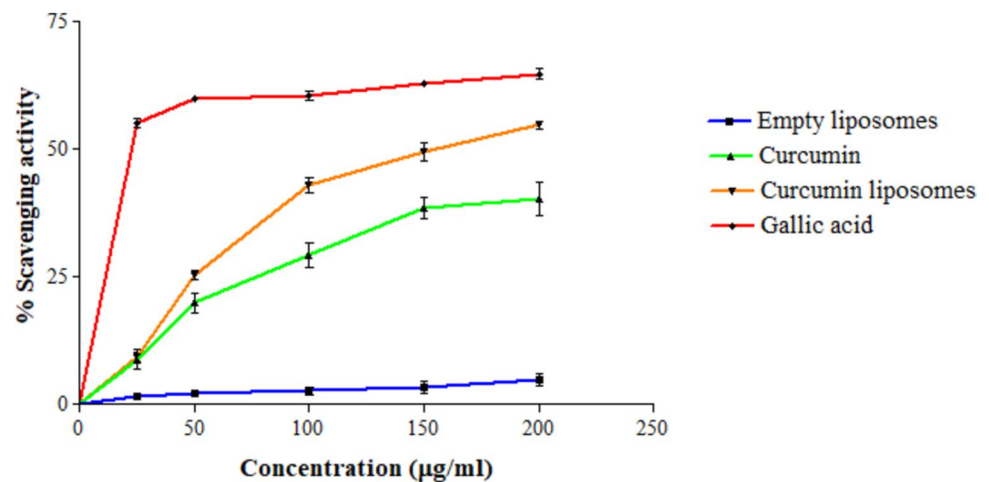


Table 3 Percent DPPH radical scavenging activity

Concentration (µg/ml)	EL		Curcumin		CL		Gallic acid	
	% SA	SEM ($\pm$)	% SA	SEM ($\pm$)	% SA	SEM ($\pm$)	% SA	SEM ($\pm$)
25	0.60	0.47	8.79	2.04	9.32	0.85	55.02	0.87
50	1.20	0.51	19.91	1.93	25.30	0.82	59.83	0.53
100	1.20	0.69	29.12	2.30	42.88	1.43	60.44	0.80
150	1.80	1.09	38.33	2.09	49.34	1.76	62.85	0.53
200	3.61	1.12	40.10	3.26	54.64	0.84	64.65	1.06

scavenging activity comes from curcumin. Gallic acid which is a standard antioxidant showed the highest scavenging activity. Till the concentration of 25 $\mu\text{g/ml}$, both curcumin and curcumin liposomes showed almost similar scavenging activity, but at higher concentrations (50–200 $\mu\text{g/ml}$), the DPPH radical scavenging activity of curcumin liposomes was greater than that of curcumin and increased with increasing concentration as can be observed from Fig. 4.

Our results showed a similar trend to the previously published reports that the level of malondialdehyde is directly proportional to age; as aging happens, the level of malondialdehyde increases. When red blood cells (RBC) were treated with curcumin (3 $\mu\text{g/ml}$) in a laboratory setting, the level of malondialdehyde (MDA) was significantly reduced in both the young and middle-aged groups. However, in the old age group, the reduction in MDA level was even more significant compared to the oxidative stress induced by t-BHP in RBC. The statistically significant ($p < 0.001$) difference in MDA reduction was observed in the young and middle age groups, and even more significant ($p < 0.0001$) in the old age group. Curcumin liposomes significantly reduced the level of MDA in all age groups compared to t-BHP (10^{-5} M), with a p-value of less than 0.0001. t-BHP (10^{-5} M) is used to induce a known amount of oxidative stress (Fig. 5). Curcumin liposomes showed a better decrease in MDA levels as compared to curcumin alone. In young and middle age groups, curcumin liposomes ($p < 0.0001$) performed better

than curcumin ($p < 0.001$) while their significance level ($p < 0.0001$) was almost similar in old age groups.

The findings of our study indicate that the level of GSH decreases with age, particularly in comparison to the old-aged and young age groups. In vitro, incubation of RBC with curcumin (3 $\mu\text{g/ml}$) and curcumin liposomes (3 $\mu\text{g/ml}$) causes a significant ($p < 0.0001$) increase in GSH level concerning the t-BHP-induced oxidative stress RBC. t-BHP 10^{-5} molar is used to induce a known amount of oxidative stress in RBC (Fig. 6). Curcumin liposomes showed better GSH activity as compared to curcumin but no significant difference is observed.

In silico biological target prediction of curcumin revealed that curcumin mainly targets monoamine oxidase A (MAOA), histone acetyltransferase p300, toll-like receptor 7 and 9, beta-amyloid A4 protein, and prostaglandin E synthase with a prediction score equal to 1 (Table 4). We predicted the active binding sites in the MAOA using COACH and found a consensus binding pocket (C-score = 0.99) comprising residues 8, 9, 11, 12, 13, 31, 32, 33, 34, 39, 40, 54, 55, 56, 57, 58, 231, 232, 233, 261, 262, 263, 292, 386, 391, 395, 397, 423, 424, 432, 433, 434, and 437 (Fig. 7A). Molecular docking shows that curcumin binds to the MAOA in its active binding site with binding energy equal to -2523.24 kcal/mol and estimated ΔG equal to -6.58 kcal/mol (Fig. 7B).

Monoamine oxidases (MAOs) control the amounts of neurotransmitters like dopamine, norepinephrine, and serotonin

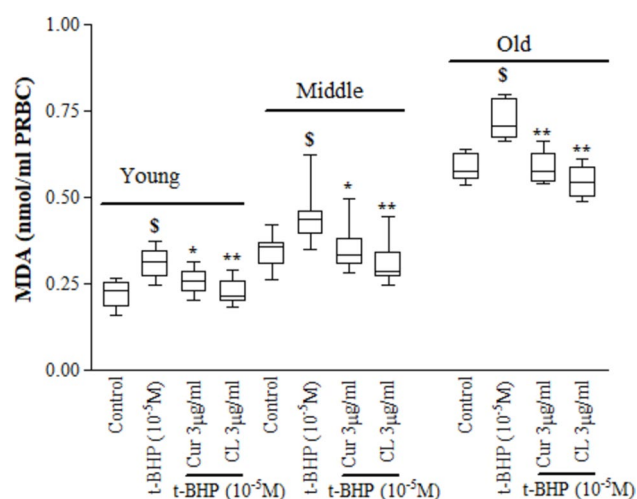


Fig. 5 The effect of curcumin and curcumin liposomes treatment (3 μ g/ml) on MDA content in t-BHP-induced oxidative stress in red blood cells with different age groups, i.e., young (20–35 years; $n=9$), middle (36–60; $n=16$), and old (>60; $n=6$) people. MDA levels were measured in nmol/ml PRBC. PRBC incubated with t-BHP increased MDA level significantly ($S_p<0.0001$) compared to the control group. Curcumin and curcumin liposome treatment decreased MDA levels significantly ($*p<0.001$; $**p<0.0001$) for the oxidative stress-induced group. Cur stands for curcumin, CL stands for curcumin liposomes, and t-BHP stands for tert-butyl hydroperoxide

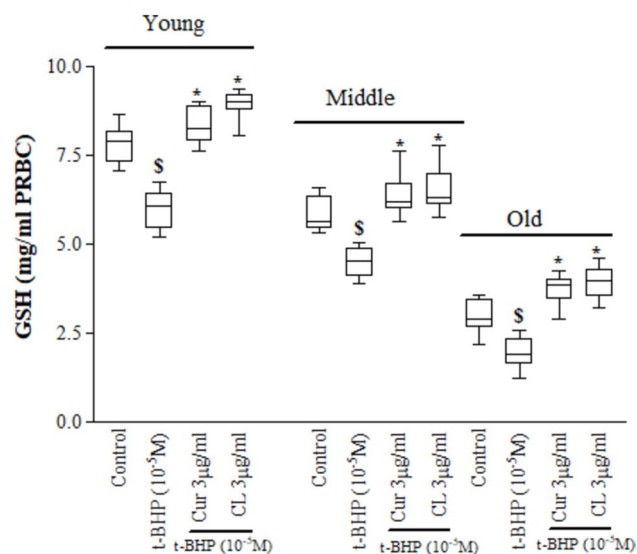


Fig. 6 The effect of curcumin and curcumin liposomes treatment (3 μ g/ml) on GSH content in oxidatively stressed red blood cells of different age groups, i.e., young (20–35 years; $n=16$), middle (36–60 years; $n=11$), and old (>60 years; $n=11$) people. GSH levels were measured in mg/ml PRBC. t-BHP (10^{-5} M) taken as oxidative stress inducer. t-BHP induced a significant ($S_p<0.0001$) decreased GSH level as compared to control. Curcumin and curcumin liposomes increased a significant amount of GSH ($*p<0.0001$) concerning their oxidative stress-induced group. Cur curcumin, CL curcumin liposomes, t-BHP tert-butyl hydroperoxide

in specific areas, making them a focus of medication development for treating certain central nervous system problems [28]. Recent studies have demonstrated that as individuals age, the activity of these enzymes increases in neurological and cardiac tissues. This upregulation may contribute to the degeneration of these tissues, as the metabolic process of these enzymes generates hydrogen peroxide, resulting in oxidative stress. Therefore, directing efforts toward these enzymes could serve as a promising approach to combat aging. Due to the vital function of MAOs in regulating neurotransmitter levels, the consequences of their irregular activity have been accompanied by various neurological disorders [29]. A recent study has identified an unexpected role of MAOs in the progression and spread of tumors as well [29].

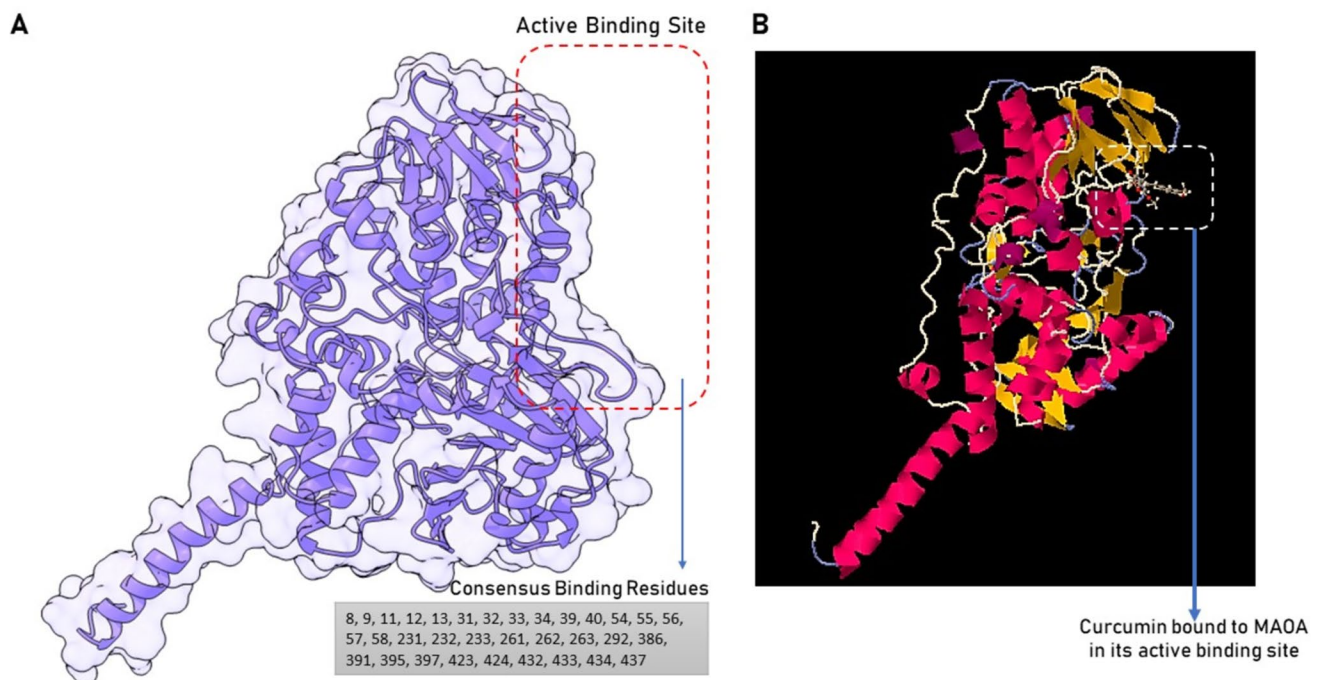
Pharmacologically inhibiting MAOs could be a beneficial therapeutic strategy for treating aging and neurological conditions. Aside from FDA-approved medications, the potential of natural items has been investigated to suppress MAO. Phenolic dietary substances like resveratrol, eugenol, quercetin, piperine, and curcumin do not serve as substrates for MAO [30–34]. However, they do have the ability to inhibit MAO. Curcumin is known to suppress the activity of MAOA; however, it needed higher inhibitory concentration ($IC_{50}=500.46$ nM) when compared to the widely recognized MAO-B inhibitor selegiline. Curcumin has been found to decrease MAO activity through both noncompetitive and competitive types of inhibition [35, 36].

11 Discussion

RBCs are perpetually exposed to free radicals, and there should be some mechanism to guard them from the deleterious effects of free radicals. Here, we propose that the liposomal formulation of curcumin will help in combating the effect of free radicals by decreasing the effect of MDA levels and increasing GSH levels. The exact mechanism is not well-known, but it is hypothesized that liposomal formulations help in the quenching of free radicals as compared to free curcumin. Curcumin possesses a robust antioxidant potential due to its chemical structure, surpassing vitamin C by 2.75 times and vitamin E by 1.6 times. Curcumin acts as an effective scavenger of ROS in the organism and also promotes the synthesis of glutathione (GSH) a natural antioxidant, which shields cells from potential harm caused by free radicals [37]. Liposomes improve the solubility of curcumin, facilitating enhanced absorption in the gastrointestinal tract [38]. This results in elevated levels of curcumin in the bloodstream and tissue, augmenting its antioxidant potential. The liposomal formulation can enhance the rate of interactions with ROS. The lipid bilayer of liposomes can modify the local milieu, facilitating curcumin's interaction with free radicals [39]. Liposomes also facilitate a continuous release of curcumin, extending its antioxidant properties in contrast to the quick degradation of free curcumin. This prolonged-release

Table 4 High-confidence protein/enzyme targets of curcumin

Sr. no	Target	Common name	Target class	Probability*
1	Monoamine oxidase A	MAOA	Oxidoreductase	1
2	Beta amyloid A4 protein	APP	Membrane receptor	1
3	Histone acetyltransferase p300	EP300	Writer	1
4	Prostaglandin E synthase	PTGES	Enzyme	1
5	Toll-like receptor (TLR7/TLR9)	TLR9	Toll-like and Il-1 receptors	1
6	Beta-secretase 1	BACE1	Protease	0.807322834
7	DNA topoisomerase II alpha	TOP2A	Isomerase	0.505302626
8	Glyoxalase I	GLO1	Enzyme	0.241303241
9	Nuclear factor erythroid 2-related factor 2	NFE2L2	Unclassified protein	0.217290828
10	Arachidonate 5-lipoxygenase	ALOX5	Oxidoreductase	0.217290828
11	Cyclooxygenase-1	PTGS1	Oxidoreductase	0.217290828
12	Inhibitor of NF-kappa-B kinase (IKK)	IKBKG IKBKB CHUK	Kinase	0.185218735
13	Epidermal growth factor receptor erbB1	EGFR	Kinase	0.121287003

**Fig. 7** **A** Active binding site prediction in the monoamine oxidase A. **B** Molecular docking between curcumin and monoamine oxidase A

may result in an extended duration of efficacy against oxidative damage. Liposomes encapsulated curcumin can more easily incorporate into cell membranes as compared to free curcumin, enabling it to neutralize radicals in situ where they are generated. The lipid composition of liposomes can enhance cellular endocytosis, resulting in elevated intracellular levels of curcumin, which is crucial for efficient radical scavenging [40]. The RBC membrane possesses a diverse range of mechanisms to protect against the effects of oxidative stress. MDA is a consequence of oxidation of polyunsaturated fatty acid (PUFA) peroxidation [41]. The current research assumed that stress is

induced when cells are stimulated with t-BHP. Peroxidation takes place in the PUFA found in the walls of the RBC membranes, leading to an increase in the content of malondialdehyde (MDA) within the cells. MDA serves as an indicator of lipid peroxidation. Liposome-encapsulated curcumin is expected to diminish MDA level more efficiently than free curcumin owing to its enhanced capacity to neutralize radicals prior to the onset of lipid peroxidation. This leads to less oxidative damage to cellular membranes. Oxidative stress biomarkers are observable on the PRBC membrane. Red blood cells possess a non-enzymatic antioxidant mechanism, namely glutathione (GSH). The

sulfhydryl (SH) group of cysteine in glutathione (GSH) assists in preserving the reduced state of the thiol group in hemoglobin, membrane proteins, and enzymes. GSH is an essential intracellular antioxidant. Liposome-encapsulated curcumin may enhance or sustain GSH levels more efficiently owing to its capacity to penetrate cells and provide a protective impact against oxidative stress. Free curcumin may not maintain GSH levels as efficiently due to its fast metabolism and excretion from the body [42]. Because of the robust connection between oxidative stress, getting older, and many diseases, controlling MDA and GSH levels through curcumin liposomes is a promising idea. While the increase in GSH suggests a strengthened cellular defense against oxidative assaults, the decrease in MDA suggests a possible reason for the slowing in aging-related cellular damage.

Monoamine oxidase-A is an enzyme that degrades neurotransmitters, such as serotonin, dopamine, and norepinephrine. We believe that curcumin acts as an inhibitor of monoamine oxidase A and prevents the degradation of neurotransmitters. Monoamine oxidase A is already associated with aggression, mood disorders, and neurodegenerative illnesses, and lower levels are observed in individuals with antisocial personality disorder. We believe that this interaction of Curcumin with Monoamine Oxidase is also associated with aging. The same has been incorporated in the manuscript.

12 Conclusion

Based on the results obtained, it is possible to conclude that curcumin liposomes have a high antioxidant capacity in comparison to free curcumin and may be utilized to delay aging to a certain extent. Curcumin liposomes offer a potential way to overcome curcumin's bioavailability restrictions and realize its full potential in slowing down the aging process. This work also discusses the use of computational methods for prediction of biological targets, a way toward designing and development of selective MAOA inhibitors. This novel strategy has significant implications for creating anti-aging treatments that are effective by using curcumin as a primary therapeutic agent.

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Author Contribution A.K. Conceptualization and Original draft preparation, S.B. Editing and Reviewing, K.M. Editing and Reviewing, S.S. Methodology and Validation, T.C.D. Methodology and Validation and P.K.M. Supervision and Reviewing.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Research Involving Humans and Animals Statement The study protocols were designed according to the Central University of Haryana, Institutional Human Ethic Committee (Ref. No. CUH/2020/IHEC/04).

Consent for Publication Not applicable.

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Conflict of Interest The authors declare no competing interests.

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