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Hypothesis Paper

MULTIPLE ACTIONS OF SUPEROXIDE DISMUTASE: WHY CAN IT BOTH INHIBIT AND STIMULATE REDUCTION OF OXYGEN BY HYDROQUINONES?

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Abstract—Superoxide dismutase can either inhibit or stimulate autoxidation of different hydroquinones. suggesting multiple roles for $O_2\cdot^-$. Inhibitory actions of superoxide dismutase include termination **of** 0,---propagated reaction chains and metal chelation by the apoprotein. Together, chelation of metals and termination of 0,---propagated chains can effectively prevent reduction of oxygen. Chain termination by superoxide dismutase can thus account for negligible accumulation of H_2O_2 without invoking a superoxide:semiquinone oxidoreductase activity for this enzyme. One *stimulatory* action **of** superoxide dismutase is to decrease thermodynamic limitations to reduction of oxygen. Whether superoxide dismutase inhibits or accelerates an autoxidation depends on the reduction potentials of the quinone and the availability of metal coordination for inner sphere electron transfers.

Keywords—Hydroquinone autoxidation, Superoxide dismutase, Oxygen reduction, Metal coordination, Inner sphere electron transfers. Chain propagation, Semiquinone, Free radicals

INTRODUCTION

Cadenas and coworkers have recently compiled a wealth of data on multiple effects of superoxide dismutase in hydroquinone autoxidations.[1,2] These data complement data of Winterbourn on autoxidation of pyrimidines.[3] The diverse, paradoxical, and sometimes contradictory actions of superoxide dismutase have prompted an equally wide range of explanations, including the novel suggestion that superoxide dismutase has superoxide:semiquinone oxidoreductase activity.[1] This suggestion rests on the observation that superoxide disniutase inhibits autoxidation of the hydroquinone derivatives of 2-hydroxy-*p*-benzoquinone and 1,4-naphthoquinones but prevents accumulation of H_2O_2, the product of superoxide dismutation. Arguing against this suggestion however, superoxide dismutase only inhibits autoxidation of 1,4-naphthohydroquinone in the early phase.[1] Superoxide dismutase *stimulates* the autoxidation when comproportionation of hydroquinone with quinone product becomes available to propagate the autoxidation.[4] Superoxide dismutase also does not inhibit, but accelerates oxidation of 1.2-naphthohydroquinone and 5-hydroxy-1,4-naphthohydroquinones.[1,2]

This wealth of recent data brings a need for some generalizations as to mechanisms by which superoxide dismutase can slow or accelerate autoxidations. Inhibitory actions of superoxide dismutase include termination of free radical chains and metal chelation by the apoprotein. Superoxide dismutase can also *accelerate* oxidations by at least three different mechanisms. We outline here some of the situations where superoxide dismutase might accelerate or slow hydroquinone oxidation. and offer possible explanations for some of the contrasting effects on different naphthohydroquinones.

METAL CHELATION CAN ACT SYNERGISTICALLY WITH SUPEROXIDE DISMUTASE ACTIVITY TO INHIBIT AUTOXIDAL'IONS

Most metal-chelating agents sensitize autoxidation of hydroquinone compounds to further inhibition by superoxide dismutase.[5,6] Metal chelators often inhibit autoxidations by decreasing the ability of the metal to

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gain or lose electrons. With a strong reductant such as 6-hydroxydopamine however, EDTA stimulates the trace metal catalyzed autoxidation[6] presumably by accelerating the (rate limiting) autoxidation of reduced iron. Desferrioxamine, a stronger chelator of ferric ions (thus decreasing the reduction potential further), slows autoxidation of 6-hydroxydopamine. Either chelator sensitizes 6-hydroxydopamine autoxidation to inhibition by superoxide dismutase.[6] By preventing formation of a ternary reductant-metal-oxygen complex, these chelators prevent sequential "inner sphere" transfer of 2 electrons and force release of $O_2\cdot^-$ and $Q\cdot^-$.[5,6] The $O_2\cdot^-$ and $Q\cdot^-$ released then serve as reaction propagators:

$$QH_2 + O_2\cdot^- \longrightarrow Q\cdot^- + H_2O_2$$

and

$$Q\cdot^- + O_2 \longrightarrow Q + O_2\cdot^-.$$

Since EDTA accelerates autoxidation of ferrous ions (and thus $O_2\cdot^-$ release) but slows reduction of ferric ions (and thus $Q\cdot^-$ formation), $O_2\cdot^-$ is the major propagator which accelerates the overall autoxidation in the presence of EDTA.

With 1,2,4-benzenetriol, both EDTA and desferrioxamine stimulated the autoxidation (although desferrioxamine caused a slight lag) (Fig. 1). Despite stimulating autoxidation of 1,2,4-benzenetriol when present alone, EDTA or desferrioxaiiiine sensitized the autoxidation to inhibition by small, catalytic quantities of superoxide dismutase. Together, metal chelation and superoxide dismutase activity can prevent consumption of oxygen (Fig. 2). Metal binding by proteins, including superoxide dismutase apoprotein, can also play this sensitizing role"' (Figs. 2 and 3). Such inhibition favors the hydroquinone form and also prevents accumulation of products, including H_2O_2, as found by Cadenas et al.[1] The concentrations of superoxide dismutase and total protein in the experiments of Cadenas et al.[1] on autoxidation of 1,2,4,-benzenetriol (present at 20 μM) were 3 μg/mL (9 U/mL) and 6.5 μg/mL (3.5 μg/mL NAD(P)H:quinone oxidoreductase stabilized with albumin). Trace metals were thus competitively bound by proteins in these experiments, and the autoxidation was sensitive to inhibition by superoxide dismutase. A superoxide:semiquinone oxidoreductase activity could prevent the autoxidation, but so could

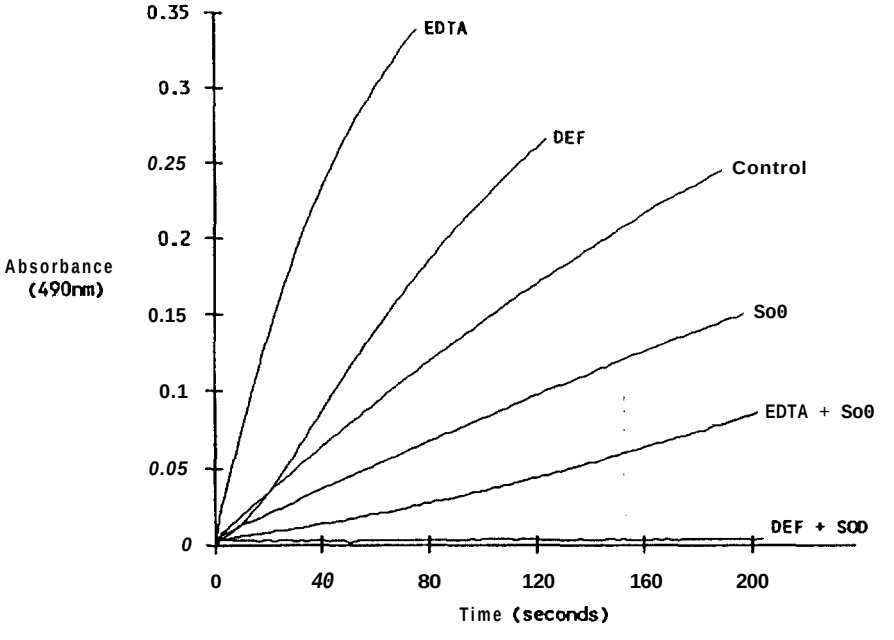

Fig. 1. 1,2,4-Benzenetriol autoxidation: Interactions between superoxide dismutase and metal chelators. Reactions were conducted in air-saturated, 50 mM phosphate buffer; pH 7.5; 25°C. Where indicated. EDTA or desferrioxaiiiine (DEF) were present at 0.5 mM; superoxide dismutase (SOD) at 0.5 μg/mL (2 U/mL). Reactions were initiated by addition of an aliquot from an anaerobically prepared stock solution of 1,2,4-benzenetriol to give an initial concentration of 250 μM. Formation of the p-quinone product of oxidation was followed spectrophotometrically at 490 nm.

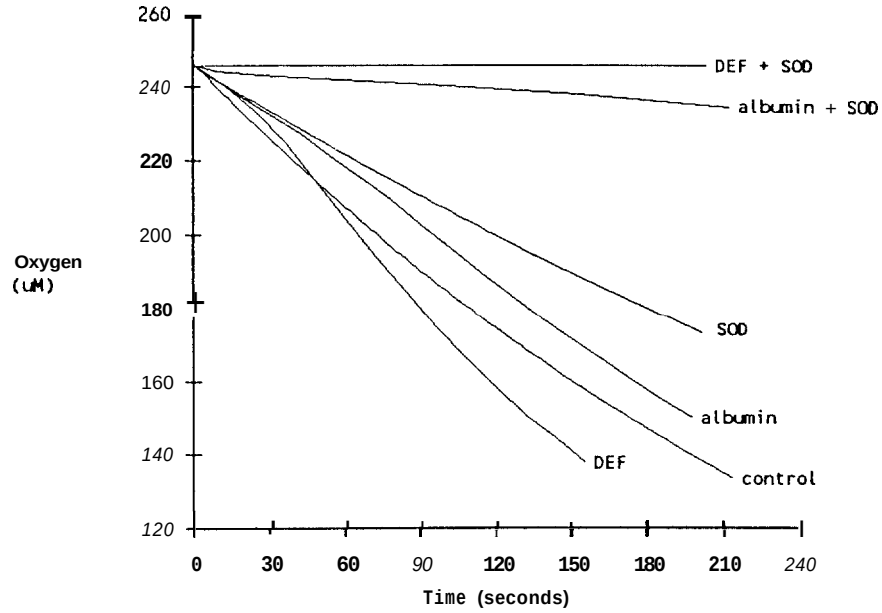

Fig. 2. 1,2,4-Benzenetriol autoxidation: Oxygen consumption in the presence of superoxide dismutase and metal chelators. Reaction conditions were as in Figure 1. Where indicated, albumin was present at 26 μg/mL. Other reagents were at the same concentrations as in Figure 1. Oxygen consumption was monitored polarographically upon addition of 1,2,4-benzenetriol.

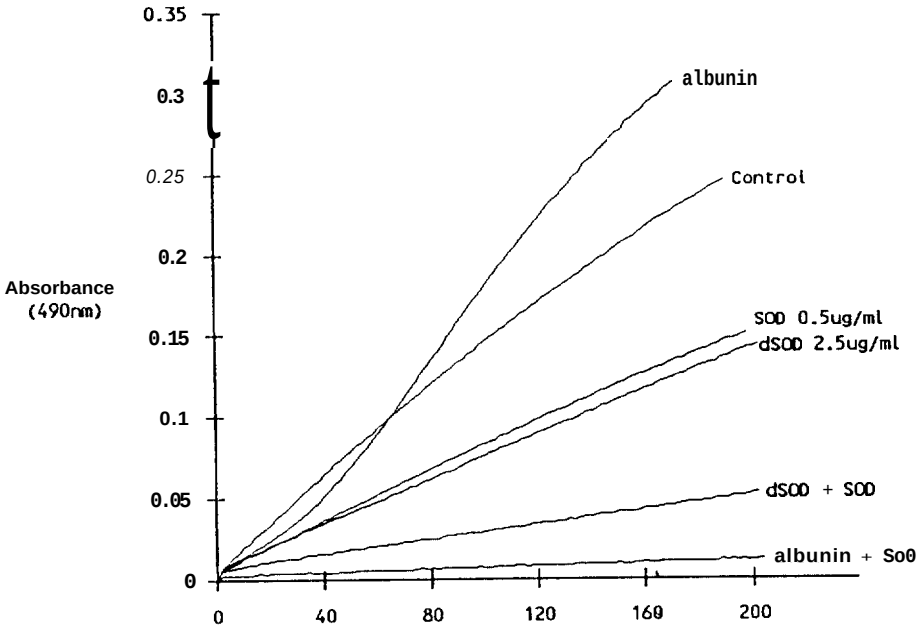

Fig. 3. 1,2,4-Benzenetriol autoxidation: Interactions between superoxide dismutase and proteins. Reactions were conducted in air-saturated, 50 mM phosphate buffer; pH 7.5; 25°C. Where indicated, superoxide dismutase (SOD) was present at 0.5 μg/mL (2 U/mL), heat-denatured (98% inactivated) superoxide dismutase (dSOD) at 2.5 μg/mL, and albumin at 26 μg/mL. Reactions were initiated by addition of an aliquot from an anaerobically prepared stock solution of 1,2,4-benzenetriol to give an initial concentration of 250 μM. Formation of the *p*-quinone product of oxidation was followed spectrophotometrically at 490 nm.

dismutation of $O_2\cdot^-$, by preventing propagation of the radical chain.

SUPEROXIDE DISMUTASE CAN INHIBIT AUTOXIDATIONS AND H_2O_2 FORMATION BY CHAIN TERMINATION

Inhibitory actions of superoxide dismutase can result from accelerated termination of free radical chains. Most if not all autoxidations are chain reactions. The main chain propagating action of superoxide in hydroquinone oxidation is:

$$QH_2 + O_2\cdot^- \longrightarrow Q\cdot^- + H_2O_2.$$

Inhibition by superoxide dismutase in autoxidations is usually considered evidence that superoxide is an intermediate in the free radical chain.[5,8] To the extent that oxidation of adrenaline is typical, superoxide has an amplification factor of up to 10.[5] In other words, one molecule of superoxide can stimulate oxidation of 10 molecules of adrenaline before its participation is ended through some chain termination reaction. On this basis, superoxide dismutase prevents the formation of 10 molecules for every half-molecule of H_2O_2 it manufactures. Thus, one explanation for the phenomena described[1] is that superoxide dismutase diminishes formation of H_2O_2 by facilitating chain termination. The combined effects of metal binding and chain termination can prevent oxygen consumption[9] and thus decrease H_2O_2 production to negligible levels.

METAL CHELATION BY PROXIMAL HYDROXYL GROUPS ALLOWS INNER SPHERE AUTOXIDATION

Why does superoxide dismutase not inhibit 1,2-naphthohydroquinone[1] or 5-hydroxy-1,4-naphthohydroquinone[1] oxidation? That the semiquinone is not a substrate for superoxide:semiquinone oxidoreductase was offered as one explanation for the lack of inhibition of the 1,2-naphthohydroquinone autoxidation by superoxide dismutase.[1] An alternative explanation is the difference in metal-binding capabilities of the different hydroquinones. The o-quinol with its adjacent phenolic hydroxyls binds metals more avidly than the (para) 1,4-napthohydroquinone. Thus the o-quinol can autoxidize by an inner sphere mechanism and is less influenced by relatively weak chelators such as proteins. The much higher concentrations of superoxide dismutase needed to inhibit autoxidation of the 2-hydroxy-1,4-napthohydroquinone (12 μg/mL, K_i = 540.8 nM)[1,2] than the 1,4-napthohydroquinone (0.3 μg/mL, K_i = 2.1 nM)[1,2] suggest more effective competition for binding of metals by the adjacent phenolic hydroxyls of 2-hydroxy-1,4-napthohydroquinone. Thus, as with autoxidation of 1,2,4-benzenetriol, sufficient protein to compete for trace metals can liniit inner-sphere electron transfers and sensitize the autoxidation to inhibition by superoxide dismutase. The 5-hydroxy-1,4-naphthohydroquinone derivatives form a six-membered ring with cations (thus showing strong intramolecular hydrogen bonding).[11] These derivatives would bind *metal cations* avidly, and thus react primarily by concerted inner sphere transfer of electrons to oxygen.

SUPEROXIDE DISMUTASE CAN ACCELERATE AUTOXIDATIONS BY SEVERAL MECHANISMS

Why might superoxide dismutase *stimulate* autoxidation of 1,2-naphthohydroquinone[1] and 5-hydroxy-1,4-naphthohydroquinones?[2] Stimulatory effects of superoxide disniutase on autoxidations are sometimes reported.[1,2,11,12,13] In general, they occur in reactions:

a) in which superoxide is a net terminator of free radical chains

$$Q\cdot^- + O_2\cdot^- + 2H^+ \longrightarrow Q + H_2O_2,$$

b) in which hydrogen peroxide is a better oxidant than oxygen

$$\text{eg. } QH_2 + H_2O_2 \longrightarrow Q\cdot^- + \cdot OH + OH^- + 2H^+$$
$$Q\cdot^- + H_2O_2 \longrightarrow Q + \cdot OH + OH^-.$$

or c) in which semiquinone or quinone formation is limited by accumulation of superoxide

$$QH_2 + O_2 \rightleftharpoons Q\cdot^- + O_2\cdot^-$$

or

$$Q\cdot^- + O_2 \rightleftharpoons Q + O_2\cdot^-.$$

These stimulatory effects require that they are not overpowered by metal chelation and chain termination by superoxide dismutase. Stimulatory effects of superoxide dismutase are most apparent when $O_2\cdot^-$-dependent propagation becomes less significant, such as when accumulation of quinone allows comproportionation with the hydroquinone.[4] By inhibiting chain propagation by $O_2\cdot^-$ and stimulating semiquinone autoxidation,[2,14] one biologically important effect of superoxide dismutase is to limit formation of semiquinones.

SUPEROXIDE DISMUTASE CAN ACCELERATE OXIDATIONS IN WHICH EQUILIBRIA FORMING SEMIQUINONES OR QUINONES ARE LIMITED BY THERMODYNAMIC CONSIDERATIONS

Superoxide disniutase can accelerate an autoxidation by displacing the equilibria: $Q\cdot^- + O_2 \leftrightarrow Q + O_2\cdot^-$ in the direction of quinone[2,13,14] or QH, $+ O_2 \leftrightarrow Q\cdot^- + O_2\cdot^-$ in the direction of semiquinone." This effect is not seen for strong reductants with reduction potentials low enough to minimize product inhibition by $O_2\cdot^-$ (such as 6-hydroxydopamine, 1,2,4-benzenetriol or 2-hydroxy-1,4-naphthohydroquinone). 1,2-Naphthohydroquinone is a weaker reductant than 1,4-naphthohydroquinone or 2-hydroxy-1,4-naphthohydroquinone ($E^{\circ\prime}(Q/QH_2) = +143$ mV vs. +36 mV and −139 mV)[15] and thus is more unfavorable for one-electron reduction of oxygen, increasing product inhibition and favoring acceleration by superoxide disinutase. Similarly, 5-hydroxy-1,4-naphthohydroquinone is a weaker reductant than 1,4-naphthohydroquinone ($E_{1/2} = -140$ mV vs. −180 mV)[16] and additionally binds metals strongly to favor inner-sphere electron transfers. 1,4-Benzohydroquinone would not bind metals as strongly as 1,2,4-benzenetriol, but is a much weaker reductant ($E^{\circ\prime}(Q/QH_2) = +280$ mV versus +106 mV),[15] and autoxidation was stimulated by superoxide dismutase."

The contrast between 1,2-naphthoquinone and 2-hydroxy-1,4-naphthoquinone resembles the contrast between the stimulation by superoxide dismutase of autoxidation of gallic acid[12] and inhibition of autoxidation of pyrogallol.[17] These compounds have identical o-hydroxy substitutes capable of binding metal ions. However, an electron withdrawing p-carboxyl substituent on gallic acid raises the reduction potential compared to pyrogallol, making it a weaker reductant. This effect slows autoxidatioii, and increases product inhibition.

Thus on the one hand, superoxide dismutase can stitiiulate 1,2-naphthohydroquinone autoxidation by decreasing product inhibition (i.e., the 2-hydroxy-1,4-napthohydroquinone is less affected by product inhibition because of the additional electron-donating hydroxy group). On the other hand, superoxide dismutase can inhibit autoxidation of 1,4-napthohydroquinone and 2-hydroxy-1,4-napthohydroquinone by metal chelation and chain terniination.

REDUCTION POTENTIALS FAVOR *OXIDATION* (RATHER THAN *REDUCTION*) OF SEMIQUINONE BY SUPEROXIDE

Finally, the proposed novel enzyme activity might be questioned on thermodynamic grounds. Semiquinone:superoxide oxidoreductase activity may be more feasible than the proposed superoxide:semiquinone oxidoreducfase activity. This is because enzymes have no effect on the overall free energies of the reactions they catalyze and the most stable products are the quinone and H_2O_2. On the ***Principle of Microscopic Reversibility,***[18] to the extent that superoxide dismutase accelerates the transfer of an electron from $O_2\cdot^-$ to $Q\cdot^-$, it will also facilitate transfer of an electron from $Q\cdot^-$ to $O_2\cdot^-$. In a reaction mixture in which both $O_2\cdot^-$ and $Q\cdot^-$ are present, the direction of the reaction, and thus the product distribution, depend on thermodynamic considerations. Such considerations more strongly favor the formation of Q and H_2O_2 than the reverse reaction. Although reduction of the semiquinone by superoxide may be thermodynamically feasible, other things being equal, oxidation of the semiquinone by superoxide is more favorable." Unless the enzyme were to act by a mechanism which favored one half-reaction over another (e.g., by specifically providing protons to one product), the thermodynaniics favor formation of quinone and H_2O_2.

CONCLUSION

The diversity **of** actions of superoxide disrnutase reflects the diversity of roles played by $O_2\cdot^-$. Superoxide propagates or terminates free radical chains, accelerates or retards oxidation of hydroquinones or semiquinones. The availability and coordination state of metals and the reduction potentials of the quinone and semiquinone all influence the roles which $O_2\cdot^-$ plays. Such diversity offers a range of explanations for paradoxical effects of superoxide dismutase which deserve exploration. In general, we conclude:

1. To the extent that reduction of oxygen occurs by sequential inner sphere electron transfers, superoxide dismutase will be without effect.
2. To the extent that reduction of oxygen by semiquinone is thermodynamically unfavorable, superoxide dismutase will accelerate hydroquinone autoxidation.
3. To the extent that reduction of oxygen by semiquinone is thermodynamically favorable, and

'An example is 1,4-naphthohydroquinone, the weakest reductant tested inhibitable by superoxide dismutase (therefore most thermodynamically favored for a semiquinone reductase activity). The two-electron reduction potential at pH 7 is +36 mV.[15,19] The one-electron reduction potentials for 1,4-naphthoquinone are also known ($E^{\circ\prime}(Q\cdot^-/Q^{2-}) = +212$ mV and $E^{\circ\prime}(Q/Q\cdot^-) = -140$ mV).[3] Thus, *reduction* of the semiquinone by superoxide ($E^{\circ\prime}(O_2/O_2\cdot^-) = -155$ mV at unit concentration) has a delta$E^{\circ\prime}$ of +367 mV. However, *oxidation* of the semiquinone by superoxide ($E^{\circ\prime}(O_2\cdot^-/H_2O_2) = +865$ mV) has a delta$E^{\circ\prime}$ of +1005 mV.

$O_2\cdot^-$ is the major propagating species, superoxide dismutase will inhibit hydroquinone autoxidation.

4. To the extent that comproportionation of hydroquinone and quinone is the major propagating pathway, superoxide dismutase will accelerate autoxidation.

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REFERENCES

1. Cadenas, E.; Mira. D.; Brumark, A.; Lind, C.; Segura-Aguilar. J; Ernster. L. Effect of superoxide dismutase on the autoxidation of various hydroquinones—a possible role of superoxide dismutase as a superoxide:semiquinone oxidoreductase. *Free Radical Biol. Med.* 5:71–79; 1988.
2. Ollinger, K.; Buffington. G. D.; Ernster. L.; Cadenas, E. Effect of superoxide dismutase on the autoxidation of substituted hydro- and semi-naphthoquinones. *Chem. Biol. Interact.* 73:53–76; 1990.
3. Winterbourn, C. C. Inhibition of the autoxidation of divicine and isouramil by the combination of superoxide dismutase and reduced glutathione. Arch. *Biochem. Biophys.* 271:447–455; 1989.
4. Ishii. T.: Fridovich, I. Dual effects of superoxide dismutase on the autoxidation of 1,4-naphthohydroquinone. *Free Radical Biol. Med.* 8:21–24; 1990.
5. Misra. H. P.; Fridovich, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide disniutase. *J. Biol. Chem.* 247:3170–3175; 1972.
6. Bandy, B.; Davison, A. I. Interactions between metals. ligands and oxygen in the autoxidation of 6-hydroxydopamine: mechanisms by which metal chelation enhances inhibition by superoxide dismutase. *Arch. Biochem. Biophys.* 259:305–3 15; 1987.
7. Lovstad, R. A. Copper catalyzed oxidation of ascorbate (vitamin C). Inhibitory effect of catalase, superoxide dismutase, serum proteins (ceruloplasmin. albumin, apotransferrin) and amino acids. frit. *J. Biochem.* 19:309–313; 1987.
8. Gee. P.: Davison, A. J. Intermediates in the aerobic autoxidation of 6-hydroxydopamine: relative importance under different reaction conditions. *Free Radical Bid. Med.* 6:271–284; 1989.
9. Gee. P.: Davison. A. I. 6-Hydroxydopamine does not reduce oxygen directly but requires a co-rcdrictnnt. *Arch. Biochem. Biophys.* 231:164–168; 1984.
10. Mukherjee. T. One-electron reduction of juglone (5-hydroxy-1,4-naphthoquinone): a pulse radiolysis study. *Radiat. Phys. Chem.* 29:455–462; 1987.
11. Greenlee, W. F.; Sun, J. D.; Bus. J. S. A proposed mechanism of benzene toxicity: formation of reactive intermediates from polyphenol metabolites. *Toxicol. Appl. Pharmacol.* 59:187–195; 1981.
12. Fatur. D. The effect of naturally occurring phenolics on the activity of ornithine decarboxylase in C3H IOT 1/2 cells: possible involvement of active species of oxygen. Simon Fraser University. Burnaby. B.C., Canada. 1988. 99–116. M.Sc. Thesis.
13. Ishii. T.; Iwahashi. H.; Sugata, R.: Kido. R.: Fridovich. I. Superoxide dismutases enhance the rate of autoxidation of 3-hydroxyanthranilic acid. *Arch. Biochem. Biophys.* 276:248–250; 1990.
14. Winterbourn. C. C. Cytochrome *c* reduction hy seiiiiquinone radicals can be indirectly inhihited by superoxide disniutase. *Arch. Biochem. Biophys.* 209:159–167; 1981.
15. Loach. P. A. Oxidation-reduction potentials. In: Fasman, G. D., ed. *Handbook of biochemistry and molecular biology*. Physical and Chemical Data. 3rd ed. vol. 1. Cleveland: Chemical Rubber Company; 1976:122–130.
16. Buffington, G. D.; Ollinger. O.: Brunmark. A.; Cadenas. E. DT-diaphorase-catalysed reduction of 1,4-naphthoquinone derivatives and glutathionyl-quinone conjugates. Effect of substituents on reaction rates. *Biochem. J.* 257:561–571: 1989.
17. Marklund. S.; Marklund, G. Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide-disniutase. *Eur. J. Biochem.* 47:469–474; 1974
18. Moore. W. J. *Physical chemistry*, 4th ed. Englewood Cliffs, NJ: Prentice-Hall Inc.; 1972:342.
19. Morton. R. A. Introductory account of quinones. In: Morton. R. A.. ed. *Biochemistry of quinones*. London: Academic Press: 1965: 1–22.