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Analyzing the *in vivo* antioxidant activity in mice

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Abstract
Analyzing the *in vivo* antioxidant activity in mice is fundamental in elucidating the physiological relevance and therapeutic potential of antioxidant compounds. Oxidative stress, stemming from the imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms, underlies the pathogenesis of numerous diseases, including cancer, cardiovascular ailments, and neurodegenerative disorders. This abstract underscore the importance of *in vivo* studies in preclinical research and drug development, particularly focusing on mice as valuable experimental models. Mice offer genetic similarity to humans, facile handling, and a plethora of genetic and pharmacological tools for mechanistic inquiries.

Keywords: Antioxidant, pathogenesis, neurodegenerative, mechanistic

Introduction
Assessing the *in vivo* antioxidant activity in animal models, particularly mice, is crucial for understanding the physiological relevance and therapeutic potential of antioxidant compounds. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, plays a significant role in the pathogenesis of various diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions.

In vivo studies provide valuable insights into the effectiveness of antioxidants in mitigating oxidative damage and preserving cellular integrity under physiological conditions. Mice are commonly used as experimental models due to their genetic similarity to humans, ease of handling, and availability of various genetic and pharmacological tools for mechanistic investigations.

Literature review
Diogo Matias *et al*. (2015) [15]. We optimised the preparation procedure and created phytosomes using a bioactive *Plectranthus madagascariensis* extract and studied their properties. Process parameters and various formulations were investigated. Using acetone as the solvent, a two-hour reaction period, and the addition of a 2.5% molar concentration of cholesterol resulted in smaller and more uniform particles. The best-formed phytosomes appeared amorphous, having a polydispersity index of 0.243±0.18 and a diameter of 191.3±75.3 nm. Compared to the main component of the extract, 7α, 6β-dihydroxyroyleanona, these nanosystems were able to encapsulate 92.8% of the extract, as per HPLC assessments. This study's findings suggest the possible use of phytosomes for the transport of bioactive compounds with medicinal potential in the near future.

Parul A. Ittadwar *et al*. (2016). A phytosome was effectively formed by complexing umbelliferone with phosphatidylcholine. Umbelliferone was positively identified in the preformulation trials. After optimizing the batch, the building was effectively completed created using the gas removal from a solvent technique in accordance with the Box-Behnken experimental design. Estimates of medicinal content, complexation rate, and practical output in percentage were within the range when the optimised batch was assessed. In both tests, the complex was more soluble than the medicine. At the same doses, the compound performed better than the *in vitro* antioxidant tests conducted on medication alone. The compound was successfully formed, according to the HPTLC, DSC, FT-IR, SEM, XRD, and NMR analysis. The phytosomal complex had superior release compared to the medication, according to the outside of living organisms permeation assays.

In order to assess the complex's photoprotective efficacy, researchers conducted an animal study and estimated the levels of antioxidant enzymes. Phytosomal complexes outperformed pharmaceuticals in protecting skin and subsequent antioxidant enzymes. There were no discernible alterations to the formulation during the three-month duration as shown by the stability study. Therefore, According to the findings of this study, the phytosomal form of umbelliferone, a new drug delivery method, has a more effective therapeutic impact compared to the medication itself.

Ahmed N. Allam *et al*. (2015) [11] A newly designed semisolid formulation was used to improve the curcumin content in soft gels, and a straightforward solvent evaporation approach was used to make curcumin phytosomes, which included both free-flowing powder and the procedure. The zeta potential and the phytosomal powder's medication content were examined. With the use of hydrophilic vehicles like Cremophor EL and KLS P 124 are bioactive surfactants that may be used with PEG 400, thirteen distinct soft gel formulations were created utilising oils like Ingredients: oleic acid, castor oil, and miglyol 812. Curcumin solubility *in vitro* was used to characterise the chosen formulations. Curcumin phytosomes in complex formulations showed excellent reliability and a round, self-contained shape, according to TEM examination. Curcumin dissolving patterns were shown to be steady in stability experiments of selected formulations made utilising the hydrophilic vehicle. When phytosomes were formed in oily carriers, however, curcumin dissolution was significantly reduced.

Rashmi Vankudri *et al*. (2017) [12] created a quarcetin-phospholipids complex and investigated its pharmacokinetics and therapeutic efficacy in rats. The goal of this project was to enhance the delivery of quarcetin, a compound that is weakly soluble, by preparing phyto-phospholipid copmlex. Utilizing A research of the solubility and dissolution of the compound was conducted an investigation was conducted on the quarcetin-phospholipids complex (QPC) employing imaging microscopy, thermal analysis, and Fourier transform infrared spectroscopy, among other physico-parameters. In addition to studying the effects of QT and QPC (50 and 100 mg/kg b.w. po. o., respectively) on STZ-induced diabetes, the anti-diabetic activity of OGIT was investigated in normoglycemic and diabetic rats at different intervals.

Singh Rudra Pratap *et al*. (2018) [13] I developed to evaluate a phytosome containing gingerol to treat respiratory infections. By combining gingerol with to produce phytosomes (GPs) from soy lecithin by an anti-solvent precipitation method manufactured. A variety of criteria were used to characterize and evaluate the LPC, which was produced by loading phytosomes a chitosan aqueous solution with phytosomes (GLPC). Physical compatibility experiments using DSC and FT-IR confirmed that GLPC was compatible with chitosan and soy lecithin. With an average the optimized GLPC had a particle size of 254.01±0.05 nm (-13.11 mV) while the other was 431.21±0.90 nm (-17.53 mV) GP had spherical and irregular particle shapes. The results showed that GLPC had an 86.02% trapping efficiency and a medication loading of 8.26%, whereas GP had an entrapment efficiency of 84.36% and a drug loading of 8.05%, respectively. Using the model of diffusion (Korsmeyer Peppas), the rate of GLPC release in a pH 7.4 phosphate buffer *in vitro* was 88.93±0.33% was faster than that of GP (86.03±0.06%) for up to 24 hours. When compared to GP, GLPC had far stronger protective against free radicals, microorganisms, and inflammation effects. In addition to correlating the haematological GLPC has considerably improved the bioavailability on rabbit blood in terms of values opposed to the growth of bacteria (*S. aureus* and *E. coli*) during incubation. Gingerol exhibited enhanced sustained-release profile and longer oral absorption rate in the nanoparticle-based complex of phytosome loaded with phyto-constituent medication, which exhibited excellent antibacterial action. Additionally, it showed improved stability when subjected to various storage conditions. The compound combined the effects of chitosan and phytosome.

Methodology

Lipid Peroxidation

One useful indicator for measuring the degree of peroxidation reaction is malondialdehyde, which is formed when polyunsaturated fatty acids are broken down. The reaction between malondialdehyde and thiobarbituric acid (TBA) yields a pink species with a wavelength of 532 nm. After properly mixing, 1 millilitre The TCA-TBA-HCl reagent was mixed with 2 millilitres of liver homogenate. After being heated in a water bath to 95 °C, the solution was allowed to boil for an additional hour. The mixture was then mixed with 5 mL of distilled water and 5 mL of a butanol: pyridine (15:1) solution once it had cooled mixture. Centrifugation was used at 3000 rpm for 10 minutes to remove the flocculent precipitate. A blank containing butanol: pyridine / 15:1 was used to test the supernatant's absorbance at 532 nm. With 4. A value of 1.56 x 10^-1 cm^-1 for extinction, we can determine the sample's malondialdehyde content.

Superoxide Dismutase

An enzyme's capacity to block the reduction of nitroblue tetrazolium dye by superoxide via the phenazine methosulphate-mediated pathway is the basis of the test. After the reaction is complete, at 560 nm, the solution's absorbance is measured. Antioxidants, which are included in popular drinks, compete with NBT for the opportunity to react with superoxide when small amounts of these drinks are introduced to the reaction. One way to measure superoxide-scavenging is by looking at the percentage inhibition of NBT reduction. For fifteen minutes at four degrees Celsius, I spun a mixture of phosphate buffer (0.1 M) or 0.15 M Tris HCl that included ten percent weight-to-volume tissue homogenate. The following were mixed and left to incubate for 90 seconds at 30°C: Here are the ingredients: The following ingredients were used: 0.1 ml of supernatant, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186 µM), 0.3 ml of 300 µM Nitroblutetrazolium, and 0.2 ml of NADH (750 µM). Mix 0.1 mL of glacial acetic acid with 4.0 mL of n-butanol. The solution was centrifuged after standing for 10 minutes. We compared the absorbance at 560 nm after butanol layer separation to a blank.

Catalase

Aebi (1984) [14] was used to evaluate CAT activity. The interaction between 126 CAT and a known amount of H2O2 is inhibited by CAT inhibitor after precisely 1 minute. The

liver tissue was ground together in a pH 7.0 M/150 phosphate buffer at rotating at 5,000 rpm after being heated to a temperature of 1-40 C in a centrifuge. In addition to the enzyme extract, the reaction mixture also included a 0.01 M phosphate buffer with 2 millimolar H2O2 and a pH of 7.0. The spectrophotometric analysis was performed when the absorbance dropped at 240 nm. Units per milligramme of liver tissue were used to represent the specific activity of catalase. In order to compare the absorbance values, a standard curve was created using known CAT values.

Reduced Glutathione (GSH)

The Ellman technique was used to measure reduced glutathione (GSH). Following the addition of 2.4 ml of a 0.02M EDTA solution to 0.1 ml of each tissue homogenate, the mixture was cooled in an ice bath for 10 minutes. Combine 600 millilitres of distilled water with 500 millilitres of TCA, a solution that is 50% concentrated. Following a 10-to fifteen-minute period on ice, the mixture was centrifuged at 3000 rpm for fifteen minutes. After vigorously mixing, 1 millilitre of supernatant, 2 millilitres of Tris-Hcl buffer, and 0.05 millilitres of DTNB solution (Ellman's reagent) were added. In contrast to a blank reagent, the absorbance was measured at 412 nm within two to three minutes after administering DTNB.

Glutathione Peroxidase (GPx)

After being incubated for 0, 30, 60, and 90 seconds, The following ingredients were removed from the reaction mixture: 4 millilitres of a 0.4 M sodium phosphate buffer (pH 7.0), 1 millilitre of a 10 mM sodium azide, 2 millilitres of reduced glutathione, 1 millilitre of a 2.5 mM H2O2, 2 millilitres of water, and half a millilitre of an enzyme. Include 1 millilitre of DTNB reagent (0.04% DTNB in 1% sodium citrate), 2 millilitres of phosphate buffer, and 3 millilitres of centrifugation-removed supernatant after adding half a millilitre of 10% TCA to halt the reaction. At 412 nm, we recorded the colour development. Many organic peroxides, including H2O2, are controlled by the selenium enzyme GPx, which has a well recognised function.

Data Analysis

***In vivo* antioxidant activity**

The results of several blood biochemical estimates for CP, KP, and BP.

Enzyme-based biochemical test for oxidative stress

Lipid Peroxidation

Rats suffering with hepatic cirrhosis liver, researchers found that CCl4 significantly increased hepatic lipid peroxidation as a result of free radical damage, with a value of 72.78±3.987 nmol MDA/mg tissue. The amounts of these dropping dramatically components to 42.43±1.211, 47.75±0.447, and 45.19±2.520 nmol MDA/mg tissue respectively in the liver was seen in the groups treated with CP, KP, and BP in addition to CCl4, suggesting that phytosomes have an anti-lipid peroxidative action.

Table 1: Effect of phytosomes on LPO levels in rats

S. N.	Group	LPO (nmol MDA/mg tissue)
1.	Control	25.31±3.688
2.	CCl4 treated	72.78±3.987
3.	Standard	34.42±2.802
4.	SCE	55.43±2.212
5.	SPE	60.35±1.232
6.	SBE	58.75±2.447
7.	CP	42.43±1.211
8.	KP	47.75±0.447
9.	BP	45.19±2.520

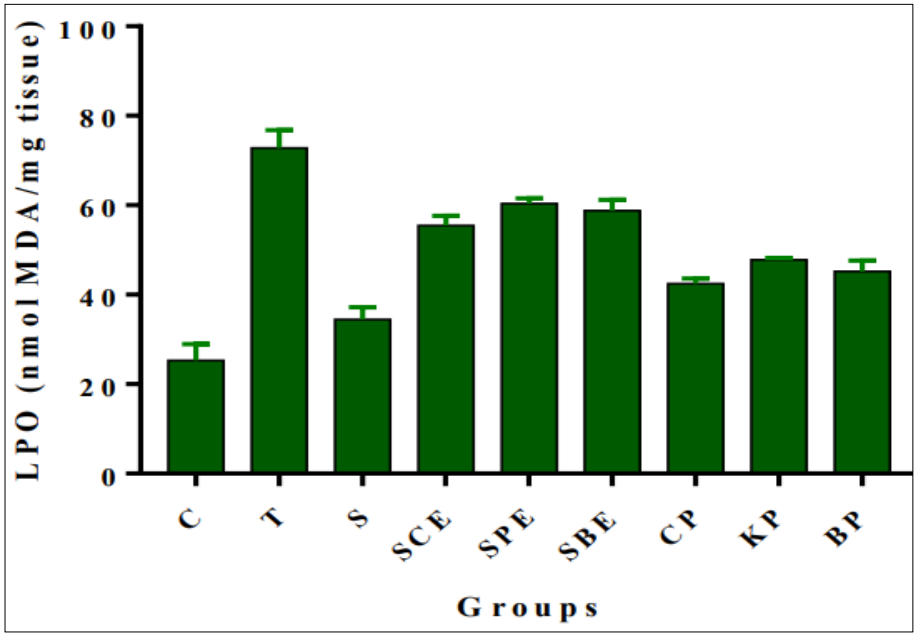

Fig 1: Effect of phytosomes on LPO levels in rats

Superoxide Dismutase

This test relies on the enzyme's capacity to block the superoxide-induced blueing of nitrobluetetrazolium dye via the phenazine methosulphate-mediated reduction pathway. The decrease in how much of an antioxidant enzyme, like SOD, is present, which was seen in rats treated with CCl4, is an obvious sign of an overabundance to activate the lipid peroxidation pathway and release free radicals, which leads to tissue damage. In rat livers that were given CP, KP, and PP, respectively, the concentrations of these components increased significantly (p<0.001) to 145.52±1.071 IU/mg tissue, 137.11±8.883 IU/mg tissue, and 140.11±0.343 IU/mg tissue, indicating the antioxidant action of phytosomes.

Table 2: Effect of phytosomes on SOD levels in rats

S. N.	Group	SOD (unit/mg tissue)
1.	Control	202.53±11.287
2.	CCl_4 treated	56.17±6.485
3.	Standard	166.53±10.325
4.	SCE	79.11±11.883
5.	SPE	82.41±1.233
6.	SBE	89.32±2.065
7.	CP	145.52±1.071
8.	KP	137.11±8.883
9.	BP	140.11±0.343

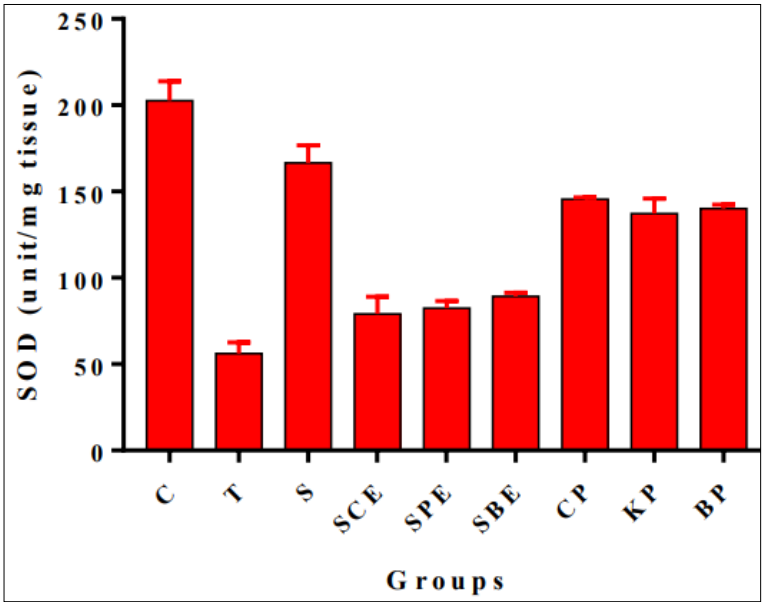

Fig 2: Effect of phytosomes on SOD levels in rats

Catalase

Aebi's method was used to assess CAT activity. After precisely one minute of reacting with a known amount of H_2O_2, CAT inhibitor stops the process. From 45.17±6.462 IU/mg tissue to 10.90±1.058 IU/mg tissue, the amount of antioxidant enzymes like catalase was significantly reduced by CCl4. The concentrations of KP and BP significantly increased to 26.11±2.999, 28.14±0.822, and 29.54±1.654 IU/mg tissue, respectively, in the groups treated with CP (p<0.001).

Table 3: Effect of phytosomes on CAT levels in rats

S. N.	Group	CAT (U/mg wet tissue)
1.	Control	45.17±6.462
2.	CCl4 treated	10.90±1.058
3.	Standard	36.93±6.802
4.	SCE	19.75±2.125
5.	SPE	20.31±1.543
6.	SBE	17.14±0.822
7.	CP	26.11±2.999
8.	KP	28.14±0.822
9.	BP	29.54±1.654

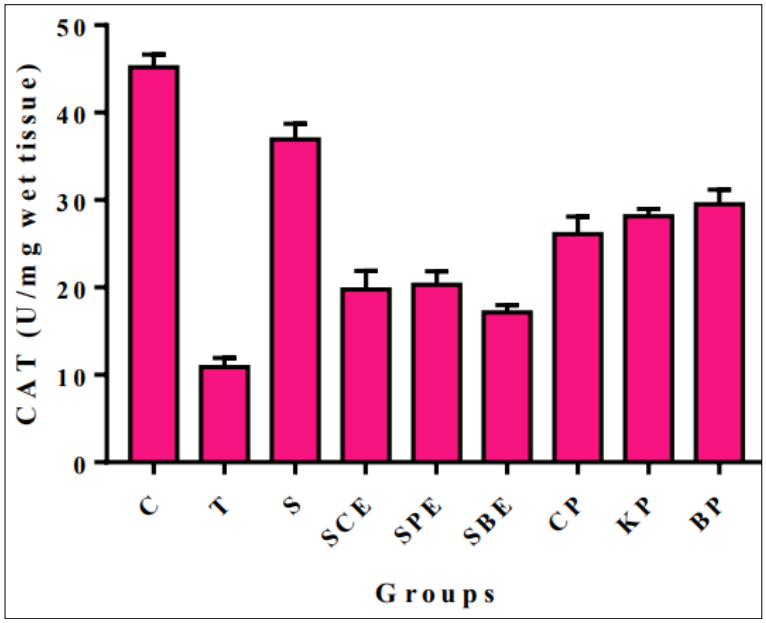

Fig 3: Effect of phytosomes on CAT levels in CCl_4 intoxicated rats

Reduced Glutathione (GSH)

The Ellman technique was used to measure reduced glutathione (GSH). After administering, there was a notable decline in glutathione activity CCl_4 0.38±0.039 mmol/mg connective tissue. The glutathione activities were considerably (p<0.05) elevated to 0.69±0.025, 0.66±0.025, and 0.67±0.025mmol/mg tissue when CP, KP, and PP were administered with CCl4.

Table 4: Effect of phytosomes on GSH levels in rats

S. N.	Group	GSH (mmol/mg tissue)
1.	Control	0.89±0.018
2.	CCl_4 treated	0.38±0.039
3.	Standard	0.77±0.048
4.	SCE	0.56±0.042
5.	SPE	0.57±0.075
6.	SBE	0.59±0.008
7.	CP	0.69±0.025
8.	KP	0.66±0.025
9.	BP	0.67±0.025

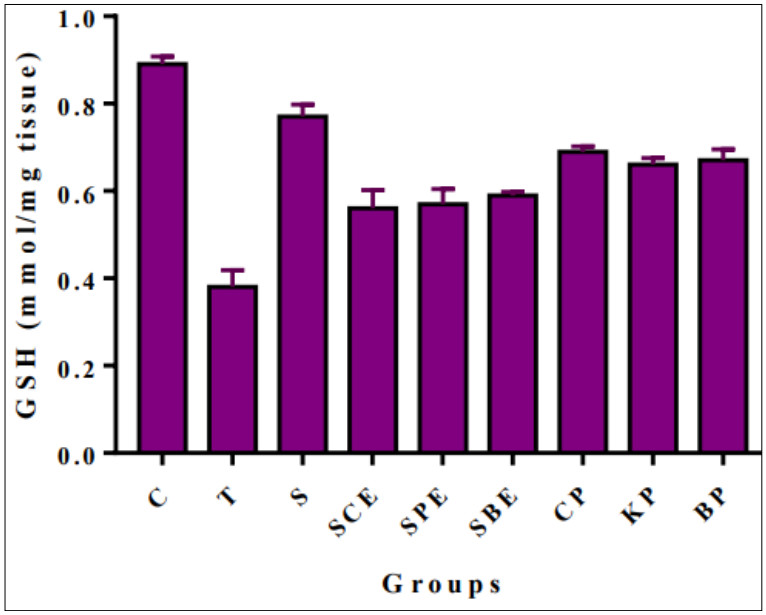

Fig 4: Effect of phytosomes on GSH levels in rats

Glutathione Peroxidase (GPx)

It was noted that the glutathione peroxidase level in CCl_4 was low, at 112.83±1.757 nmol/min. It is well-known that the selenium enzyme GPx is crucial for controlling the levels of H_2O_2 and other organic peroxides.Concentrations of 166.12±0.497, 164.27±1.14, and 168.27±1.497 nmol/min

Groups treated with had a substantially higher incidence (p<0.001) of CP, KP, and PP in addition to CCl_4.

Table 5: Effect of phytosomes on GPx levels rats

S. N.	Group	GPx Level nmol/min
1.	Control	183.09±3.551
2.	CCl_4 treated	112.83±1.757
3.	Standard	179.88±4.330
4.	SCE	151.52±2.213
5.	SPE	150.21±5.321
6.	SBE	153.75±8.401
7.	CP	166.12±0.497
8.	KP	164.27±1.142
9.	BP	168.27±1.497

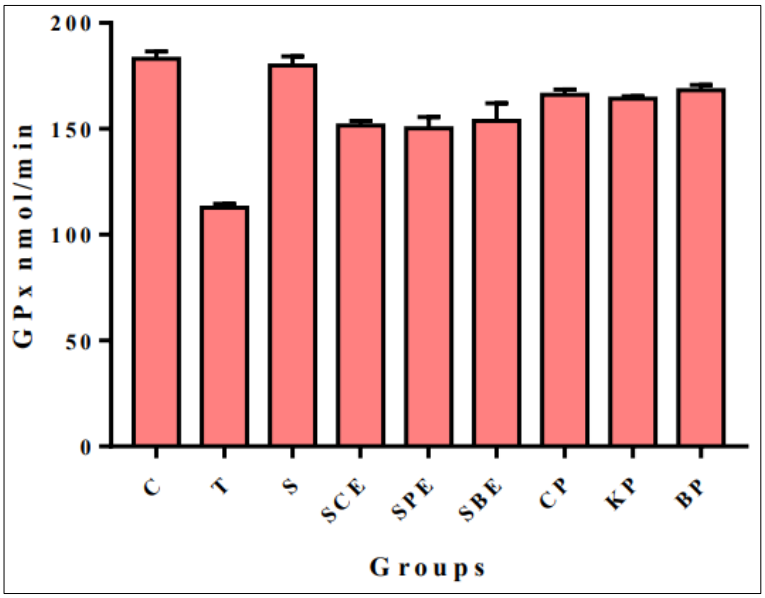

Fig 5: Effect of phytosomes on GPx levels in CCl_4 intoxicated rats

Conclusion

In vivo studies in mice serve as critical bridges between *in vitro* experiments and clinical trials, providing essential preclinical data to inform translational research efforts. By assessing antioxidant enzyme activity, oxidative stress biomarkers, antioxidant capacity, and histological changes *in vivo*, researchers can comprehensively evaluate the antioxidant properties of compounds and their impact on biological systems.

The findings from *in vivo* antioxidant studies in mice contribute to the advancement of antioxidant-based therapies and the development of novel therapeutic strategies for oxidative stress-related disorders. Moreover, these studies facilitate the identification of potential biomarkers for monitoring antioxidant efficacy and optimizing treatment regimens in clinical settings.

In conclusion, the analysis of *in vivo* antioxidant activity in mice plays a pivotal role in advancing our understanding of oxidative stress biology and harnessing the therapeutic potential of antioxidants for improving human health. Continued research in this area holds promise for the development of effective antioxidant interventions and the prevention or treatment of a wide range of oxidative stress-related diseases.

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