PROTECTIVE EFFECT OF MELATONIN AGAINST FLUORIDE-INDUCED OXIDATIVE STRESS IN THE MOUSE OVARY

Sunita L Chawla,[a] Mandava V Rao[a,b]
Ahmedabad, India

SUMMARY: The protective effect in female mice by intraperitoneal injection of melatonin (10 mg/kg bw/day) against ovarian oxidative stress induced by oral administration of sodium fluoride, NaF (10 mg/kg bw/day), was studied. After 30 days, NaF treatment resulted in a significant reduction in body and ovary weights. Increased production of malondialdehyde in the NaF-treated group was accompanied by marked alterations in the levels of total proteins, total ascorbic acid, reduced glutathione, and the activities of superoxide dismutase and catalase. The ovarian histology was also abnormal, thereby indicating deterioration of ovarian function. Compared to NaF alone, the combined treatment of F with melatonin significantly lowered the level of lipid peroxides and enhanced the antioxidant status. Likewise, the histopathological changes of ovary were also revived by the combined treatment. The second group of mice given melatonin without exposure to NaF exhibited no significant changes in the above indices. Thus the results from this study showed that melatonin exerted a protective action against F-induced oxidative stress and disturbance of ovarian functions in the mouse.

Keywords: Fluoride ovary toxicity; Melatonin as antioxidant; Mouse ovary; Ovarian oxidative stress.

INTRODUCTION

Fluoride (F) has been found to interfere with the reproductive system of animals. In male animals the effects include decreased activity of the steroidogenic enzymes, decreased serum testosterone levels, reduced sperm count and motility, testicular damage, structural defects in spermatozoa, and oxidative stress.[1-7] In female Sprague-Dawley rats with exposure to NaF in drinking water, significant decreases in the number of viable fetuses and increases in the resorption rate were observed.[8] F has also been shown to induce free radical toxicity in mice ovary.[9,10] In prepubescent gerbils, it inhibits synthesis of melatonin (N-Acetyl-5-methoxytryptamine), a secretory product of the pineal gland as well as other select organs, thereby causing an earlier onset of pubertal development in human females and decreasing testes weights in males.[11] Exposure to high F concentrations in drinking water is also associated with decreased human birth rates.[12]

As a scavenger of free radicals, directly detoxifying both reactive oxygen and nitrogen species, and indirectly increasing the activity of the antioxidant defense systems,[13,14] melatonin has been shown in our laboratory to exert protective effects against F-induced hepato- and nephrotoxicity.[15,16] It also reduces chromosomal anomalies induced by F *in vitro*.[17] Recently, Bharti and Shrivastava have reported ameliorative effects of pineal proteins and melatonin against high F intake,[18] and in our laboratory we have found that melatonin has ameliorative effects *in vitro* on F-induced haemolysis.[19]

MATERIALS AND METHODS

Animals: Forty healthy adult female Swiss-strain albino mice (*Mus musculus*) weighing between 35 and 40 g were obtained from Alembic Pharmaceuticals,

[a]Zoology Department, School of Sciences, Gujarat University, Ahmedabad 380 009, India.
[b]For correspondence: Dr MV Rao, Professor and Head, Zoology Department, School of Sciences, Gujarat University, Ahmedabad 380 009, India. E-mail:zooldeptgu@satyam.net.in.

Vadodara, India, under the Animal Maintenance and Registration No. 167/1999/ CPCSEA, from the Ministry of Social Justice and Empowerment, Government of India Committee for the purpose of Control and Supervision of Experiments on Animals, Chennai, India. The mice were acclimatized for seven days prior to the commencement of the treatment and were housed in an air-conditioned animal house at 26±2ºC with exposure to 10–12 hr of daylight. They were fed a standard mouse chow according to the guidelines of the National Institute of Occupational Health (NIOH), Ahmedabad, and were given water (0.6–1.0 ppm F) *ad libitum*.

Exposure: The animals were divided into four groups of ten with a 30-day treatment period for each group. The first group served as control. The second group was intraperitoneally injected with melatonin (MLT, Hi-media, Mumbai) at a dose of 10 mg/kg bw/day. Sodium fluoride, NaF (Qualigens Fine Chemical, Mumbai, 99% purity) was administered orally (10 mg/kg bw/day) with a feeding tube attached to a hypodermic syringe to the mice in Group III. The fourth group was given both MLT and NaF with MLT preceding NaF by 30 minutes. At the end of the 30-day treatment period, the mice were weighed on an animal weighing balance (Ohaus, USA) and sacrificed by cervical dislocation. The ovaries were dissected out carefully, blotted free of blood, weighed to the nearest milligram, and used for the estimation of total proteins,[20] total ascorbic acid,[21] reduced glutathione,[22] and the activities of superoxide dismutase[23] (E.C. 1.15.1.1), and catalase[24] (E.C.1.11.1.6). Histology of the ovary was conducted by standard haematoxylene-eosin (HE) staining on 5-µ sections.

Statistical analysis: For all biochemical parameters, at least 6 to 8 replicates were performed. The data were analyzed statistically using Student's test and Analysis of Variance (ANOVA). A level of $p<0.05$ was accepted as significant.

RESULTS

Body and ovary organ weights: As shown in Figures 1 and 2, NaF treatment brought about a significant ($p<0.001$) reduction in the body and ovary weight ($p<0.05$).

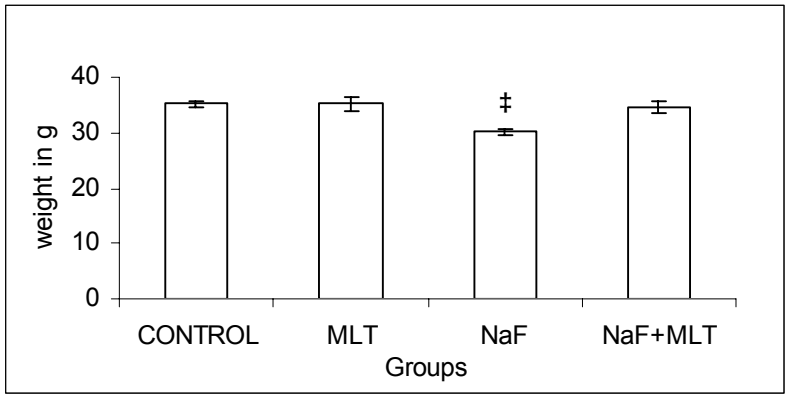

Figure 1. Body weights of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; ‡$p<0.001$ when compared to control.

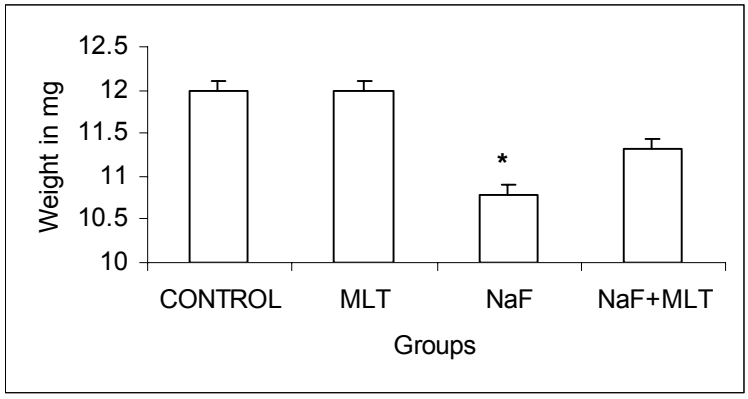

Figure 2. Ovarian weights of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; *p<0.05 when compared to control group.

SOD and catalase: The activities of superoxide dismutase (SOD) and catalase declined significantly (p<0.01) following NaF exposure (Figures 3 and 4).

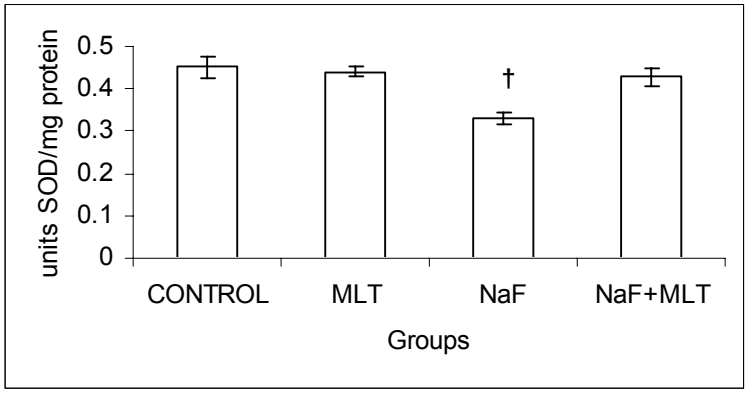

Figure 3. Activity of SOD in ovary of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; †p<0.01 when compared to control.

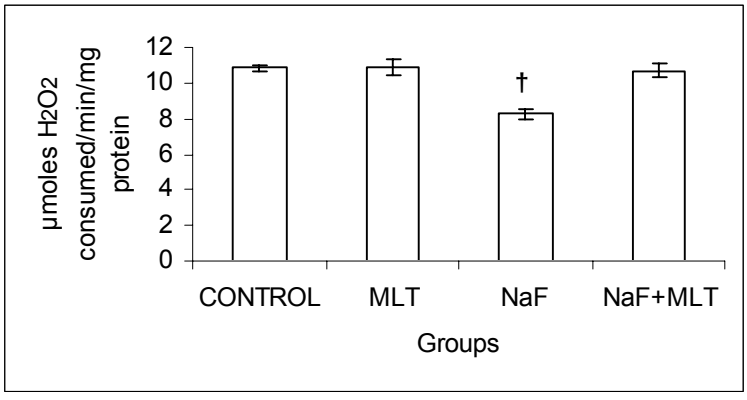

Figure 4. Activity of catalase in ovary of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; †p<0.01 when compared to control.

Lipid peroxidation (LPO), glutathione (GSH), total ascorbic acid (TAA), and protein: Lipid peroxide levels recorded a steep elevation ($p<0.001$), whereas GSH, TAA and protein levels registered a significant depletion ($p<0.05$, $p<0.001$, $p<0.001$) in the ovary following NaF exposure (Figures 5–8).

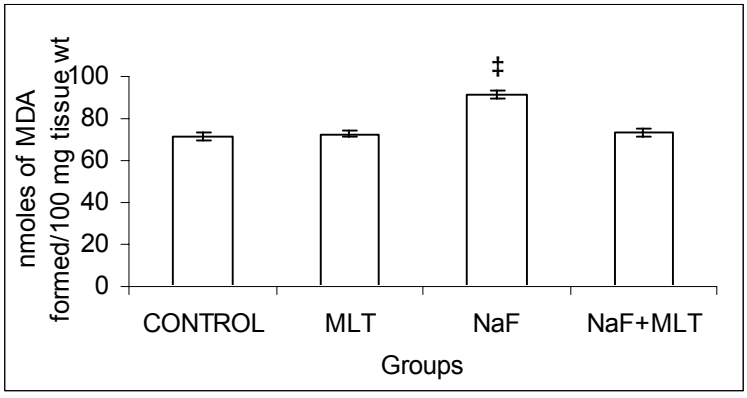

Figure 5. Malondialdehyde levels in ovary of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; ‡$p<0.001$ when compared to control.

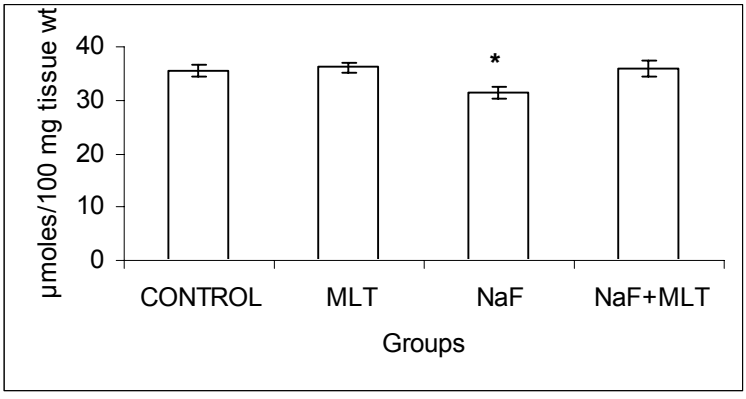

Figure 6. GSH levels in ovary of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; *$p<0.05$ when compared to control group.

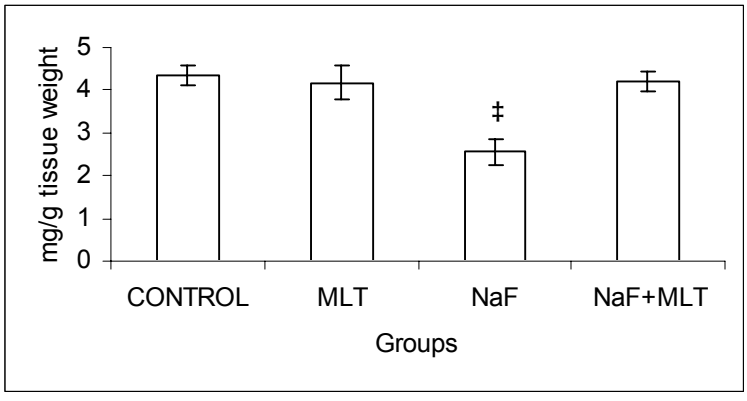

Figure 7. Levels of total ascorbic acid in ovary of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; ‡$p<0.001$ when compared to control group.

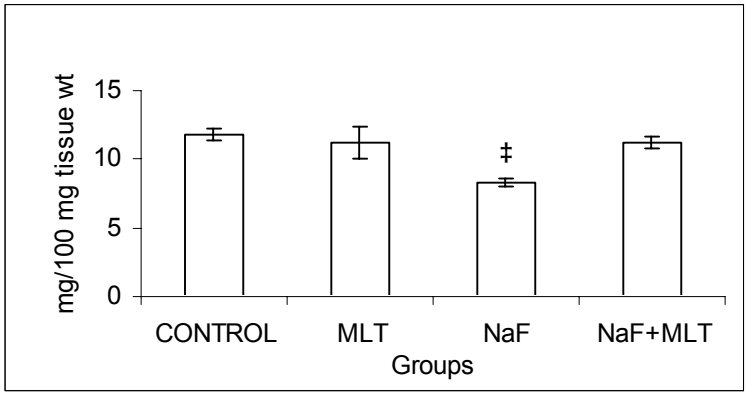

Figure 8. Levels of protein in ovary of control, melatonin (MLT), and NaF-treated groups. Data are mean ± SEM of 10 mice in each group; ‡p<0.001 when compared to control group.

MLT effects: As with the body and ovary weights, the above-mentioned parameters exhibited no significant changes in the Group II mice treated with MLT alone. Similarly, pretreatment with MLT of the Group IV NaF-MLT-treated mice revealed no significant changes in these indices as compared to the controls in Group I.

Histology: The ovaries of the control mice (Group I) exhibited all the characteristic features of normal tissue (Figure 9). After NaF treatment, follicular atresia, and degenerated stromal tissue were observed (Figure 10). The combined treatment brought about significant recovery (Figure 11).

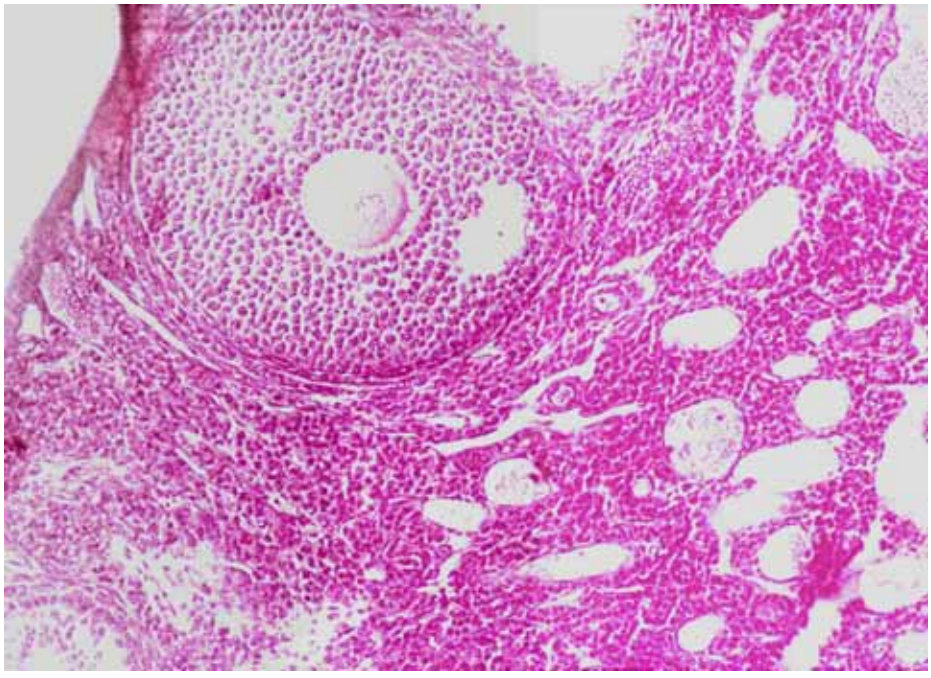

Figure 9. Transverse section of ovary of control mice showing oocyte in a secondary follicle. HE staining (× 250).

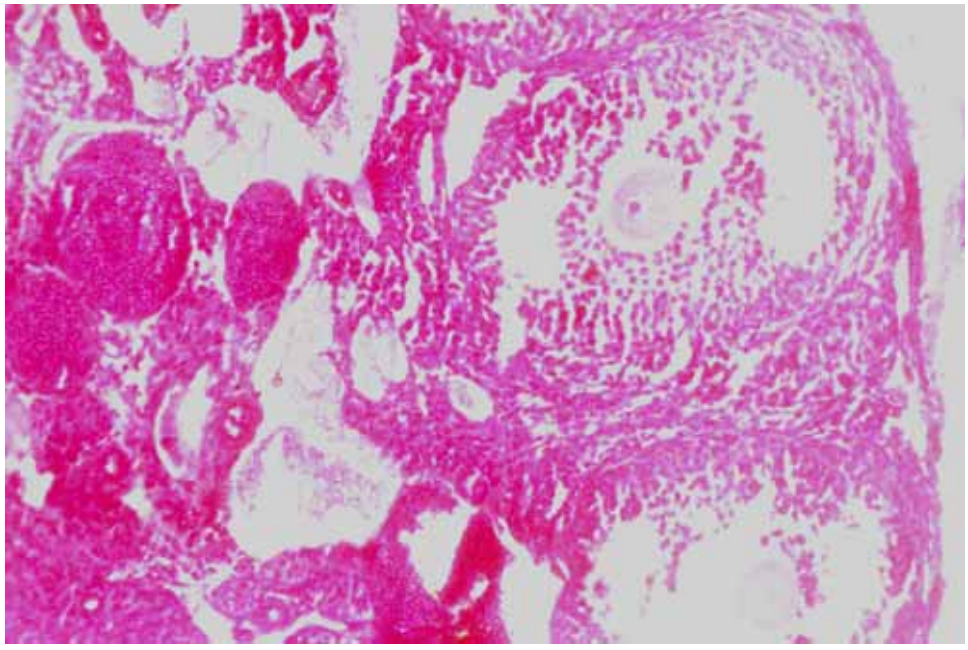

Figure 10. Transverse section of mouse ovary showing follicular atresia and degenerated stromal tissue after NaF treatment. HE staining (× 250).

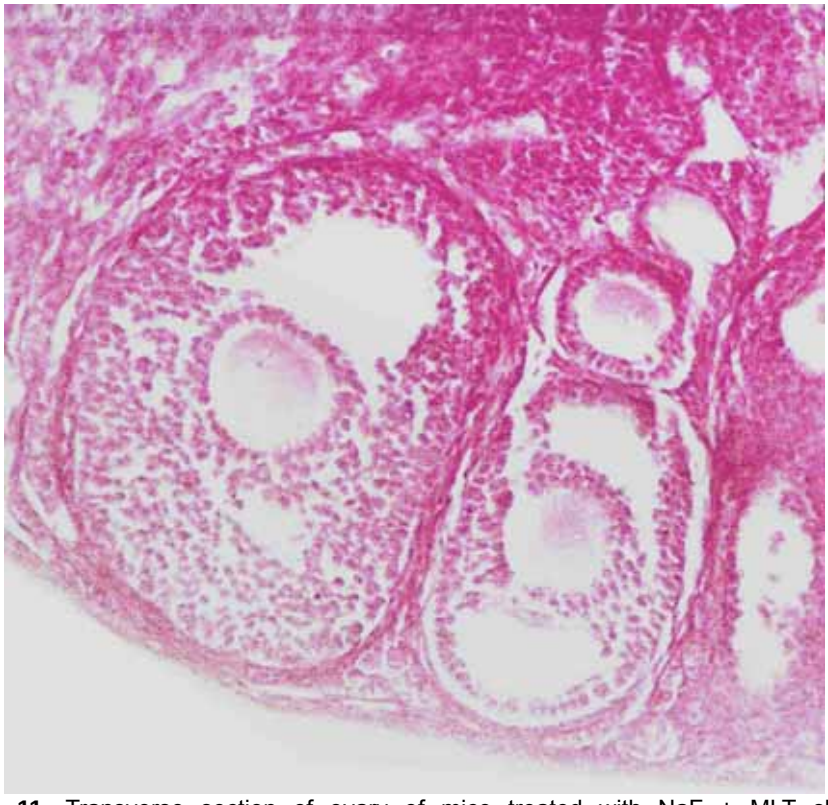

Figure 11. Transverse section of ovary of mice treated with NaF + MLT showing recovery and normal secondary follicle. HE staining (× 250).

DISCUSSION

A decrease in ovarian protein levels occurred following NaF exposure in agreement with data reported by others.[8,9] This reduction could be due to impaired protein synthesis known to be caused by F.[25] In the present study, the levels of enzymatic and non-enzymatic antioxidants were significantly decreased, whereas lipid peroxide levels were enhanced in the ovary of NaF-exposed mice. These results indicate that F enhanced lipid peroxidation and impaired the antioxidant system in this organ. The histopathology of ovary revealed loss of follicular maturation and other atrophic conditions as its protein levels were reduced, resulting in weight loss by the organ. Our findings are therefore in agreement with those of other workers.[8,9]

The pineal gland contains the highest concentration of F in any part of the human body.[26] An excessively high local concentration of F within the pineal gland would be expected to affect the functions of the gland, i.e., the synthesis of hormonal products, especially MLT. In agreement with this view, female gerbils treated with F have been shown to have lower levels of circulating MLT as reflected by reduced levels of MLT and its metabolites in the urine.[10] MLT is also known to have direct effects on ovarian function,[27] and its receptors have been identified in both female and male gonads.[28,29] Thus, Tamura et al. have demonstrated that MLT curtails oxidative mutilation of essential molecules in oocytes.[30] As a hormone, MLT also helps protect the body from cell damage caused by free radicals. Decreases in MLT levels therefore cause tissues to be more susceptible to oxidative stress. In the present study, MLT supplementation along with NaF, reduced the toxic effects of F in the ovary as shown by the fact that all the above-mentioned biochemical indices were comparable to the controls.

Mechanisms involved in the protective effects of MLT against oxidative stress involve direct free radical scavenging activity and indirect antioxidant actions including stimulating antioxidative enzymes,[12,13] increasing the efficiency of mitochondrial oxidative phosphorylation, reducing electron leakage with a decrease in free radical generation,[31] and augmenting the efficiency of other antioxidants.[32] In conclusion, the present study has shown that MLT ameliorates F-induced oxidative stress in the mouse ovary as it exerts its potential to maintain normal antioxidant status and gonadal function.

This work was presented at The XXVIIIth Conference of the International Society for Fluoride Research, August 7–11, 2008, Toronto, Canada.

ACKNOWLEDGEMENT

MVR thanks the University Grants Commission (UGC), New Delhi for financial assistance.

REFERENCES

1 Narayana MV, Chinoy NJ. Effect of fluoride on rat testicular steroidogenesis. Fluoride 1994;27(1):7-12.
2 Huang C, Nui RY, Wang JD. Toxic effects of sodium fluoride on reproductive function in male mice. Fluoride 2007;40(3):162-8.
3 Zhang JH, Liang C, Ma JJ, Niu RY, Wang JD. Effects of sodium fluoride and sulfur dioxide on sperm motility and serum testosterone in male rats. Fluoride 2006;39(2):126-31.
4 Chinoy NJ, Sharma A. Amelioration of fluoride toxicity by vitamins E and D in reproductive functions of male mice. Fluoride 1998;31(4):203-16.

5 Krasowska A Włostowski T, Bonda E. Zinc protection from fluoride-induced testicular injury in the bank vole (*Clethrionomys glareolus*). Toxicol Lett 2004;147:229-35.
6 Kumar A, Susheela AK. Ultrastructural studies of spermiogenesis in rabbit exposed to chronic fluoride toxicity. Int J Fertil Menopausal Stud 1994;39(3):164-71.
7 Ghosh D, Das Sarkar S, Maiti R, Jana D, Das UB. Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reprod Toxicol 2002;16(4):385-90.
8 Al-Hiyasat AS, Elbetieha AM, Darmani H. Reproductive toxic effects of ingestion of sodium fluoride in the female rats. Fluoride 2000;33(2):79-84.
9 Chinoy NJ, Patel D. Influence of fluoride on biological free radicals in ovary of mice and its reversal. Environ Sci 1998;6:171-84.
10 Jhala DD, Chinoy NJ, Rao MV. Mitigating effects of some antidotes on fluoride and arsenic induced free radical toxicity in mice ovary. Food Chem Toxicol 2008;46(3):1138-42.
11 Luke J. Is the pineal gland a target for fluoride toxicity? [abstract].Fluoride 2008;41(3)240–1.
12 Freni SC. Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. J Toxicol Environ Health 1994;42:109-21.
13 Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, et al. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger [review]. Curr Top Med Chem 2002;2:181-97.
14 Rodriquez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, et al. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004;36:1-9.
15 Chawla SL, Yadav R, Shah D, Rao MV. Protective action of melatonin against fluoride-induced hepatotoxicity in adult female mice. Fluoride 2008;41(1):44-51.
16 Rao MV, Chawla SL, Patel N. Melatonin reduction of fluoride-induced nephrotoxicity in mice. Fluoride 2009;42(2):110-6.
17 Rao MV, Tiwari H. Amelioration by melatonin of chromosomal anomalies induced by arsenic and/or fluoride in human blood lymphocyte cultures. Fluoride 2006;39(4):255-60.
18 Bharti VK, Srivastava RS. Effect of pineal proteins and melatonin on certain biochemical parameters of rats exposed to high-fluoride drinking water. Fluoride 2011;44 (1):30-6.
19 Rao MV, Vyas DD, Meda RB, Chawla SL. *In vitro* protective role of melatonin against hemolysis induced by sodium fluoride in human red blood cells. Fluoride 2011;44(2)77-82.
20 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
21 Roe JH, Kuether CA, The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl hydrazine derivative of dehydroascorbic acid. J Biol Chem 1943;147: 399-407.
22 Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
23 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
24 Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
25 Hongslo JK, Holland RI. Effect of sodium fluoride on protein and DNA synthesis, ornithine decarboxylase activity, and polyamine content in LH cells. Acta Pharmacol Toxicol (Copenh) 1979;44:350-3.
26 Luke J. The effect of fluoride on the physiology of the pineal gland. [doctoral dissertation]. Guildford: University of Surrey; 1997.
27 Woo MM, Tai CJ, Kang SK, Nathwani SP, Pang SF, Leung PC. Direct action of melatonin in human granulosa-luteal cells. J Clin Endocrinol Metab 2001;86:4789-97.
28 Frungieri MB, Mayerhofer A, Zitta K, Pignataro OP, Calandra RS, Gonzalez-Calvar SI. Direct action of melatonin on Syrian hamster testes: melatonin subtype 1a receptors, inhibition of androgen production, and interaction with local corticotropin-releasing hormone system. Endocrinology 2005;146:1541-52.
29 Tamura H, Nakamura Y, Korkmaz A, Manchester LC, Tan DX, Sugino N, et al. Melatonin and the ovary: physiological and pathophysiological implications. Fertil Steril 2009;92(1):328-43.
30 Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, et al. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rates. J Pineal Res 2008; 44:280-7.
31 Acuńa-Castroviejo D, Escames G, Carozo A, Leon J, Khaldy H, Reiter RJ. Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. Curr Top Med Chem 2002;2:133-52.
32 Gitto E, Tan DX, Reiter RJ, Karbownik M, Manchester LC, Cuzzocrea S, et al. Individual and synergistic actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferrioxamine (desferoxamine) in rat liver homogenates. J Pharm Pharmacol 2001;53:1393-401.

Copyright © 2012 The International Society for Fluoride Research Inc.
www.fluorideresearch.org www.fluorideresearch.com www.fluorideresearch.net
Editorial Office: 727 Brighton Road, Ocean View, Dunedin 9035, New Zealand.