World Journal of Medical Sciences 7 (4): 270-275, 2012
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2012.7.4.1111

The Role of Cadmium Exposure on Spontaneous Abortion

[1]Aziza A. Saad, [2]Noha M. Hegazy [2]Nagat Amer, [3]Khaled Gaber,
[1]Amany I. Youssef, [2]Nevin E. Sharaf and [2]Naela A.M. Diab

[1]Applied Medical Chemistry, Medical Research Institute, Alexandria University, Egypt
[2]Envionmental and Occupational Medicine, National Research Center, Dokki, Giza, Egypt
[3]Prenatal Genetic Diagnosis, Department of Genetics, National Research Centre, Dokki, Giza, Egypt

Abstract: Cadmium is extremely toxic metal occurring in the environment naturally and as a pollutant emanating from industrial and agricultural sources. Overexposure may occur even in situations where trace quantities of cadmium are found. Food is the main source of cadmium intake in the non-smoking population. The toxicity is affected by several factors including nutritional status. The study aimed to evaluate the potential effect of environmental exposure to cadmium on pregnancy outcome and to elucidate the mechanism of action, by which cadmium induces spontaneous abortion. A total of one hundred and twenty volunteer women were recruited from the prenatal genetic diagnosis clinic of the National Research Center, Dokki, Egypt. The study subjects were divided into four groups. The first group included 30 healthy fertile women (control group), had no history of spontaneous abortion. Group II, III, IV involved 27, 23 and 40 subjects respectively with medically unexplained recurrent spontaneous abortion. The determination of (i) blood cadmium level; (ii) plasma malondialehyde (MDA) as a biomarker for detection of oxidative stress; (iii) serum glutathione (GSH) and glutathione peroxidase (GPx) as a biomarkers of antioxidant defense mechanisms, were measured for all the women of the study. The three studied groups of aborted women were suffering from cadmium exposures an environmental pollutant. This exposure induced elevated levels of plasma malondialdehyde associated with decreased levels of antioxidants glutathione and glutathione peroxidase. In the studied groups, the female reproductive health status had been deteriorated by cadmium exposure; this exposure could enhance lipid peroxidation, which may end in spontaneous abortion. Lipid peroxidation may occur as a direct effect of cadmium, through, displacement of redox metal ion or decreases in glutathione, glutathione peroxidase and the pre-implanted embryos are very sensitive to conditions that cause oxidative stress. Pregnant women should avoid exposure to a state of oxidative stress and to be supplemented by antioxidants, vitamin C and E.

Key words: Spontaneous abortion · Cadmium · Oxidative stress · Antioxidant enzymes

INTRODUCTION

Miscarriage, generally defined as losses of pregnancy before 20 weeks gestation, is a devastating event with both physical and emotional impacts. A loss of pregnancy after 20 weeks, however, is considered a stillbirth. More than 80 % of miscarriage occurs in the first 12 weeks and the rate decreases rapidly. The causes of miscarriage are multiple ranging from genetic, structural, infectious, immunological, metabolic, endocrine and environmental factors [1]. The environmental pollutants play an important role in the manifestations of reproductive-associated disorders. One of these pollutants was cadmium (Cd). It is a heavy metal widely dispersed throughout the modern environment and has contributed to several deleterious effects. The Agency for Toxic Substances and Disease Registry (ATSDR) has listed Cd among the top seven of the 275 most hazardous substances in the environment [2]. Cadmium is confirmed as environmental toxin and exposure to it could contribute to pregnancy loss [3, 4]. Basically there are three possible routes of cadmium entry into the body: the gastrointestinal route; the respiratory route and the dermal route, given that the general population and people living near hazardous waste sites may be exposed to cadmium in contaminated food, dust, or

Corresponding Author: Nevin E. Sharaf, Environmental and Occupational Medicine, National Research Center, Dokki, Giza, Egypt.

water from unregulated releases or accidental releases [5, 6]. In addition, to the study of Nawrot *et al.* [7], in which they stressed on the house dust as an additional relevant exposure route for the general population.

The glutathione redox system is also involved in embryogenesis. Preimplanted embryos are very sensitive to conditions that cause oxidative stress. Their glutathione status changes dramatically during development. Glutathione in reproductive tract fluid may help protection of pre-implanted embryos from the adverse effects of toxicants [8]. Usefulness of glutathione in embryo production has been demonstrated in culture system. Increased embryonic fragmentation and a slow cleavage rate may be partially attributed to the early exposure of embryos to high reactive oxygen species (ROS) levels in intra-cytoplasmic sperm injection cycles [9, 10]. The causative factors associated with recurrent loss of pregnancy can be varied and multiple. In about 50%-60% of recurrent pregnancy losses, a causative factor cannot be identified and are therefore, classified as idiopathic. So, exposure to environmental genotoxic pollutants, like cadmium may participate in the induction of idiopathic spontaneous abortion [5].

The present study aimed to evaluate the potential effect of environmental exposure to cadmium on pregnancy outcome and to elucidate the mechanism of action, by which cadmium induces spontaneous abortion.

MATERIALS AND METHODS

Subjects: After the agreement of the ethical committee of the National Research Center on this study, a total of one hundred and twenty volunteer women were recruited from the prenatal genetic diagnosis clinic of the National Research Center, Dokki, Egypt. Informed consent was taken from all of them. The studied subjects were divided into four groups. The first group included 30 healthy fertile women (control group) with an average age of (25-35 years) and weighted from 60-90 kg and had no history of recurrent spontaneous abortion. Group II, III, IV involved 27, 23 and 40 subjects, respectively with medically unexplained recurrent spontaneous abortion, with an average age of (22-37 years) and weighted from 62-90 kg and with a history of one, two and three (or more) miscarriages respectively, including abortion up to 20 weeks gestational age.

The demographic, medical and clinical data were collected from all the women, based on personal interviews and medical reports at the clinic. Detailed medical, surgical, genetic and menstrual histories were taken. Patients were questioned about their use of drugs, tobacco, alcohol and caffeine and whether they have been exposed to occupational hazard. Also, their food habits especially fish consumption were taken in concern. Thyroid dysfunction, diabetes, uterine anomalies and ovarian disease were excluded referring to medical reports by the hospital. The exclusion criteria which could be interpreted as a cause of miscarriage were:

- Infectious diseases involving toxoplasmosis, rubella, cytomegalovirus and herpes (TORCH).
- anatomical obstetric complications
- Immunological factors and hereditary chromosomal aberrations.

Sampling: Ten ml blood sample was withdrawn from every women included in this study. Each blood sample was divided into three tubes, one of which was blank (non-heparinized) and the other two on heparin. One of the heparinized tubes was used for the determination of blood cadmium (Cd), using Graphite Furnace Atomic Absorption Spectroscopy (GF-AS) for assay of blood cadmium [11] and the second tube was centrifuged for 10 minutes at 30000 rpm and the separated plasma was used for determination of plasma concentration of malondialdehyde (MDA) as a biomarker for detection of oxidative stress [12]. The blank one was used for determination of the blood reduced glutathione (GSH), as well as glutathione peroxidase (GPx) as biomarkers of antioxidant defense mechanisms, by the colorimetric determination of whole blood reduced glutathione (GSH) [13)] and the enzymatic estimation of glutathione peroxidase (GPx) in whole blood [13].

Statistical Analysis: The obtained data of the present study were statistically analyzed using SPSS version 10. Independent t-test was used to compare each two individual groups separately and correlation coefficient was used to study the relationship between the Cd and the oxidative-antioxidant parameters. Significance was considered at $p< 0.05$.

RESULTS

Data of the present study revealed that there is a significant increase in blood level of cadmium concentration in groups of the aborted women (II, III, IV), in comparison to that of the control healthy women of group I (Table 1). However, within the three groups

Table 1: Comparison of blood cadmium (Cd), MDA, GSH, GPx, between the four studied groups

	Studied groups			
Parameters	Group I N=30	Group II N=27	Group III N=23	Group IV N=40
Blood cadmium (Cd) mg/L Mean ±SD	0.49± 0.04	1.45±0.14(gp I**)	1.3±0.13(gp I**)	1.58±0.1(gp I**)
Malondialdehyde (MDA) nmol/ml Mean ±SD	3.36±0.18	6.31±0.32(gp I**, IV*)	6.95±0.51(gp I**)	7.48±0.35(gp I**, II*)
Blood glutathione (GSH) mg/dl Mean ±SD	64.33±3.24	33±1.27(gp I**, IV*)	34.3±1.47(gp I**, IV*)	29.38±1.36(gp I**, II*, III*)
Blood glutathione peroxidase (GPx) mU/ml Mean ±SD	50.7±1.46	33±1.75(gp I**)	34.6±1.75(gp I**)	33.1±1.2(gp I**)

* P<0.05, * * P = 0.001; Group I = Women with no previous history of miscarriage, Group II = Women with a history of one miscarriage
Group III = Women with a history of two miscarriages, Group IV = Women with a history of three or more miscarriage

Table 2: Correlation coefficient between cadmium and different biochemical parameters in the studied women

Cadmium	R	p-value
Malondialdehyde (MDA) nmol/ml	0.47	0.001
Blood glutathione (GSH) mg/dl	-0.516	0.001
Blood glutathione peroxidase (GPx) mU/ml	-0.478	0.001

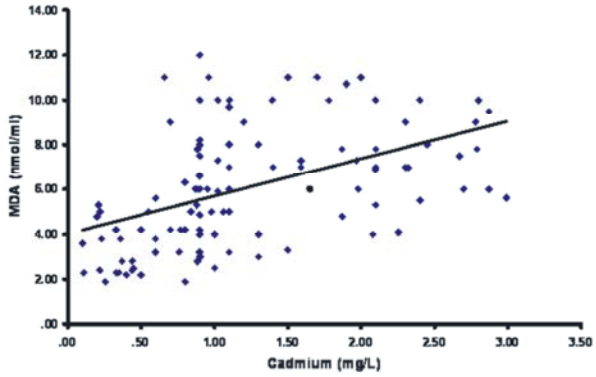

Fig. 1: General correlation coefficient between cadmium and Malondialdehyde (MDA)

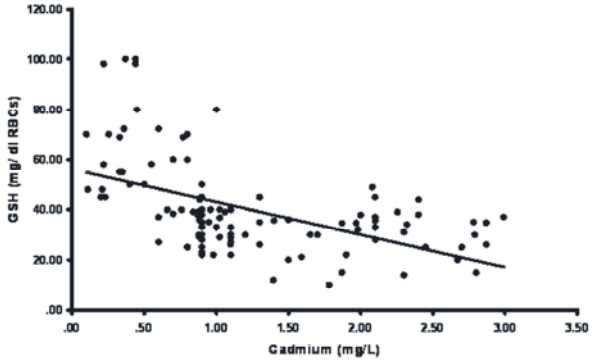

Fig. 2: General Correlation coefficient between cadmium and GSH

of the aborted women (Gp II, Gp III, Gp IV), no significant difference in blood cadmium concentrations were

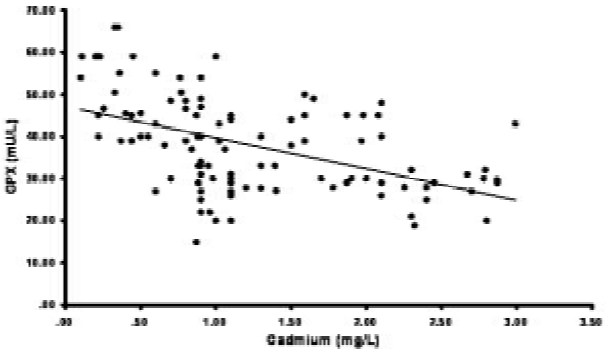

Fig 3: General correlation coefficient between cadmium and glutathione peroxidase activity

observed. It is obvious that the plasma levels of lipid peroxidation product, malondialdehyde (MDA) measured in the present study were significantly elevated in groups of the aborted women as compared to the control one (Table 1). In addition, the present study also revealed a significant positive correlation between the blood cadmium and the elevation in plasma MDA concentration (Table 2 and Fig. 1). The changes in the blood glutathione (GSH) of women suffering from abortion and control subjects were represented in Table 1. The mean blood levels of glutathione show a significant decrease in groups II, III, IV compared to their control counterparts ($p > 0.01$). Within these three groups of abortion, there were significant decrease in the mean of GSH in group IV in comparison to groups II and III. The blood glutathione (GSH) was found to be negatively correlated with Cd (Table 2 and Fig. 2).

Data in Table 1 demonstrates the mean value of blood glutathione peroxidase (GPx) activity. In this regard, the glutathione peroxidase activity was significantly diminished in groups II, III and IV, when compared to the control one. The present study shows, that the activity of the glutathione peroxidase enzyme was negatively affected by cadmium exposure (Table 2 and Fig. 3).

DISCUSSION

The results of the present study revealed, that there was significant increase in the level of blood cadmium in the three studied groups of spontaneous abortions than that of the corresponding control, and this is in agreement with that reported by Marouf *et al.* [14]. This finding proved that the patients recruited in the present study were exposed to cadmium as an environmental pollutant. This metal has no known beneficial or biological function. Prolonged exposure to it has been linked to toxic effects in both humans and animals [6, 15]. Also, exposure to cadmium during gestation is known to cause placental necrosis and hemorrhage, with an increased rate of fetal death [4, 16]. Several mechanisms were postulated to describe cadmium-induced toxicity. One of them depends on the binding of cadmium with S-H groups [17], which means, that the cell membrane proteins, cytoplasmic proteins and enzymes, as well as glutathione are targets for cadmium toxicity. Cadmium may also interact with metal moieties of the antioxidant enzymes and causes inhibition of their activities. Beside cadmium can replace zinc in superoxide dismutase [18] and selenium in selenium-dependent glutathione peroxidase [19].

Stimulation of lipid peroxidation is another mechanism for cadmium-induced toxicity. Lipid peroxidation is an early intracellular event after cadmium exposure. Cadmium interacts with critical subcellular sites, such as the mitochondria and results in the generation of free radicals. Thus, lipid peroxidation has long been considered as the primary mechanism for cadmium toxicity. Although cadmium induced lipid peroxidation has been extensively studied, its peroxidative mechanism is a controversial matter, as cadmium does not undergo redox cycling and does not participate in the Haber-Weiss reaction [20]. However, Hussain *et al.* [21] reported that the increase in lipid peroxidation could be the consequence of a direct effect of cadmium, a cadmium-induced displacement of redox metal ions or a decrease in glutathione content. Under normal conditions, ROS are counteracted by protective mechanisms, which are increased throughout pregnancy and protect the fetus. Nevertheless, potential damage may occur as soon as those mechanisms become insufficient. Accumulation of the toxic metal in placental tissue may result in abnormal placental function, leading to impaired transport of essential metals, such as zinc, copper, selenium and iron [22]. This undesirable condition leads to an imbalance between toxic and essential metals in favor of the formers, with detrimental effects on enzymatic protective systems, such as selenium-dependent glutathione peroxidase and copper-zinc-dependent superoxide dismutase, which require these essential metals as co-factors.

Therefore, the presence of cadmium in placentas may be detrimental for placental GPx activity and as a result the fetus is subjected to some degree of oxidative stress, which may result in potential damage [23]. Zhang *et al.* [24] confirmed on that by its conclusion that environmental exposure to cadmium significantly lower neonatal birth height. Glutathione peroxidase (GPx) is a member of a family of enzymes, whose function is to detoxify organic peroxides or hydrogen peroxide by reducing them to alcohols or water, respectively, mainly by preventing lipid peroxidation [25]. The observed decrease in glutathione (GSH) might be a result of its utilization in the scavenging of free radicals. It is also suggested, that glutathione is utilized for metallothionein synthesis in response to cadmium. Metallothionein is involved in the detoxification of cadmium and the scavenging of free radicals [26]. Cadmium is one of the most powerful inducers of hepatic and renal metallothionein synthesis [27]. Reactions of metals with glutathione might lead to either the formation of complexes or oxidation of glutathione. Similarly, formation of a chemical complex between cadmium and selenium at the active site of glutathione peroxidase is the postulated mechanism for glutathione peroxidase decrease [28]. The decreased activity of glutathione peroxidase (GPx) probably resulted from decreased transcription of the genes, which encode glutathione peroxidase, as well as post-transcriptional effects [29]. Ookawara *et al.* [30] found that, while mRNA expression for glutathione peroxidase, were decreased in preeclamptic placentas, their enzymatic activities were decreased to a greater extent. Possible mechanisms of post-transcriptional inhibition of antioxidant activity by oxidative stress include depletion of cellular glutathione, a necessary cofactor for glutathione peroxidase (GPx) [31]. Moreover, glutathione has been reported to interact with aldehydic lipid oxidation products, such as malondialdehyde to protect tissues from oxidation, since aldehydes can form adducts with DNA, proteins, enzymes causing alterations in their biological activity [19]. This finding was in agreement with our results, as the aborted women in groups II, III, IV were exposed to heavy cadmium burden, manifested by increase in plasma MDA of the three mentioned groups, with a decrease in GSH and GPx

CONCLUSION

The finding of this study established, that the three studied groups of aborted women were suffering from

exposure to cadmium as an environmental pollutant. This exposure induced elevated levels of plasma malondialdehyde associated with decreased levels of antioxidants, glutathione; glutathione peroxidase and it may be suggested, that this exposure could enhance lipid peroxidation, which may end in spontaneous abortion. It is postulated, that lipid peroxidation may occur as a direct effect of cadmium, via cadmium induced displacement of redox metal ion or decreases in glutathione, glutathione peroxidase. As, the glutathione redox system is also deeply involved in embryogenesis. The pre-implanted embryos are very sensitive to conditions that cause oxidative stress. There is no margin of safety exists for cadmium exposure levels, in the general population. Therefore, measures should be put in place to reduce exposure to a minimum, through:

- Subjection of the pregnant women to regular antenatal care and laboratory investigations, especially those in the vicinity of industrial and agricultural sources and to identify, populations, who are at special risk for Cd toxicity.
- Avoidance of the pregnant women to reside in areas of high environmental pollution, as eating food crops grown on cadmium containing soils, constitute a major source for exposure, in addition to exposure to cigarette smoking, especially the passive one.

REFERENCES

1. Michels, T.C. and A.Y. Tiu, 2007. Second trimester pregnancy loss. Am. Fam. Physician, 76(9): 1341-6.
2. ATSDR, 2001. Agency for Toxic Substances and Disease Registry Toxicological profile for cadmium; Atlanta, GA: US Department of health and human services, Public Health service, 244.
3. Kumar, S., 2004. Occupational exposure associated with reproductive dysfunction. J. Occup. Health, 46: 1-9.
4. Wu, S.Y., J. Tian, M.Z. Wang, B.J. Pan, H.D. Lü, Z.M. Wang and H.Y. Li, 2004. The effect of cadmium pollution on reproductive health in females, Zhonghua Liu Xing Bing XueZaZhi., 25(10): 852-5.
5. Godt, J., F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich and D. Groneberg, 2006. The toxicity of cadmium and resulting hazards to human health. Journal of Occupational Medicine and Toxicity, 1: 22.
6. Järup, L. and A. Akesson, 2009. Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharmacol., 238(3): 201-8.
7. Nawrot, T.S., J.A. Staessen, H.A. Roels, E. Munters, A. Cuypers, T. Richart, A. Ruttens, K. Smeets, H. Clijsters and J. Vangronsveld, 2010. Cadmium exposure in the population: from health risks to strategies of prevention. Biometals, 23(5): 769-82.
8. Tatemoto, H., N. Sakurai and N. Muto, 2000. Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during *in vitro* maturation: role of cumulus cells. Biol. Reprod., 63: 805-810.
9. Bedaiwy, M.A., T. Falcone, M.S. Mohamed, A.A. Aleem, R.K. Sharma, S.E. Worley, J. Thorton and A. Argawal, 2004. Differential growth of human embryos *in vitro*: role of reactive oxygen species. Fertil. Steril., 82: 593-600.
10. Fujii, J., Y. Iuchi and F. Okada, 2005. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. Reproductive Biology and Endocrinology, 3: 43.
11. Zeneli, L., N.M. Daci, M.N. Daci-Ajvazi and H. Paçarizi, 2008. Effects of pollution on lead and cadmium concentration and correlation with biochemical parameters in blood of human population nearby Kosovo thermo power plants. American Journal of Biochemistry and Biotechnology, 4(3): 273-276.
12. Bakan, E., S. Taysi, M. FevziPolat, S. Dalga, Z. Umudum, N. Bakan and M. Gumus, 2002. Nitric Oxide Levels and Lipid Peroxidation in Plasma of Patients with Gastric Cancer Japanese Journal of Clinical Oncology, 32: 162-166.
13. Ashour, M.N., S.I. Salem, H.M. El-Gadban, N.M. Elwan and T.K. Basu, 1999. Antioxidant status in children with protein-energy malnutrition. Europ. J. Clin. Nutr., 53(8): 669-673.
14. Marouf, T., F. Othman, T. Mohamed and E. El Sayed, 2011. Role of lead and cadmium in early fetal demise: A cross sectional study. EBWHJ, 1(3): 130-135.
15. Zadorozhnaja, T.D., R.E. Little, R.K. Miller, N.A. Mendel, R.J. Taylor, B.J. Presley and B.C. Gladen, 2000. Concentrations of arsenic, cadmium, copper, lead, mercury and zinc in human placenta from two cities in Ukraine. J. Toxicol. Environmental Health, 61: 255-263.
16. Levin, A.A., R.W. Klipper and R.K. Miller, 1987. Fetal toxicity of cadmium chloride: The pharmacokinetics in the pregnant Wistar rat. Teratology, 36: 163-170.
17. Thomas, P. and H.W. Wofford, 1983. Effects of metals and organic compounds on hepatic glutathione, cysteine and acid soluble thiol levels in the mullet. Toxicol. Appl. Pharmacol., 76: 172-179.

18. Bauer, R., I. Demeter, V. Hasemann and J.T. Johansen, 1980. Structural properties of the zinc sites in Cu, Zn superoxide dismutase. Perturbed angular correlation of gamma ray spectroscopy on the Cu-Cd superoxide dismutase derivative. Biochem. Biophys. Res. Commun., 94: 1296-1302.
19. El-Maraghy, S., M.Z. Gad, A.T. Fahim and M.A. Hamdy, 2001. Effect of cadmium and aluminium intake on the antioxidant status and lipid peroxidation in rat tissues. J. Biochem. Molecular Toxicology, 15: 207-214.
20. Casalino, E., C. Sblano and C. Landriscina, 1997. Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. Arch. Biochem. Biophys, 346: 171-179.
21. Hussain, T., G.S. Shukla and S.V. Chandra, 1987. Effects of cadmium on superoxide dismutase and lipid peroxidation in liver and kidney of growing rats. *In vivo* and *in vitro* studies. Pharmacol. Toxicol., 60: 355-358.
22. Casanueva, E. and F.E. Viteriy, 2003. Iron and oxidative stress in pregnancy. J. Nutr., 133: 1700S-8S.
23. Masso, E.L., L. Corredor and M.T. Antonio, 2007. Oxidative damage in liver after perinatal intoxication with lead and/or cadmium. J. Trace Elem. Med. Biol., 21: 210-6.
24. Zhang, Y.L., Y.C. Zhao, J.X. Wang, H.D. Zhu, Q.F. Liu, Y.G. Fan, N.F. Wang, J.H. Zhao, H.S. Liu, L. Ou-Yang, A.P. Liu and T.Q. Fan, 2004. Effect of environmental exposure to cadmium on pregnancy outcome and fetal growth: a study on healthy pregnant women in China. J. Environ. Sci. Health A Tox. Hazard Subst Environ. Eng., 39(9): 2507-15.
25. Kankofer, M., 2001. Antioxidative defense mechanisms against reactive oxygen species in bovine retained and not retained placenta: activity of glutathione peroxidase, glutathione transferase, catalase and superoxide dismutase. Placenta, 22: 466-72.
26. Richards, M.P., 1989. Recent developments in trace element metabolism and function role of metallothionein in copper and zinc metabolism. J. Nutr., 119: 1062-1070.
27. Yamada, H. and S. Koizumi, 1991. Metallothionein induction in human peripheral blood lymphocytes by heavy metals. Chem. Biol. Interact., 78: 347-354.
28. Gambhir, J. and R. Nath, 1992. Effect of cadmium on tissue glutathione peroxidase in rats. Influence of selenium supplementation. Indian J. Exp. Bio., 30: 597-601.
29. Wang, Y. and S.W. Walsh, 1996. Antioxidant activities and mRNA expression of superoxide dismutase, catalase and glutathione peroxidase in normal and preeclamptic placentas. J. Soc. Gynecol. Invest., 3: 179-184.
30. Ookawara, T., N. Kawamura, Y. Kitagawa and N. Taniguchi, 1992. Site-specific and random fragmentation of Cu, Zn-superoxide dismutase by glycation reaction. Implication of reactive oxygen species. J. Biol. Chem., 267: 18505-18510.
31. McCord, J.M. and I. Fridovich, 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem., 244: 6049-6055.