Role of non-enzymatic antioxidants in stimulation of Metallothionein against metal toxicity

Dharmendra Kumar
Toxicology Lab, Dept. of Zoology, Govt. P.G. College, Lohaghat -262524, (Champawat), Uttarakhand, India.
dkrathod71@gmail.com
doi:10.6088/ijes.00202030044

ABSTRACT

Metallothionein (MT) is a low molecular-weight protein considered to be protective in function. It has been estimated in the liver and kidney of cadmium, mercury, copper and zinc treated rats offered antioxidative protection by selenium (Se), vitamin E and glutathione (GSH). These antioxidants stimulated induction of MT in liver and kidney of metal treated rats. Induction was greater in liver than kidney. α-tocopherol induced maximum synthesis of MT in the liver of mercury treated rats during lipid peroxidation, whereas maximum induction of MT in Cd fed rats was observed after GSH treatment. Minimum accumulation of mercury in comparison to other metals was observed in liver as well as kidney in glutathione treated rats. It is suggested that different metal species induced specific metallothionein(s) that express specific effects. The mechanism for maintaining high levels of MT might result from increased translation and transcription of MT. Present results suggest that these proteins not only scavenge free radicals (FR) but also decrease accumulation of metals in soft tissue.

Keywords: Metallothionein (MT), Glutathione (GSH), α –Tocopherol (Vitamin E), Selenium (Se), Cadmium (Cd), Mercury (Hg), Copper (Co), Zinc (Zn).

1. Introduction

A wide variety of inorganic and organic chemicals viz: cadmium, mercury, zinc carbon-tetrachloride, t-butylhydroperoxide (t-BHP), adriamycin as well as radiation and other oxidant stressors are known to induce metallothionein (Clarke 1986, Min 1991, Koropatnick 1989, Bauman 1991,). Metallothioneins (MT) are low molecular weight cysteine rich metal binding proteins that play an important role in homeostasis of trace elements and detoxication of poisonous metals i.e. cadmium and mercury (Cousins 1985, Bremner 1990). A few investigations have postulated that MT can effectively scavenge reactive oxygen species (ROS) (Thornalley 1985, Sato 1993,). Thus, MTs also share this property with antioxidants viz: selenium, α-tocopherol reduced glutathione (GSH), ascorbic acid and carotenoids that manifest protective influences by scavenging free radicals. The possibility of a relationship between antioxidants and metallothionein deserves further attention. Therefore, a study on the effects of a few well established antioxidants i.e. selenium, GHS and α-tocopherol on induction of MT against metal toxicity in liver and kidney of laboratory rats (Rattus rattus albino) chronically treated with few environmentally significant metals i.e. cadmium (Cd), mercury (Hg), copper (Cu) and zinc(Zn) was undertaken.

2. Materials and Method

2.1 Chemicals
Reduced glutathione (GSH), α-tocopherol, thiobarbituric acid, BSA and 1,1,3 tetramethoxypropane were procured from Sigma Chemical Co. (USA). Sodium selenite was supplied by the loba chemie. Cadmium chloride, mercuric chloride, zinc chloride and copper sulphate were obtained from Merck (India). All other chemicals used in this study were of AR grade or of highest purity available commercially.

2.2 Experimental procedure

Male Wistar rats (body weight 150 ± 10 gm) were produced from the Animal Facility of All India Institute of Medical Science, New Delhi. Each rat was maintained under standard laboratory condition (room temperature, 25 ± 5°C and relative humidity, 60 ± 10%) in a polypropylene cage and offered commercial food pellets (Lipton, India) and tap water *ad libitum*.

After brief acclimatization to laboratory conditions, the rats were divided at random into four major groups (A,B,C,D), each containing 25 rats. Each group from A to D was further divided into subgroups 1 to 5 and subjected to treatment with metals and antioxidants as described earlier (Rana, 1996).

Briefly, the rats of subgroups 2-5 of group A were administrate predetermined sublethal dose of cadmium chloride (5mg /100gm body weight) by gavage on each alternate day for 30 days. Similarly, rats of groups of B were administered mercuric chloride (5mg /100gm body weight). Rats of group C were treated with copper sulphates (5mg /10gm body weight) and rat of group D were few fed on Zinc chloride (5mg /10gm body weight) on each alternate day for 30 days in morning hours. Rat of subgroup (1) of all the groups served as controls.

Three antioxidants selected for present investigations were carefully administered to the rats of subgroups (iii) – (v) of each group. Briefly, the rats of subgroup (iii) of all the groups were intraperitoneally injected reduced glutathione (30 mg/ 100g body weight) alternate to the day of mettle treatment. Similarly all the rats of subgroups (iv) were offered α – tocopherol (0.4 IU/100g body weight as a suspension in sucrose) on each alternate day by gavage. Rats of subgroup (v) of all the groups were administered selenium as sodium selenite by gavage (15mg/100g body weight) alternate to the day of metal treatment. Rats of subgroups (i) of all the groups were offered saline only through gavage and treated as controls. Each treatment lasted 30 days, while rats of subgroup (ii) of all groups were offered selected metal accordingly.

After scheduled treatment, the rats were starved overnight and decapitated next morning. Samples of liver and kidney were carefully removed and processed for the estimation of MT in microsomal lipid peroxidation, tissue metal load and metallothionein.

3. Estimations

3.1 Microsomal Lipid Peroxidation

Microsomal lipid peroxidation was assayed following the method of Jordan and Schenkman (Jordan, 1982). The microsomes were separated following the calcium method (Schenkman, 1978). The formation of thiobarbituric acid reactive substances (TBARS) was screened at 532nm using a spectrophotometer (Systronics, India). 1,1,3 tetramethoxypropane was used as the standard. Microsomal protein was determined following the method suggested by Lowry and coworkers (Rana, 1978).
3.2 Induction of Metallothionein (MT)

Metallothionein concentration was determined by the silver saturation method (Rana, 1997). The reproducibility and accuracy of this method was tested by analyzing purified horse kidney metallothionein (Sigma, USA).

3.3 Accumulation of heavy metals

1. Cadmium, zinc and copper analyses

Samples of 0.5 - 1.0g dried tissue (liver and kidney) were acid digested in 10ml concentrated nitric acid on a hot plate by first soaking and 100°C for two hours and boiling at 120°C for 20 min. Samples were diluted to 15ml using double distilled water. Metal concentration were analysed by inductively coupled plasma emission spectrophotometry at USIC, University of Roorkee.

2. Mercury analyses

Total mercury in acid digested samples were determined by a cold vapour technique using a mercury analyzer.

4. Statistical Analyses

Inter groups comparisons were made using student’s “t” test (Henry, 1994).

Table 1: Influence of metallothionein on microsomal lipid peroxidation (n moles MDA / mg) in metal fed rats

| Group No. | Treatments                        | Liver            | Kidney           |
|-----------|----------------------------------|------------------|------------------|
| A (i)     | Control                           | 0.512 ± 0.08     | 0.275 ± 0.012    |
| (ii)      | Cadmium                           | 1.812 ± 0.04***  | 0.842 ± 0.10***  |
| (iii)     | Cadmium + GSH                     | 0.463 ± 0.01*    | 0.280 ± 0.04 **  |
| (iv)      | Cadmium + α - tocopherol          | 1.181 ± 0.05***  | 0.526 ± 0.03**   |
| (v)       | Cadmium + Selenium                | 0.585 ± 0.04 NS  | 0.235 ± 0.1**    |
| B (i)     | Control                           | 0.535 ± 0.13     | 0.305 ± 0.09     |
| (ii)      | Mercury                           | 1.640 ± 0.12***  | 0.784 ± 0.07***  |
| (iii)     | Mercury + GSH                     | 0.428 ± 0.02*    | 0.362 ± 0.05 NS  |
| (iv)      | Mercury + α - tocopherol          | 1.184 ± 0.11***  | 0.460 ± 0.03**   |
| (v)       | Mercury + Selenium                | 0.729 ± 0.04***  | 0.395 ± 0.01**   |
| C (i)     | Control                           | 0.498 ± 0.10     | 0.298 ± 0.09     |
| (ii)      | Copper                            | 0.960 ± 0.15*    | 0.460 ± 0.04***  |
| (iii)     | Copper + GSH                      | 0.263 ± 0.07**   | 0.230 ± 0.03 NS  |
| (iv)      | Copper + α - tocopherol           | 1.513 ± 0.14***  | 0.395 ± 0.04*    |
| (v)       | Copper + Selenium                 | 0.856 ± 0.09**   | 0.265 ± 0.01 NS  |
| D (i)     | Control                           | 0.525 ± 0.13     | 0.363 ± 0.03*    |
| (ii)      | Zinc                              | 0.860 ± 0.05***  | 0.230 ± 0.05 NS  |
| (iii)     | Zinc + GSH                        | 0.263 ± 0.01***  | 0.395 ± 0.04*    |
| (iv)      | Zinc + α - tocopherol             | 0.955 ± 0.18*    | 0.362 ± 0.01**   |
| (v)       | Zinc + Selenium                   | 0.428 ± 0.02*    |                  |

Values are mean ± SE of 5 observations in each group.
‘p’ = * < 0.02, ** < 0.01, *** < 0.001 (Control versus experimental rats). NS denotes not significant.

### Table 2: Influence of antioxidants on induction of metallothionein (Cd ppm) in liver and kidney fed rats

| Group No | Treatments | Liver       | Kidney       |
|----------|------------|-------------|--------------|
| A (i)    | Control    | 0.95 ± 0.130| 1.09 ± 0.38  |
| (ii)     | Cadmium    | 1.80 ± 0.120***| 2.15 ± 0.54***|
| (iii)    | Cadmium + GSH | 2.20 ± 0.140***| 3.15 ± 0.07***|
| (iv)     | Cadmium + α - tocopherol | 1.98 ± 0.060***| 2.39 ± 0.12***|
| (v)      | Cadmium + Selenium | 2.55 ± 0.095***| 2.95 ± 0.75***|
| B (i)    | Control    | 0.80 ± 0.45  | 0.90 ± 0.58  |
| (ii)     | Mercury    | 0.95 ± 0.021NS| 1.15 ± 0.82NS|
| (iii)    | Mercury + GSH | 0.98 ± 0.070NS| 2.30 ± 0.96***|
| (iv)     | Mercury + α - tocopherol | 2.45 ± 0.045***| 2.40 ± 0.10***|
| (v)      | Mercury + Selenium | 5.90 ± 0.130***| 2.75 ± 0.10***|
| C (i)    | Control    | 0.10 ± 0.14  | 1.30 ± 0.36  |
| (ii)     | Copper     | 2.00 ± 0.09***| 4.85 ± 0.09***|
| (iii)    | Copper + GSH | 2.25 ± 0.11***| 2.28 ± 0.09***|
| (iv)     | Copper + α - tocopherol | 2.15 ± 0.07***| 3.95 ± 0.11***|
| (v)      | Copper + Selenium | 1.55 ± 0.08***| 3.15 ± 0.08***|
| D (i)    | Control    | 0.12 ± 0.35  | 1.03 ± 0.80  |
| (ii)     | Zinc       | 2.10 ± 0.45* | 5.00 ± 0.22***|
| (iii)    | Zinc + GSH | 2.75 ± 0.24**| 3.05 ± 0.09***|
| (iv)     | Zinc + α - tocopherol | 3.15 ± 0.30***| 3.65 ± 0.10***|
| (v)      | Zinc + Selenium | 2.15 ± 0.41**| 4.75 ± 0.11***|

Values are mean ± SE of 5 observations in each group.

‘p’ = * < 0.02, ** < 0.01, *** < 0.001 (Control versus experimental rats). NS denotes not significant.

### 5. Results

1. Observations on microsomal lipid peroxidation suggest that treatments with selected metals could cause significant peroxidative damage to liver and kidney of rats. Highest concentration of microsomal peroxides was recorded after cadmium treatment followed by mercury, copper and zinc respectively. Antioxidants offered protection by inducing MT, however, it varied with different antioxidants. Glutathione (GSH) offered maximum protection by synthesizing MT in the liver of cadmium fed rats followed by mercury, copper and zinc. In kidney too, MT also provide maximum defence in mercury fed rats on administration with GSH, whereas selenium offered highest protection in liver of copper fed rats. (Table -1).

2. Treatments of rats with antioxidants at the selected dose and duration with cadmium, mercury, copper and zinc stimulated the induction of metallothionein in liver as well kidney. Zinc promoted maximum induction of this protein in liver as well as kidney. Zinc promoted maximum induction of this protein in kidney as well as liver. Co-treatments with antioxidants further stimulated the induction of MT. Selenium resulted into maximum induction of MT in the liver of cadmium and mercury fed rats.
In zinc fed rats, GSH caused maximum induction of MT, while, α-tocopherol also express maximum influence of MT in zinc fed rats.

Induction of metallothionein was found to be greater in liver than kidney. Moreover, influence of antioxidants of MT in kidney was also differ from liver. Selenium induces maximum synthesis of MT in the kidney of zinc and kidney of mercury treated rats. However, maximum synthesis of MT in kidney of cadmium fed rat was observed after glutathione treatment. α-Tocopherol offered maximum protection in the kidney of copper fed rats by synthesizing MT (Table-2).

3. Present observations show preferential accumulation of cadmium in kidney than liver. Treatment with selected antioxidants restricted the accumulation of heavy metals by producing MT. However, most favorable results in induction of MT were obtained after selenium treatment. Minimum accumulation of mercury in the liver was recorded after glutathione treatment, followed by selenium and α-tocopherol. However, in kidney, minimum accumulation was observed after selenium treatment followed by glutathione and α-tocopherol in mercury fed rats. Copper behaved differently than cadmium and mercury. Accumulation of copper was higher in liver than kidney. Minimum accumulation of copper in liver was noticed after selenium treatment. However, in kidney, minimum accumulation of copper occurred after glutathione treatment. Similarly liver was also found to be a preferential organ for zinc than kidney. Highest restriction to its accumulation in liver and kidney both was offered by selenium. These result suggest that antioxidants change the cumulative behavior of these elements by synthesizing of MT at different levels. (Table-3).

Table 3: Influence of metallothionein on accumulation (µ gm / gm dry tissue) of heavy metals in liver and kidney rats

| Group No. | Treatments | Liver | Kidney |
|-----------|------------|-------|--------|
| A (i)     | Control    | ND    | ND     |
| (ii)      | Cadmium    | 6.22 ± 0.55 | 9.11 ± 0.64 |
| (iii)     | Cadmium + GSH | 1.25 ± 0.25 | 1.56 ± 0.36 |
| (iv)      | Cadmium + α-tocopherol | 1.56 ± 0.36 | 1.71 ± 0.57 |
| (v)       | Cadmium + Selenium | 1.51 ± 0.28 | 1.62 ± 0.45 |
| B (i)     | Control    | ND    | ND     |
| (ii)      | Mercury    | 3.03 ± 0.71 | 7.63 ± 2.65 |
| (iii)     | Mercury + GSH | 0.55 ± 0.05 | 0.92 ± 1.02 |
| (iv)      | Mercury + α-tocopherol | 2.15 ± 0.62 | 4.37 ± 1.15 |
| (v)       | Mercury + Selenium | 0.68 ± 0.50 | 0.82 ± 0.95 |
| C (i)     | Control    | 2.03 ± 0.10 | 1.83 ± 0.12 |
| (ii)      | Copper     | 4.19 ± 0.18*** | 3.27 ± 0.15*** |
| (iii)     | Copper + GSH | 2.37 ± 0.12NS | 1.78 ± 0.08NS |
| (iv)      | Copper + α-tocopherol | 1.77 ± 0.06* | 1.85 ± 0.06NS |
| (v)       | Copper + Selenium | 1.38 ± 0.09** | 1.99 ± 0.08* |
| D (i)     | Control    | 2.42 ± 0.13 | 3.27 ± 0.19 |
| (ii)      | Zinc       | 5.14 ± 0.21*** | 4.75 ± 0.23* |
| (iii)     | Zinc + GSH | 2.65 ± 0.18NS | 2.87 ± 0.17NS |
| (iv)      | Zinc + α-tocopherol | 1.86 ± 0.15** | 2.16 ± 0.11*** |
| (v)       | Zinc + Selenium | 1.04 ± 0.09*** | 1.14 ± 0.13*** |
Values are mean ± SE of 5 observations in each group.

'p' = * < 0.02, ** < 0.01, *** < 0.001 (Control versus experimental rats).

NS denotes not significant.

Group A: Distribution of Cadmium in liver and kidney.
Group B: Distribution of Mercury in liver and kidney.
Group C: Distribution of Copper in liver and kidney.
Group D: Distribution of Zinc in liver and kidney.

5.1 Discussion

Present results emphasize that antioxidants not only inhibit lipid peroxidation but also do modify the metal component and induced MT synthesis in soft tissue like liver and kidney. Involvement of antioxidative activity in the mobilization of metal component has been suggested earlier also (Hamer, 1986). However, the antioxidants seemingly do not follow identical mechanisms. Se manifests protection by forming an antagonistic interaction with the metal (Piscator, 1964). This interaction is brought about by endogenous glutathione that reduces selenite to a selenide compound (Nordberg, 1972). The high lipo-affinity of this compound may alter their distribution in critical tissue. We further postulate that GSH can work as a chelating agent like methionine as suggested earlier (Kojima, 1987). Peptide bonds analogous to the biuret reaction can be formed. A low metal GSH ratio allow the metal to associate with sulphhydryl groups, possible as GS-Me-GS (Sato, 1994). Vitamin E functions as a specific lipid soluble antioxidant 9Dun, 19870. Like other biological reductants, α-tocopherol might function through redox-cycles which deliver reducing equivalents for antioxidant reactions and maintained membrane α-tocopherol. Related studies indicate that the enzyme mediated antioxidant action of GSH in rat liver Microsomes depends, at least in part, on the α-tocopherol status of the membranes. Experimental evidences for the reaction of GSH with the α-tocopherol semiquinone radical has also been reported (Lowry, 1951). Further study on GSH-dependent antioxidant activity in biological membranes may reveal a system whose function complements that of α-tocopherol.

There exists large evidence suggesting that these metals deplete GSH in target tissue (Onosaka 1982, Fisher 1950). A large reduction in GSH could be related to MT synthesis. Species and tissue differences in MT are known in the literature (Fliss, 1992). The mechanism for maintaining high level of metallothionein might result from increased transcription and translation of MT. There may also be differences in the degradation rate of MT-mRNA or the protein itself in different species. Further, it has been suggested that metallothionein play a role as free radical scavengers (Sato, 1993). This property is supported by the fact that one-third of the amino acids found in MT are cyssteines. MT has the abundance of sulphhydryl groups which are extremely important in the functioning of these proteins (Rana, 1992). Metal-thiolate clusters on MT might scavenge hydroxyl radicals (Thornalley, 1985). The metal ions are ligated in cysteine on clusters. Further a role of MT on amino acid transport analogous to GSH has been proposed. It has also been postulated that Metallothionein acts as a detoxifying agent for to metal (Iwata 1981, Chaberel 1959). MT functions homeostatically as a reservoir, able to sequester and/or donate its metal e.g. Zn or Cu intracellularly to metals requiring metallothionein (Albro, 1986).

Yet there is no direct explanation for the increased of MT by antioxidants. Interleukin-1 and interferon treatments also induce MT (Chen, 1993). Harmonal changes including glucagons and gluco-corticoids also increase MT (Chen, 1993). Hormonal changes including glucagon and gluco-corticoids also increase MT synthesis (Niki, 1991). All these experimental
Role of Non-Enzymatic Antioxidants in Stimulation of Metallothionein against Metal Toxicity

Dharmendra Kumar
International Journal of Environmental Sciences Volume 2 No.3, 2012

evidences tend to suggest that these proteins are a part of protective system, however, the primary role of MT still remain controversial.

6. References

1. Albro P.W., Corbett. J.T., and Shroeder, J.L., (1986), Generation of hydrogen peroxide by incidental metal ion catalyzed autoxidation of glutathione, J. Inorg. Biochem., 27, pp 191-203.

2. Bauman J.W., Liu P., and Klaassen C.D., (1991), Increase in metallothionein produced by chemicals that induce oxidative stress, Toxicol. Appl. Pharmacol., 110, pp 347-354.

3. Bremner J., and Beattie. J.H., (1990), Metallothionein and trace minerals, Annu. Rev. Nutr, 10, pp 63-83.

4. Chen H.M., and Cherian M.G., (1993), Metallothionein isoforms on different mammalian species, Toxicologist, 13, pp 575.

5. Chaberal S., and Mertell A. E., (1959), Organic sequestering agents, John Wiley & Sons, N.Y. pp 548.

6. Clarke J.S., and Lui, E.M.K., (1986), Interaction of metallothionein and carbon-tetrachloride on the protective effect of zinc on hepatotoxicity, Can. J. Physol. Pharmacol., 64, pp1104-1110.

7. Cousins R.J., (1985), Absorption, transport, and hepatic metabolism of copper and zinc. Special reference to metallothionein and ceruloplasmin, Physiol. Rev., 65, pp 238-309.

8. Dunn M.A., Blalock T.A., and Cousins, R.J., (1987), Metallothionein, Proc. Soc. Exp. Biol. Med., 185, pp 107-119.

9. Fisher R. A., (1950), Statistical methods for research workers 11th ed. Oliver and Boyd, London.

10. Fliss H., and Menard M., (1992), Oxidant induced mobilization of zinc from metallothionein, Arch. Biochem. biophys, 293, pp 195-199.

11. Hamer D.HJ., (1986), Metallothionein. Annu. Rev. Biochem., 55, pp 913-951.

12. Henry R.B., Liu J., Choudhari S., and Klaassen C.D., (1994), Species variation in hepatic metallothionein, Toxicology Letters., 74, pp 23-33.

13. Iwata H. et. Al., (1981), Involvement of tissue sulphydryl in the formation of a complex of methyl mercury with selenium, Biochem. Pharmacol., 30, pp 3159-3163.

14. Jordan R. A. and Shenkman J.B., (1982), Relationship between malondialdehyde production and arachidonic consumption during NADPH – supported microsomal lipid peroxidation, Biochem. Pharmacol, 31, pp 1393-1400.
15. Kojima Y., and Kagi J.H.R., (1987), Metallothioneins II, Experientia, Suppl., Birkhauser, Basel.

16. Koropatnick J., Leibrandt M., and Cherain M.G., (1989), Organ specific metallothionein induction in mice by X-irradiation, Radiant Res., 119, pp 356-365.

17. Lowry O.H., Rosebrough, N. J., Farr. A. L., and Randall R. J., (1951), Protein measurement with folin phenol reagent, J. Biol. Chem., 193, pp 265-275.

18. Min K., Terano Y., Onosaka S., and Tanaka K., (1991), Induction of hepatic metallothionein by non-metallic compounds associated with acute phase response in inflammation, Toxicol. Appl. Pharmacol., 111, pp 152 -162.

19. Niki, E., (1991), Antioxidants compounds. In K.A.J. Davies (Ed). Oxidative damage and repair. Chemical, biological and medical aspects. Elmsford. N.Y. Pergamon Press pp 57-64.

20. Nordberg G.F., (1972), Cadmium metabolism and toxicity, Environ. Physiol. Biochem., 2, pp 7-36.

21. Onosaka S., and Cherian M.G., (1982), Comparison of metallothionein determination of polarographic and cadmium saturation methods, Toxicol. Appl. Pharmacol., 63, pp 270-274.

22. Piscator M., (1964), On cadmium in normal human kidney together with a report on the isolation of metallothionein from liver of cadmium exposed rabbits, Nord. Hy. Tidskr, 45, pp 76-82.

23. Rana S.V.S., (1997), Oxidative stress and liver injury by environmental xenobiotics. In “Liver and Environmental Xenobiotics” Rana S.V.S., and Taketa K. eds. Springer Verlag, Berlin, Heidelberg and New York, pp. 114-134.

24. Rana S.V.S., and Boora P.R., (1992), Anti-peroxidative mechanisms offered by selenium against Liver injury caused by cadmium and mercury in rat, Bull. Env. Cont. Toxicol., 48, pp 120-124.

25. Rana S.V.S., and Verma S., (1996), Protective effect of GSH, vitamin E, and selenium on lipid peroxidation in Cd fed rats, Trace. Elem. Res., 51, pp 161-168.

26. Rana S.V.S., and Verma S., (1997), Protective effects GSH, α-tocopherol and selenium on lipid peroxidation in liver and kidney of copper fed rats, Bull. Env. Cont. & Toxicol., 59, pp 152-158.

27. Sato M., and Bremner I., (1993), Oxygen free radicals, and metallothionein, Free Rad. Biol. Med., 14, pp 325-337.

28. Sato M., Sasaki M., and Hojo H., (1994), Differential induction of metallothionein synthesis by interlukin-6 and tumor necrosis factor- α, Int. J. Immunopharmacol., 16, pp 187-195.

29. Schenkman J.B., and Cinti D.L., (1978), Preparation of microsomes with calcium. Methods in Enzymology, 52, pp 83-88.
30. Thornalley P. J., and Vasak M., (1985), Possible role for metallothionein in protection against radiation induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals, Biochem. Biophys. Acta., 872, pp 448-461.

31. Valko.M, H. Morris, M.T.D., (2005), Cronin, Metals, Toxicity and Oxidative Stress, Current Medicinal Chemistry, 12, 1161-1208.