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Role of Antioxidants in Protection Against Potassium Bromate Induced Lipid Peroxidation in the Rat Thyroid Gland

KEYWORDS: potassium promate, melatonin, ascorbic acid, thyroid, lipid peroxidation

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ABSTRACT *Potassium bromate (KBrO3) has been classified as possible human carcinogen. Oxidative stress plays a major role in the carcinogenic action of KBrO3. So, the aim was to prove the prooxidative effect of KBrO3 and to evaluate and compare the possible protective effect of melatonin and ascorbic acid against lipid peroxidation in the thyroids. Study was done on six groups of rats: (control group) treated with 0.9% NaCl: ethanol (v/v 10:1), or KBrO3 in group II, or melatonin in group III, or ascorbic acid in group IV. Rats in groups V and VI were received either melatonin, or ascorbic acid combined with KBrO3. The results prove lipid peroxidation in the rat thyroid, followed KBrO3 injection. Treatment with melatonin or ascorbic acid did not affect the basal lipid peroxidation. However, the pre-treatments with either antioxidant ameliorate the lipoperoxidative changes and increase the expression of antioxidant enzymes in rat thyroid. In Conclusion, it is possible that these antioxidants may be of great value as protective agents against KBrO3-induced tumor induction in the thyroid. Melatonin is more powerful antioxidant than ascorbic acid.*

Introduction:
Potassium bromate ($KBrO_3$) was used as a food additive in flour and barley processing and as a component in cold-wave hair lotions (8). Bromate is frequently detected in tap and bottled water, since it is an inorganic oxyhalide by-product, formed during water disinfection by ozonation (30). Potassium bromate has been classified by International Agency for Research on Cancer (IARC) as a compound belonging to the group 2B of carcinogens (a possible human carcinogen) (12). It has been demonstrated to cause renal tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid in rats (34).

Oxidative stress plays a major role in the carcinogenic action of $KBrO_3$ in kidneys (19). $KBrO_3$ is known to increase the formation of free radicals and reactive oxygen species such as: peroxynitrite anion ($ONOO^-$), nitric oxide ($NO^\cdot$) and superoxide anion radical ($O_2^{\cdot}$) and to decrease the activity of an important antioxidative enzyme—glutathione peroxidase (32). Reduction of $KBrO_3$ by sulfhydryl compounds yields oxobromate ions (BrO^-), bromine oxides (BrO, BrO_2), and bromine radicals ($Br^\cdot$), which oxidatively damage DNA and are responsible for carcinogenic effects of $KBrO_3$ (19).

Due to the prooxidative action of $KBrO_3$, antioxidants are expected to protect against carcinogenicity, caused by this compound. So, it can be hypothesized that antioxidants may act against thyroid oxidative stress and hyperproliferative response mediated by KBrO3. In fact, certain antioxidant substances, like ebselen (35) and kolaviron (9), and melatonin (7), have been shown to prevent oxidative damage to macromolecules caused by $KBrO_3$ in kidney.

Melatonin (*N*-acetyl-5-methoxytryptamine), the pineal hormone, is a direct free radical scavenger, and an indirect antioxidant when stimulating antioxidant enzymes (24). It has the ability to increase the efficiency of mitochondrial electron transport chain (ETC) thereby reducing free radical generation (1).

Fruits and vegetables are good sources of natural antioxidants for the human diet and have been strongly associated with reduced risk of many chronic diseases and age-related functional decline in addition to other health benefits (31). Ascorbic acid (vitamin C), one of famous antioxidant, is an electron donor by donating its electrons; it prevents other compounds from being oxidized. However, ascorbic acid itself is oxidized in the process (22).

No studies except Karbownik et al. (16) have yet been carried out on the thyroid gland, which is the target tissue for the carcinogenic action of $KBrO_3$.

So, the aim of this study was to prove the prooxidative effect of $KBrO_3$ and to evaluate and compare the possible protective effects of melatonin and ascorbic acid against lipid peroxidation in the thyroids, collected from rats pretreated with $KBrO_3$.

Material and Method:
Experimental design
A total of 48 male Wistar albino rats (weighing about 160 -200 g each) aged 6-8 months old were used in the study. The animals were randomized into six groups each consists of 8 rats. The rats, were treated with freshly prepared 0.9% NaCl: ethanol (v/v 10:1) (control group) in group I, or $KBrO_3$ (110 mg/kg, i.p. on the 10th day of the experiment) in distilled H_2O in group II (16), or ascorbic acid (500 mg/kg, daily, for 10 days) in distilled H_2O in group III (33), or melatonin [15 mg/kg, two times daily, for 10 days in freshly prepared 0.9% NaCl: ethanol (v/v 10:1)] in group IV (16).

Rats in groups V and VI were received either ascorbic acid, or melatonin respectively combined with $KBrO_3$ (on the 10^{th} day) [all chemicals were in the same dose as previous groups]. All the substances (except for ascorbic acid that was given in gavage) were administered i.p. in volumes of 0.5 ml/injection. Chemicals (Melatonin, ascorbic acid and KBrO3) were purchased from Sigma-Aldrich (powder).

All rats were received water ad libitum. The rats will be killed by decapitation 24 h after $KBrO_3$ injection. The thyroids will be collected and prepared for biochemical analysis and gene expression. All sacrificed animals were disposed in a safety cabinet in the Medical Experimental Research Centre in Mansoura University. This research was approved by the Medical Research Ethics Committee of Mansoura University.

Tissue Homogenate preparation: Thyroid was perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg / ml heparin to remove any red blood cells and clots. Then, thyroid was homogenized in 5 – 10 ml cold buff-

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er (i.e. 50 mM potassium phosphate, pH 7.5. 1 mM EDTA). Homogenates were centrifuged at 10000 x *g* for 15 minutes at 4°C and the supernatant was kept at −80 °C till used for analysis of lipid peroxides, total antioxidants, catalase, and superoxide dismutase.

Lipid peroxides (malondialdehyde, MDA) were analyzed by the method of Buege and Aust, (5) using colorimetric kit (Bio-Diagnostics, Dokki, Giza, Egypt, Cat. No. # MD 2528).

Total antioxidants (TAO) were colorimetrically determined according to the method described by Koracevic et. al. (17), using the kit (Cat. No. # TA 25 12) supplied by Bio-Diagnostics, Dokki, Giza, Egypt.

Catalase (CAT) was estimated by the method described by Aebi (2) using colorimetric kit (Bio-Diagnostics, Dokki, Giza, Egypt, Cat. No. # CA 25 16).

Superoxide dismutase (SOD) was determined according to the method described by Nishikimi et. Al. (20) using the colorimetric kit (Bio-Diagnostics, Dokki, Giza, Egypt, Cat. No. # SD 25 20).

Gene expression: Semiquantitative Reverse transciptase-PCR

Total RNA was extracted from the selected thyroid tissue, using TriFast ™ reagent (PeqLab. Biotechnologie GmbH, Carl-Thiersch St. 2B 91052 Erlongen, Germany, Cat. No. 30-2010) according to the manufacturer's instructions. Measuring the absorbance at 260nm as RNA yield (µg/ml) = A260 X 40 X 100 (dilution factor) (23). The two bands represented 28S and 18S ribosomal RNA.

RT-PCR for extracted RNA: RT-PCR was performed using Ready-to-Go RT-PCR beads for first cDNA synthesis and PCR reaction provided by Amersham Biosciences, England. Cat. No. 27-9266-01, according to the method of Berchtold (4).

***A)* Synthesis of cDNA:**
Gene specific primers used were purchased from Biolegio. BV, PO Box 91, 5600 AB Nijmegen, Netherlands.

Gene	Primer	Reference
Catalase	Forward 5`- GCT CCC AAC TAC TAC CCC AAC-3`. Reverse 5`- CGT TTC CTC TCC TCC TCA TTC-3`.	(25)
Glutathione peroxidase	Forward 5`- GGT TTC CCG TGC AAT CAG-3`. Reverse 5`- GAA CAC CGT CTG GAC CTA CC-3`.	(25)
Cu Zn SOD	Forward 5`- AAC TGA AGG CGA GCA TGG-3`. Reverse 5`- ATC ACA CCA CAA GCC AAGC-3`.	(25)
Internal housekeeping (control) gene (GAPDH)	Forward 5`- CCATCAC-CATCTTCCAGGAG-3`. Reverse 5`- CCTGCTTCAC-CACCTTCTTG-3`.	(18)

B)* Amplification of cDNA by PCR*:
Thermal cycling reaction was performed using thermal cycler (Minicycler PTC-150)

***C)* Detection of amplified RT-PCR products:**
For semiquantitative RT-PCR, the products of amplification were subjected to agarose gel electrophoresis using 2% agarose stained with ethidium bromide and visualized via light UV Transilluminator (Model TUV-20, OWI. Scientific, Inc. 800 242-5560). The results photos were analyzed with scion image ® release Alpha 4.0.3.2. software for windows ®. Gene expression levels were determined by calculating the ratio between the square pixel value of the target gene in relation to the internal house keeping control gene (GAPDH).

Negative control tubes showed no PCR products indicating that all reagents were free from target sequence contamination.

Statistical analyses:
Statistical analysis was performed using the Statistical Package for Social Science program (SPSS, version 10.0, Chicago, IL, USA). The data were expressed as mean ± standard deviation. The significance of differences between mean values was determined using Student's t-test, and p values were considered significant (*) if values < 0.05, highly significant (**) with values < 0.01, and extremely significant (***) with values < 0.001.

Results:
The present results indicated the significant increase in MDA (indicator of lipid peroxidation) in $KBrO_3$group (GII) as compared with control group (GI) in thyroid homogenate. This high level of MDA in GII decreased significantly in groups pretreated with ascorbic acid or melatonin (GV, VI) and approached the basal level of GI, so they prevented the $KBrO_3$-induced oxidative damage to lipids in rat thyroid. Neither melatonin nor ascorbic acid (GIII, IV) could affect the basal level of MDA or lipid peroxidation in thyroid [figure1].

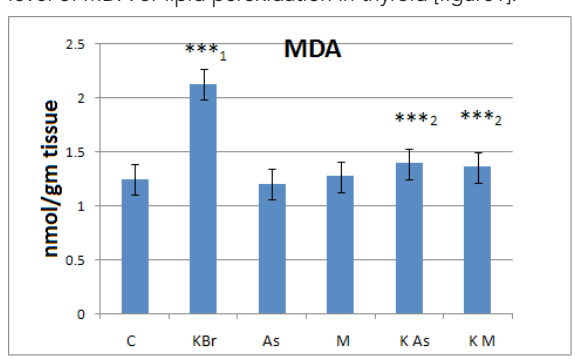

Figure (1): Effect of ascorbic acid and melatonin alone or with $KBrO_3$ on MDA in thyroid tissue. C (control), KBr ($KBrO_3$), As (ascorbic acid), M (melatonin), KAs ($KBrO_3$pretreated with ascorbic a.), KM ($KBrO_3$pretreated with melatonin). 1: significance of results as compared with control group. 2: significance of results as compared with $KBrO_3$ group.

By measuring the activity of TAO in thyroid homogenate; there were mild significant increases in G II, III and IV as compared with GI. Pretreated groups V and VI gave extremely significant increase in TAO as compared with both GII (by38% and 47%, respectively) and G I (by 58% and 64%, respectively) [figure2].

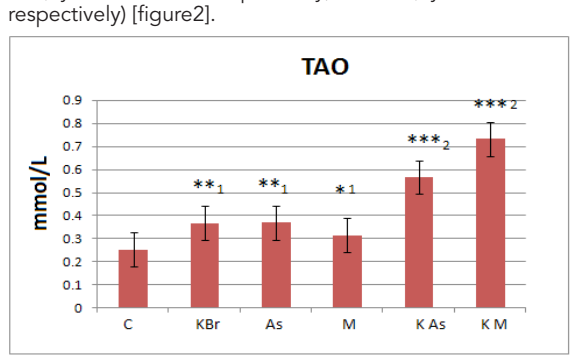

Figure (2): Effect of ascorbic acid and melatonin alone or with $KBrO_3$ on TAO activity in thyroid tissue. C (control),

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KBr ($KBrO_3$), As (ascorbic acid), M (melatonin), KAs ($KBrO_3$pretreated with ascorbic a.), KM ($KBrO_3$pretreated with melatonin). 1: significance of results as compared with control group. 2: significance of results as compared with $KBrO_3$ group

Considering SOD activity in tissues, there was mild significant increase in basal level with ascorbic acid (GIII). $KBrO_3$ caused extremely significant decrease in SOD but pretreatment groups (GV, VI) showed elevation in its level significantly when compared with either control group (by 9.8% and 14%, respectively) or $KBrO_3$ group (by 19.5% and 23%, respectively) [figure3].

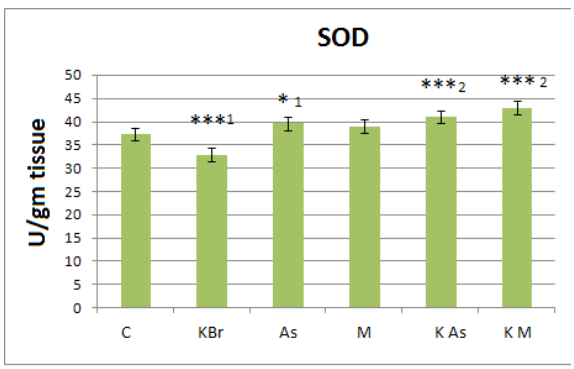

Figure (3): Effect of ascorbic acid and melatonin alone or with $KBrO_3$ on SOD activity in thyroid tissue. C (control), KBr ($KBrO_3$), As (ascorbic acid), M (melatonin), KAs ($KBrO_3$pretreated with ascorbic a.), KM ($KBrO_3$pretreated with melatonin). 1: significance of results as compared with control group. 2: significance of results as compared with $KBrO_3$ group.

By measuring CAT enzyme activity in thyroid tissues; figure (4) showed that only pretreatment groups (V, VI) gave extremely significant increase when compared either with control group (by 48.6% and 62%, respectively) or with $KBrO_3$ group (by 46% and 60%, respectively).

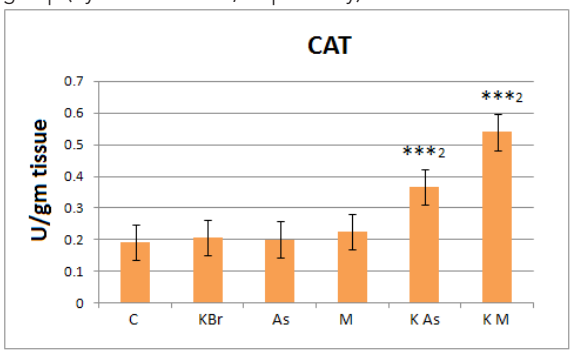

Figure (4): Effect of ascorbic acid and melatonin alone or with $KBrO_3$ on CAT activity in thyroid tissue. C (control), KBr ($KBrO_3$), As (ascorbic acid), M (melatonin), KAs ($KBrO_3$pretreated with ascorbic a.), KM ($KBrO_3$pretreated with melatonin). 1: significance of results as compared with control group. 2: significance of results as compared with $KBrO_3$ group

By genetic expression of antioxidant enzymes mRNAs; $KBrO_3$ showed mild significant decrease in both SOD and GPx mRNAs expression as compared with control group (tables 2, 3). Pretreatment with either ascorbic acid or melatonin gave extremely significant increase in the expression of CAT mRNA (by 40% and 54.5%, respectively), SOD mRNA (by 42% and 57%, respectively) and GPx mRNA (36.7% and 50.6%, respectively) in thyroid tissues as compared with $KBrO_3$ group (tables 1, 2, 3). Also, these antioxidant enzymes mRNAs expressions increased significantly with group V and group VI as compared with control group. Only melatonin gave mild significant increase in basal expression of CAT and GPx mRNAs (tables 1, 3). All results showed that melatonin is better than ascorbic acid as powerful antioxidant in thyroid tissues by stimulating the activity and expression of antioxidant enzymes mRNAs.

Table (1): Effect of ascorbic acid and melatonin alone or with $KBrO_3$ on CAT expression in thyroid tissue

	Control	$KBrO_3$	Ascorbic a.	Melatonin	$KBrO_3$ pretreated with ascorbic a.	$KBrO_3$ pretreated with melatonin
mean±S.D.	0.37±0.04	0.32±0.05	0.36±0.05	0.44±0.05	0.5±0.1	0.66±0.1
P1	-	NS	NS	=0.05	<0.01	<0.001
P2		-			=0.001	<0.001

P1: significance of results as compared with control group.
P2: significance of results as compared with $KBrO_3$ group.

Table (2): Effect of ascorbic acid and melatonin alone or with $KBrO_3$ on SOD expression in thyroid tissue

	Control	$KBrO_3$	Ascorbic a.	Melatonin	$KBrO_3$pretreated with ascorbic a.	$KBrO_3$pretreated with melatonin
mean±S.D.	0.43±0.05	0.33±0.06	0.42±0.06	0.4±0.03	0.57±0.08	0.71±0.09
P1	-	<0.01	NS	NS	<0.01	<0.001
P2		-			<0.001	<0.001

P1: significance of results as compared with control group.
P2: significance of results as compared with $KBrO_3$ group.

Table (3): Effect of ascorbic acid and melatonin alone or with $KBrO_3$ on GPx expression in thyroid tissue

	Control	$KBrO_3$	Ascorbic a.	Melatonin	$KBrO_3$pretreated with ascorbic a.	$KBrO_3$pretreated with melatonin
mean±S.D.	0.49±0.04	0.38±0.06	0.49±0.04	0.5±0.05	0.6±0.05	0.77±0.07
P1	-	<0.01	NS	<0.05	<0.001	<0.001
P2		-			<0.001	<0.001

P1: significance of results as compared with control group.
P2: significance of results as compared with $KBrO_3$ group.

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Discussion:
In thyroid, ROS and free radicals participate in physiological and pathological process in the gland (15). Much evidence gathered in the last years involves the free radicals in the mechanisms of initiation, development of neoplastic transformations *in vivo* and *in vitro*, as well as in the activity of specific oncogenes (19).

Our study is devoted to evaluate the prooxidative effects of $KBrO_3$ in the thyroid which is a well-known target organ for tumorigenic action of this compound. Worth underlining is the fact that the increase of lipid peroxidation in the rat thyroid followed $KBrO_3$ injection is spectacularly high. This finding agrees with Karbownik et al. (15) who noticed lipid peroxidation induced by $KBrO_3$ in the thyroid gland under both in vivo and in vitro conditions. Also, Murata et al. (19) suggested that oxidative stress plays a crucial role in the tumorogenic action of $KBrO_3$ in kidneys.

The present results detect that treatment with either ascorbic acid or melatonin does not affect the basal lipid peroxidation (auto-oxidation) in the examined tissues. This finding agrees with the observation of Karbownik and Lewiński (13). Also, the pre-treatments with either antioxidant ameliorate the lipoperoxidative changes induced by KBrO3 in rat thyroid. It is possible that these antioxidants would prevent KBrO3-induced tumor in the thyroid. It is observed also that pretreatment with melatonin shows more significant effects than ascorbic acid. These results are in agree with Karbownik and Lewinski (14) who noticed that melatonin can inhibit thyroid growth processes and its protection against oxidative damage in thyroid homogenates . Melatonin, which is highly lipid soluble, is believed to be widely distributed in cellular membranes (26). It has the ability to scavenge such toxic species, like $ONOO^-$, OH and $NO^{\cdot}$ (14), that being induced by $KBrO_3$.

Although ascorbic acid has a range of physiological and pharmacological functions, its solubility limits its cumulative amount in the cells and hinders its applications to reduce the reactive oxygen species (ROS) levels in vivo (21). It was suggested that ascorbic acid is an effective chemoprotective agent against nephrotoxicity induced by the antitumoral cisplatin in Wistar adult rats (3). Helen and Vijayammal (11) noticed that rats when exposed to cigarette smoke were protected by ascorbate. However, Dillard et al. (6) found that when alloxan was the oxidant stress agent, rats had increased oxidant stress when supplemented with vitamin C. Such data suggest that ascorbate can act as an antioxidant or prooxidant, dependent on its concentrations and those of the administered oxidizing agent (22).

Results reveal that melatonin is a powerful antioxidant. Many experimental researches support our results like Urata et al. (29) who detected its stimulation of glutathione synthesis and Gitto et al. (10) that noticed its ability to augment the activities of other antioxidants, as well as its protection of antioxidative enzymes from oxidative damage. Melatonin easily crosses all morphophysiological barriers and it is widely distributed in tissues that guarantee its ability to interact with toxic molecules throughout the cell (28). Unlike other antioxidants, melatonin does not undergo redox cycling. Redox cycling may allow other antioxidants (such as vitamin C) to act as pro-oxidants and promote free radical formation. Melatonin, once oxidized, cannot be reduced to its former state. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (27).

Finally, the present results support that melatonin is more powerful antioxidant than ascorbic acid and has better preventing role against $KBrO_3$ toxicity in thyroid tissues.

Conclusion: Oxidative stress plays a major role in the carcinogenic action of $KBrO_3$. It is possible that these antioxidants may be of great value as protective agents against $KBrO_3$-induced tumor induction in the thyroid. Melatonin is more powerful antioxidant than ascorbic acid and it could be considered as pharmacological agents for protection against potential carcinogens.

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Author contribution: Shereen M. Samir and Abeer F. Mostafa designed and performed research; also, wrote the paper. Shereen M. Samir also analyzed data.

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REFERENCE

1. Acuna-Castroviejo D, Escames G, Carozo A, Leon J, Khaldy H, Reiter RJ : Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. Curr. Top Med. Chem. 2, 133–52(2002). | 2. Aebi H : Catalase in vitro. Methods Enzymol. 105, 121 – 126 (1984). | 3. Antunes LMG, Darin JDC, Bianchi MLP: Protective effects of vitamin C against cisplatin–induced nephrotoxicity and lipid peroxidation in adult rat: a dose dependent study. Pharmacological Research. 41(4), 405–411(2000). | 4. Berchtold MW: A simple method for direct cloning and sequencing cDNA by the use of a single specific oligonucleotide and oligo(dT)in a polymerase chain reaction(PCR). Nucleic Acids Res. 17(1), 453 (1989). | 5. Buege JA and Aust SD: Microsomal lipid peroxidation. Methods Enzymol. 52, 302–10 (1972). | 6. Dillard CJ, Kunert KJ, Tappel AL: Effects of vitamin E, ascorbic acid and mannitol on alloxan-induced lipid peroxidation in rats. Arch. Biochem. Biophys. 216, 204– 212 (1982). | 7. El-Sokkary GH: Melatonin protects against oxidative stress induced by the kidney carcinogen KBrO3. Neuroendocrinol. Lett. 21,461–468(2000). | 8. FAO/WHO: Food and Agriculture Organization. Guide to the Safe Use of Food Additives, Second Series. Geneva. World Health Organization (1979). | 9. Farombi EO, Alabi MC, Akuru TO : Kolaviron modulates cellular redox status and impairment of membrane protein activities induced by potassium bromated (KBrO3) in rats. Pharmacological Research 45, 63–68 (2002). | 10. Gitto E, Tan DX, Reiter RJ, Karbownik M, Manchester LC, Cuzzocrea S, Fulia F, Barberi I : Individual and synergistic actions of melatonin: Studies with vitamin E, vitamin C, glutathione and desferoxamine in liver homogenates. J. Pharm. Pharmacol. 53, 1393–401 (2001a). | 11. Helen A and Vijayammal PL: Vitamin C supplementation on hepatic oxidative stress induced by cigarette smoke. J. Appl. Toxicol. 17, 289– 295 (1997). | 12. IARC : Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation. Lyon, France: IARC publication No. 40 (1986). | 13. Karbownik M and Lewinski A: The role of oxidative stress in physiological and pathological processes in the thyroid gland; possible involvement in pineal-thyroid interactions. Neuro. Endocrinol. Lett. 24(5), 293-303 (2003a). | 14. Karbownik M and Lewiński A: Melatonin reduces Fenton-induced lipid peroxidation in porcine thyroid tissue. J. Cell Biochem. 90, 806-811 (2003b). | 15. Karbownik M, Lewinski A, Reiter RJ: Anticarcinogenic actions of melatonin which involve antioxidative processes: Comparison with other antioxidants. Int. J. Biochem. Cell Biol. 33, 735–753 (2001c). | 16. Karbownik M, Stasiak M, Zasada K, Zygmunt A, Lewinski A : Comparison of potential protective effects of melatonin, indole-3-propionic acid, and propylthiouracil against lipid peroxidation caused by potassium bromate in the thyroid gland. Journal of Cellular Biochemistry 95(1), 131–138 (2005). | 17. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S and Cosic V: Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol. 54, 356 – 361 (2001). | 18. Li HS and Carayanniotis G : Induction of Goitrous Hypothyroidism by dietary iodide in SJL mice. Endocrinology 148(6),2747–2752 (2007). | 19. Murata M, Bansho Y, Inoue S, Ito K, Ohnishi S, Midorikawa K, Kawanishi S: Requirement of glutathione and cysteine in guanine-specific oxidation of DNA by carcinogenic potassium bromate. Chem. Res. Toxicol. 14, 678–685(2001). | 20. Nishikimi M, Roa NA and Yogi K: The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem. Bioph. Res. Commun. 46,849-854 (1972). | 21. Oh C, Li M, Kim EH, Park JS, Lee JC, and Ham SW: Antioxidant and Radical Scavenging Activities of Ascorbic Acid Derivatives Conjugated with Organogermanium Bull. Korean Chem. Soc. 31 (12), 3513(2010). | 22. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J-H, Chen S, Corpe C, Dutta A, Dutta SK and Levine M : Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. J. Am. Coll. Nutr. 22 (1), 18-35 (2003). | 23. Raha S, Ling M and Merante F: Extraction of total RNA from tissues and cultured cells. NJ.Ch.1,1-8 (1998). | 24. Rodriquez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ : Regulation of antioxidant enzymes: A significant role for melatonin. J Pineal Res. 44, 22-32 (2004). | 25. Ross Laybutt D, Hideaki Kaneto, Wendy Hasenkamp, Shane Grey, Jean-Christophe Jonas, Dennis C. Sgroi, Adam Groff, Christiane Ferran, Susan Bonner-Weir, Arun Sharma and Gordon C. Weir: Increased Expression of Antioxidant and Antiapoptotic Genes in Islets That May Contribute to beta-Cell Survival During Chronic Hyperglycemia. Diabetes 51,413–423 (2002). | 26. Tan DX, Manchester LC, Hardeland R, Lopez-Burillo S, Mayo JC, Sainz RM, Reiter RJ: Melatonin: A hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. J. Pineal Res. 34, 75–78 (2003). | 27. Tan, Dun-Xian, Manchester, Lucien C, Reiter, Russel J, Qi, Wen-Bo, Karbownik, Malgorzata, Calvo, Juan R : "Significance of Melatonin in Antioxidative Defense System: Reactions and Products". Neurosignals 9 (3–4), 137–59 (2000). | 28. Tan, Dun-Xian, Manchester, Lucien C, Terron, Maria P, Flores, Luis J, Reiter, Russel J: "One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species?". Journal of Pineal Research 42 (1), 28–42 (2007). | 29. Urata Y, Honma S, Goto S, Todoroki S, Ueda T, Cho S, Honma K, Kondo T: Melatonin induces gammaglutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. Free Radic. Biol. Med. 27, 838–47(1999). | 30. Urso ML and Clarkson PM: Oxidative stress, exercise, and antioxidant supplementation. Toxicology 189, 41–54 (2003). | 31. Vasquez-Garzon VR, Arellanes-Robledo J, Garcia-Roman R, Aparicio-Rautista DI, Villa- Trevino S: Inhibition of reactive oxygen species and pre-neoplastic lesions by quercetin through an antioxidant defense mechanism. Free Radical Research. 43,128–137 (2009). | 32. Velioglu YS, Mazza G, Gao L, Oomah BD : Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry. 46, 4113–4117 (1998). | 33. Wen Y, Cooke T, Feely J : The effect of pharmacological supplemen-tation with vitamin C on low density lipoprotein oxidation. Br. J. Clin. Pharma. 44,94-97 (1997). | 34. Wolf DC, Crosby LM, George MH, Kilburn SR, Moore TM, Miller RT, DeAngelo AB: Time- and dose-dependent development of potassium bromate-induced tumors in male Fischer 344 rats. Toxicol. Pathol. 26,724–729 (1998). | 35. Yoshizumi M, KogameT, SuzakiY, FujitaY, KyawM, Kirima K, Ishizawa K, Tsuchiya K, Kagami S and Tamaki T: Ebselen attenuates oxidative stress-induced apoptosis via the inhibition of the c-Jun N-terminal kinase and activator protein-1 signalling pathway in PC12 cells. Br J Pharmacol. 136(7), 1023–1032 (2002). |