SAGE-Hindawi Access to Research
International Journal of Nephrology
Volume 2011, Article ID 142896, 5 pages
doi:10.4061/2011/142896

Research Article

Cerebral Epiphyseal Proteins and Melatonin Modulate the Hepatic and Renal Antioxidant Defense of Rats

Vijay K. Bharti,[1,2] R. S. Srivastava,[1] P. Subramaian,[3] D. Warren Spence,[4] S. R. Pandi-Perumal,[5] and Gregory M. Brown[6,7]

[1] *Neurophysiology Laboratory, Division of Physiology and Climatology, Indian Veterinary Research Institute (IVRI), Izatnagar 243122, India*
[2] *Nutrition and Toxicology Laboratory, Defence Institute of High Altitude Research (DIHAR), Defence Research and Development Organization (DRDO), Ministry of Defence, C/o- 56 APO, Leh 194101, India*
[3] *Department of Biochemistry and Biotechnology, Faculty of Sciences, Annamalai University, Annamalainagar 608 002, India*
[4] *323 Brock Ave, Toronto, ON, Canada M6K 2M6*
[5] *Somnogen Inc., College Street, Toronto, ON, Canada M6H 1C5*
[6] *Department of Psychiatry, University of Toronto, 100 Bronte Road, Unit 422, Oakville, ON, Canada L6L 6L5*
[7] *Centre for Addiction and Mental Health, 250 College Street, Toronto, ON, Canada M5T 1R8*

Correspondence should be addressed to Vijay K. Bharti, vijaykbharti@rediffmail.com

Received 17 January 2011; Revised 24 March 2011; Accepted 30 March 2011

Academic Editor: Nuket Bavbek

Copyright © 2011 Vijay K. Bharti et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The cerebral epiphysis (pineal gland) secrets melatonin and number of other proteins and peptides. It was thus hypothesized that antioxidant properties of epiphyseal proteins and melatonin could potentially benefit from exogenous therapies. In view of the therapeutic potential of these proteins, the present experiment was conducted to investigate the effect of buffalo epiphyseal proteins (BEP, at 100 μg/kg BW, i.p.) and melatonin (MEL, at 10 mg/kg BW, i.p) on changes in hepatic and renal antioxidant enzymes of adult female Wistar rats. Buffalo epiphyseal proteins significantly ($P < .05$) increased hepatic lipid peroxidation (LPO), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), reduced glutathione (GSH), and renal LPO, catalase (CAT), GR, GSH, GPx levels as compared to control animals. Similarly, MEL treatment significantly ($P < .05$) up-regulated hepatic SOD and GPx activity, whereas CAT, GR, GPx, and GSH levels in renal tissues were increased while SOD and LPO remained unaffected. Buffalo epiphyseal protein treatment produced greater effects on hepatic GPx and renal CAT and GSH levels than did MEL. These findings support the conclusion that buffalo epiphyseal proteins and melatonin activate a number of antioxidant mechanisms in hepatic and renal tissues.

1. Introduction

The cerebral epiphysis (pineal gland) is broadly involved in the synchronization of bodily functions(s) with the environment and serves as a "regulator of regulators". It is known that, in addition to its synthesis of melatonin (MEL) from serotonin, this organ secretes various proteins and peptides [1, 2]. The epiphysis additionally has a rich supply of adrenergic nerve fibers that greatly influence its secretory activity. The cerebral epiphysis, through its production of MEL and its effect on serotonin, affects many neuroendocrine functions [1]. However, many researchers regard MEL as the sole mediator of epiphyseal functions. Recently, a number of studies reported that epiphyseal proteins have the ability to regulate various physiological functions in numerous animals and thus hypothesized that these proteins effectively acted as epiphyseal hormones [3–7]. In spite of these studies, the functional role of epiphyseal hormones and proteins in antioxidant defense system of vital organs remains poorly understood. Oxidative damage to vital organs, particularly to the liver and kidney, becomes very important in humans and animals when the antioxidant defense system is either absent or functioning inefficiently [8]. Inasmuch as the liver and kidney are metabolically highly

active in xenobiotic metabolism and excretion, they have, compared to other organs, a greater load of free radical activity and thus are more prone to oxidative damage [8, 9]. As a consequence, reactive oxygen species (ROS) tissue injuries are found more commonly in these organs, as are their sequelae of toxic damage, disease, and the ultimate death of the biological systems in which they occur [10, 11]. In healthy animals, there is a balance between the production of various ROS and antioxidant defenses [8]. It has been noted that the antioxidant defense systems of the organs of many species are inadequately equipped to take up an excessive load of free radicals and ROS, and that when this does take place it is associated with increased oxidative damage in the affected organs [10–12]. Having to contend with oxidative stress brought about by uncontrolled oxidation of important molecules in foods and body tissues is thus a significant biological challenge faced by most living organisms [13, 14]. Antioxidant therapies, which are based on upregulation of body antioxidant defense system, are now a commonly employed strategy for combating molecular damage in various tissues [12]. Variations of this approach, which make use of exogenously administered antioxidant agents, could potentially provide an important and inexpensive alternative treatment for diseases related to oxidative stress. Hence, the present study was based on the perceived value of investigating several molecules for their potential benefit in bolstering the antioxidant defense systems of the liver and kidney. Melatonin (*N-acetyl-5-methoxytryptamine*), which has been long known as the pineal's major secretory product, has potent antioxidant properties [15–17]. Earlier, we reported on the antioxidant action of buffalo (*Bubalus bubalis*) epiphyseal proteins (BEPs) under fluoride and arsenic-induced oxidative stress in blood, brain, and kidney [2–6, 18–20]. However, the effect of epiphyseal hormone and proteins on liver and kidney antioxidant defense system has not previously been studied. Thus in view of findings reported by ourselves and others, we hypothesized that pineal BEP and MEL might enhance levels of antioxidant defense activity (enzymatic and nonenzymatic) in the liver and kidney and could therefore be of benefit for animals undergoing oxidative stress.

2. Methods

All the procedures, conducted on the experimental animals were duly approved by the Institutional Animal Ethics Committee (IAEC) of Indian Veterinary Research Institute (IVRI) for the purpose of control and supervision of experiments on animals.

2.1. Chemicals. All chemicals used in the study were of analytical grade from HiMedia, Loba Chemie (Mumbai, India), SRL Chemicals, India. Melatonin was procured from Sigma Chemical Co. (St. Louis, USA). Buffalo (*Bubalus bubalis*) epiphyseal proteins were supplied by the Neurophysiology Laboratory, Division of Physiology and Climatology, IVRI (Izatnagar, India).

2.2. Experimental Animals. The present study was carried out on eighteen sexually mature and healthy female Wistar rats of 130–142 g body weights, procured from the Laboratory Animal Resource (LAR) Section of the IVRI. Rats were examined on arrival for any abnormality or overt ill health. Rats were housed in polypropylene cages in a light/dark (LD) cycle of 12 h, in a pathogen-free, temperature- and humidity-controlled environment (set at $21 \pm 2^{\circ}$C and relative humidity at 50 ± 10%, resp.). After an acclimatization period of 1 week, they were weighed and randomly assigned to various groups with approximately equal initial group mean body weights. Following allocation, the animals were marked with picric acid solution for individual identification. All the animals had free access to the standard laboratory animal diet and water, which were replenished on daily basis. The animals were also checked daily for the health and husbandry conditions.

2.3. Experimental Design. The experimental design for the present study, including various groups, doses, route of administration, and duration of treatment is presented in Table 1. BEPs were used as the experimental agent in as much as its safety and utility were confirmed in a study that we have previously published. Appropriate dosages of BEP and MEL were optimized from experience in our previous work and thereafter they were dissolved in a suitable vehicle before administration at exactly 16.00 hrs [6].

2.4. Sample Collection. Daily observations were taken for the behavioral changes and mortality, if any, throughout the experimental period. The samples were collected at the end of the experiment (day 28). The rats were euthanized using ether at the end of the experiments. The liver and kidney were collected, cleaned, rinsed in chilled saline, blotted, weighed, and stored at -20°C. Frozen liver and kidney tissues samples were partially thawed, and 200 mg of sample was weighed and taken in 2 mL of ice-cold saline. Another 200 mg of the samples were weighed separately and taken in 2 mL of 0.02 M EDTA for GSH estimation. Organ homogenates were prepared using an IKA homogenizer (Germany), under ice-cold conditions and collected, and then centrifuged for 10 min at 3000 rpm. Thereafter, cell-free supernatant was collected and transferred to precooled microfuge tubes in duplicate and stored at below -20°C. These supernatants were used for estimation of total proteins (organs), lipid peroxidation (LPO), and enzyme activity namely, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) as well as nonenzymatic namely, reduced glutathione (GSH) antioxidant defense level.

2.5. Analytical Procedures. Estimations of different antioxidant defense-related biochemical parameters in hepatic and renal tissues were carried out using a Double Beam UV-VIS Spectrophotometer (UV 5704 SS, ECIL, India).

Table 1: Distribution of experimental rats to different treatments.

Groups (N/group = 6)	Treatments	Dose	Route of administration
Control	Drinking water + Normal saline	*Ad libitum*	Oral Intraperitoneal
BEP	Buffalo epiphyseal proteins (BEPs)	100 μg/kg BW	Intraperitoneal
MEL	Melatonin (MEL)	10 mg/kg BW	Intraperitoneal

Lipid Peroxidation (LPO). Renal and hepatic tissues LPO was determined in terms of malondialdehyde (MDA) production by the method of Rehman [21].

Reduced Glutathione (GSH). The concentration of GSH in renal and hepatic tissues was estimated by evaluating free-SH groups, using the 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) method as described by Sedlak and Lindsay [22].

Catalase (CAT). Activities of catalase enzymes were estimated as described by Bergmayer [23] and were expressed as nM H_2O_2 utilized per minute per milligram protein.

Superoxide Dismutase (SOD). Superoxide dismutase activities were estimated using the method described by Madesh and Balasubramanian [24] and are expressed as SOD units [one unit of SOD is the amount (μg) of protein required to inhibit the MTT reduction by 50%].

Glutathione Peroxidase (GPx). Glutathione peroxidase activities were determined by the method of Paglia and Valantine [25]. The enzyme activity is expressed as U/mg of protein, and one unit of enzyme activity is defined as 1 nM of substrate (NADPH) utilized/min/mg protein at 25°C.

Glutathione Reductase (GR). The enzyme activities were assayed by the method of Goldberg and Spooner [26], and activity is expressed as nM NADPH oxidized to NADP/ min/mg protein.

Protein Assay. Protein contents in liver and kidneys homogenates were determined and calculated by the method of Lowry et al [27].

Statistical Analysis. Differences between groups were statistically analyzed by one-way ANOVA, and the differences between the means of groups were separated by the least significant difference (LSD) test. All data were presented as mean±standard error. Values differing by $P < .05$ are regarded as significant. A computer program (SPSS 10.01, SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

3. Results

All the animals were healthy, and no mortality was observed during the entire period of the experiment. The activities of hepatic and renal glutathione peroxidase (GPx), superoxide dismutase (SOD), and the levels of lipid peroxidation (LPO) and glutathione (GSH) were measured to assess the level of antioxidant defense in female rats. BEP significantly increased ($P < .05$) hepatic LPO over the control and melatonin-treated animals (Table 2). However, no effects were observed on CAT activity in hepatic of BEP- and MEL-treated groups. Interestingly, GPx and SOD activity in hepatic tissues was significantly increased ($P < .05$) in both BEP- and MEL-administered groups. No effect of MEL was recorded on hepatic GSH and GR level, however, their levels were significantly higher ($P < .05$) in BEP-treated animals.

Similarly, the renal LPO level was significantly ($P < .05$) greater in BEP administered animals (Table 3). On the other hand, renal CAT, GSH, GR, and GPx levels were markedly enhanced in MEL and BEP-supplemented groups as compared to control animals (Table 3). However, effects on CAT and GSH were greater in BEP-treated animals than in those which were given MEL (Table 3). Superoxide dismutase activity in kidney tissues was unaffected in all groups.

4. Discussion

Markers of oxidative stress damage can be found in many disease states including renal damage, chronic heart disease, and liver disease [28, 29]. Thus, excess free radical formation is associated with many disease states. Many diseases and increased toxicity are often associated with oxidative stress in different vital organs and are characterized by the reductions in the activity of specific enzymes activity, including catalase, SOD, GPx, and GR [15].

We observed marked increases in renal CAT, GSH, GR, and GPx in MEL- and BEP-supplemented animals. Interestingly, GPx and SOD activities in hepatic tissues were also significantly increased ($P < .05$) in BEP- and MEL-administered groups. However, only hepatic GSH and GR levels were significantly higher ($P < .05$) in BEP-treated animals. These pharmacological effects of BEP and MEL may be relevant to their potentiation of antioxidant defense activity in renal and hepatic tissues [17–19]. Present study findings do not support the hypothesis of increased endogenous antioxidant activity in response to the deleterious/toxic effect of BEP in the rat's liver and kidney. Since, liver and kidney protective responses to any deleterious agents are immediate and transient, and so these endogenous protective/beneficial effects cannot be for long periods (beyond 2-weeks), as in our study. Therefore, findings of the present study support the antioxidant properties of BEP. Also, BEP increased not only the catalase (kidney), SOD (liver), GPx, and GR enzymes but also GSH level. GSH levels should have decreased if BEPs had a deleterious effect.

GPx removes H_2O_2 by coupling its reduction to H_2O_2 with oxidation of reduced glutathione, GSH. It is the most important enzyme for extraperoxisomal inactivation of

Table 2: Effects of different treatments on lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) in liver of female rats.

Group	Parameters					
	LPO (nM MDA/mL)	CAT (nM/min/mg protein)	SOD (U)	GR (nM/min/mg protein)	GPx (nM/min/mg protein)	GSH (μM/g tissue)
Control	$4.61^a \pm 0.11$	$291.03^a \pm 14.93$	$6.15^a \pm 0.37$	$145.85^a \pm 7.33$	$25.67^a \pm 1.87$	$3.30^a \pm 0.06$
BEP	$5.78^b \pm 0.21$	$293.45^a \pm 9.20$	$7.94^b \pm 0.24$	$189.17^b \pm 5.79$	$37.44^c \pm 1.70$	$4.49^b \pm 0.08$
MEL	$4.89^a \pm 0.13$	$300.40^a \pm 11.68$	$7.60^b \pm 0.33$	$139.32^a \pm 13.12$	$30.28^b \pm 1.03$	$3.50^a \pm 0.15$

Values ($n = 6$; Means ± S.E) in the same column bearing no superscript $^{(a,b,c)}$ common vary significantly ($P < .05$).

Table 3: Effects of different treatments on lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) in kidney of female rats.

Group	Parameters					
	LPO (nM MDA/mL)	CAT (nM/min/mg protein)	SOD (U)	GR (nM/min/mg protein)	GPx (nM/min/mg protein)	GSH (μM/g tissue)
Control	$4.99^a \pm 0.13$	$251.70^a \pm 4.58$	$5.98^a \pm 0.17$	$126.64^a \pm 8.47$	$20.13^a \pm 1.11$	$3.50^a \pm 0.06$
BEP	$5.80^b \pm 0.11$	$327.49^c \pm 18.14$	$5.46^a \pm 0.31$	$168.14^b \pm 6.20$	$36.99^b \pm 3.69$	$5.91^c \pm 0.15$
MEL	$4.95^a \pm 0.12$	$284.10^b \pm 2.79$	$6.12^a \pm 0.34$	$152.82^b \pm 10.54$	$32.48^b \pm 1.92$	$4.19^b \pm 0.113$

Values ($n = 6$; Means ± S.E) in the same column bearing no superscript $^{(a,b,c)}$ common vary significantly ($P < .05$).

H_2O_2, especially in the liver and kidney. Since the liver is a major source of GSH, metabolism of xenobiotics in the liver, which can drastically deplete liver GSH, may also result in GSH depletion in other tissues [30]. In numerous reactions, GPx, GR, and GSH act as free radical scavenging molecules and therefore the finding in our study that these enzymatic and nonenzymatic antioxidant defense systems are upregulated in liver and kidney of animals that exhibit oxidative stress underscore the critical importance of BEP and MEL as potentiators of antioxidant activity. Melatonin, the chief secretory product of the cerebral epiphysis, is a direct free radical scavenger and indirect antioxidant [31]. In addition to its direct free radical scavenging activity, MEL also enhances the synthesis of SOD, GSH, CAT, GR, and GPx [32].

In the present study, it was shown that BEP from the cerebral epiphysis possesses antioxidant properties exceeding in some cases the effects of the MEL. The superior effects of BEPs might be due to their direct antioxidant effects, but also because BEPs have been implicated in the stimulation of MEL production [33]. In recent studies, the role of BEP in the stimulation of MEL production was demonstrated [3]. Of relevance here is that MEL has a very short half-life and is metabolized and excreted within a few minutes. However, the presence of BEP may represent a constant source of stimulation for MEL synthesis. This might also be the reason that certain enzymes have greater antioxidative effects when compared to MEL action alone. Besides increasing the activity of the antioxidative enzymes, it is known that melatonin also increases their expression. This observation suggests that the indoleamine may have a physiological role in promoting endogenous antioxidative defense activity. These findings support the hypothesis that BEP and MEL modulate antioxidant defense of liver and kidney, and also demonstrate that these agents are generally equivalent in the potency of their of antioxidant activities.

5. Conclusions

These experimental findings establish that buffalo epiphyseal proteins and melatonin are effective antioxidants and that they may play a protective role against hepatic and renal damage induced by oxidative stress. The findings suggest that epiphyseal proteins and melatonin may have therapeutic potential as antioxidants drugs for the management of oxidative stress.

Conflict of Interests and Disclosure Statement

S. R. Pandi-Perumal is a stockholder and the President and Chief Executive Officer of Somnogen Inc., a New York Corporation. He declared no competing interests that might be perceived to influence the content of this paper. All remaining authors declare that they have no proprietary, financial, professional, nor any other personal interest of any kind in any product or services and/or company that could be construed or considered to be a potential conflict of interest that might have influenced the views expressed in this paper.

Acknowledgments

This study was supported in the form of a Senior Research Fellowship (SRF) to the first author (VKB), and facilities provided by Indian Veterinary Research Institute (IVRI), India for conducting this study are duly acknowledged. The authors would like to thank the staff of their animal facility for their care of the subjects used in this study and for their assistance during the project.

References

[1] D. E. Blask, M. K. Vaughan, and R. J. Reiter, *In the Pineal Gland*, Elsevier, Amsterdam, The Netherlands, 1983.

[2] M. Tandon, R. S. Srivastava, S. K. Meur, and M. Saini, "Proteins and peptides present in pineal gland and other brain structures of buffaloes," *Indian Journal of Animal Sciences*, vol. 76, pp. 383–394, 2006.

[3] V. Sejian, *Studies on pineal-adrenal relationship in goats (Capra hircus) under thermal stress*, Ph.D. thesis, Indian Veterinary Research Institute, Izatnagar, India, 2006.

[4] M. Ramasamy, *Studies on bubaline pineal proteins/peptides below 20 kDa and their immunopotentiation in guinea pigs*, Ph.D. thesis, Indian Veterinary Research Institute, Izatnagar, India, 2006.

[5] V. K. Bharti, *Studies on buffalo (Bubalus bubalis) pineal proteins on fluoride-induced oxidative stress and apoptosis in rats*, Ph.D. thesis, Indian Veterinary Research Institute, Izatnagar, India, 2008.

[6] V. K. Bharti and R. S. Srivastava, "Protective role of pineal proteins at different dose level on fluoride-induced changes in plasma biochemicals and blood antioxidants enzymes in rats," *Biological Trace Element Research*, 2010.

[7] O. A. Zimina, R. I. Kovalenko, A. D. Nozdrachev, and E. M. Tsoi, "Effect of pineal peptides on the adrenal gland cortex glucocorticoid function and behavior in rats orally immunized with ovalbumin," *Ross Fiziol Zh Im I M Sechenova*, vol. 89, pp. 1362–1369, 2003 (Russian).

[8] N. V. Georgieva, "Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems—a review," *Bulgarian Journal of Veterinary Medicine*, vol. 8, pp. 1–11, 2005.

[9] B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, UK, 4th edition, 2006.

[10] N. F. Bence, R. M. Sampat, and R. R. Kopito, "Impairment of the ubiquitin-proteasome system by protein aggregation," *Science*, vol. 292, no. 5521, pp. 1552–1555, 2001.

[11] B. Halliwell, "Oxidative stress in cell culture: an under-appreciated problem?" *FEBS Letters*, vol. 540, no. 1-3, pp. 3–6, 2003.

[12] G. D. Zeevalk, L. P. Bernard, C. Song, M. Gluck, and J. Ehrhart, "Mitochondrial inhibition and oxidative stress: reciprocating players in neurodegeneration," *Antioxidants and Redox Signaling*, vol. 7, no. 9-10, pp. 1117–1139, 2005.

[13] R. J. Reiter, D. Melchiorri, E. Sewerynek et al., "A review of the evidence supporting melatonin's role as an antioxidant," *Journal of Pineal Research*, vol. 18, no. 1, pp. 1–11, 1995.

[14] M. R. Fernández-Santos, A. E. Domínguez-Rebolledo, M. C. Esteso, J. J. Garde, and F. Martínez-Pastor, "Catalase supplementation on thawed bull spermatozoa abolishes the detrimental effect of oxidative stress on motility and DNA integrity," *International Journal of Andrology*, vol. 32, no. 4, pp. 353–359, 2009.

[15] R. J. Reiter, D. X. Tan, E. Gitto et al., "Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage," *Polish Journal of Pharmacology*, vol. 56, no. 2, pp. 159–170, 2004.

[16] M. I. Rodriguez, G. Escames, L. C. López et al., "Melatonin administration prevents cardiac and diaphragmatic mitochondrial oxidative damage in senescence-accelerated mice," *Journal of Endocrinology*, vol. 194, no. 3, pp. 637–643, 2007.

[17] S. L. Chawla, R. Yadav, D. Shah, and M. V. Rao, "Protective action of melatonin against fluoride-induced hepatotoxicity in adult female mice," *Fluoride*, vol. 41, no. 1, pp. 44–51, 2008.

[18] V. K. Bharti and R. S. Srivastava, "Pineal proteins upregulate specific antioxidant defense systems in the brain," *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 2, pp. 88–92, 2009.

[19] V. K. Bharti and R. S. Srivastava, "Fluoride-induced oxidative stress in rat's brain and its amelioration by buffalo (*Bubalus bubalis*) pineal proteins and melatonin," *Biological Trace Element Research*, vol. 130, no. 2, pp. 131–140, 2009.

[20] V. K. Bharti and R. S. Srivastava, "Effect of pineal proteins and melatonin on certain biochemical parameters of rats exposed to high-fluoride drinking water," *Fluoride*, vol. 44, pp. 30–36, 2011.

[21] S. Rehman, "Lead-induced regional lipid peroxidation in brain," *Toxicology Letters*, vol. 21, no. 3, pp. 333–337, 1984.

[22] J. Sedlak and R. H. Lindsay, "Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent," *Analytical Biochemistry*, vol. 25, no. C, pp. 192–205, 1968.

[23] H. U. Bergmayer, "UV method of catalase assay," in *Methods of Enzymatic Analysis*, S. K. Bansal, Ed., p. 273, Weinheim Deer field Beach, Florida, USA, 3rd edition, 1983.

[24] M. Madesh and K. A. Balasubramanian, "Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide," *Indian Journal of Biochemistry and Biophysics*, vol. 35, no. 3, pp. 184–188, 1998.

[25] D. E. Paglia and W. N. Valentine, "Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase," *The Journal of Laboratory and Clinical Medicine*, vol. 70, no. 1, pp. 158–169, 1967.

[26] D. M. Goldberg and R. J. Spooner, "Glutathione reductase," in *Methods in Enzymatic Analysis*, J. Bergmeyer and M. Grassi, Eds., pp. 258–265, VCH Weinheim, Germany, 1983.

[27] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[28] X. Guo, Y. Sun, and G. Sun, "Effect of fluoride on activities of enzymes and ultrastructure in primary cultured rat hepatocytes," *Weisheng Yanjiu*, vol. 34, pp. 35–37, 2005 (Chinese).

[29] M. Oncu, K. Gulle, E. Karaoz et al., "Effect of chronic fluorosis on lipid peroxidation and histology of lung tissues in first and second generation rats," *Toxicology and Industrial Health*, vol. 22, no. 9, pp. 375–380, 2006.

[30] S. M. Deneke and B. L. Fanburg, "Regulation of cellular glutathione," *American Journal of Physiology*, vol. 257, no. 4, pp. L163–L173, 1989.

[31] J. Cabeza, V. Motilva, M. J. Martín, and C. A. De La Lastra, "Mechanisms involved in gastric protection of melatonin against oxidant stress by ischemia-reperfusion in rats," *Life Sciences*, vol. 68, no. 12, pp. 1405–1415, 2001.

[32] A. Menendez-Pelaez, B. Poeggeler, R. J. Reiter, L. Barlow-Walden, M. I. Pablos, and D. X. Tan, "Nuclear localization of melatonin in different mammalian tissues. Immunological and radioimmunoassay evidence," *Journal of Cellular Biochemistry*, vol. 53, no. 4, pp. 373–382, 1993.

[33] V. K. Bharti and R. S. Srivastava, "Protective role of buffalo pineal proteins on arsenic-induced oxidative stress in blood and kidney of rats," *Health*, vol. 1, pp. 167–172, 2009.