30 Research report
Fluoride 44(1)30–36
January-March 2011

Effect of pineal proteins and melatonin on biochemical parameters
of F-exposed rats
Bharti, Srivastava
30

# EFFECT OF PINEAL PROTEINS AND MELATONIN ON CERTAIN BIOCHEMICAL PARAMETERS OF RATS EXPOSED TO HIGH-FLUORIDE DRINKING WATER

Vijay K Bharti,[a] RS Srivastava[a]

Izatnagar, India

SUMMARY: As part of a series of experiments in our laboratory, we studied the effect of fluoride (F) alone or with buffalo (*Bubalus bubalis*) pineal proteins (BPP) and melatonin (MEL) on certain plasma biochemical parameters in rats. Six groups of six adult female Wistar rats weighing 123-142 g with an untreated group as Control were administered BPP (100 µg/kg BW, i.p.), MEL (10 mg/kg bw, i.p.), F (from NaF at 150 ppm F ion in their drinking water), F+BPP, and F+MEL daily for 28 days. Blood samples were collected at the end of the experiment to estimate plasma glucose, proteins, sodium ($Na^+$), potassium ($K^+$), urea, creatinine, cholesterol, blood urea nitrogen (BUN), serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP) activity. There was no significant variation in glucose, protein, and $K^+$ level among the Control, BPP, and MEL groups. However, significantly ($p < 0.05$) higher levels of plasma glucose were observed in the F+BPP and F+MEL-treated rats. Plasma creatinine, urea, BUN, cholesterol, $K^+$, and $Na^+$ concentrations and also SGPT, SGOT, and ALP activities were significantly higher ($p < 0.05$) in the F-treated animals as compared to the Control group. Administration of BPP and MEL in F-treated rats caused significant ($p < 0.05$) reduction of these adverse changes. BPP and MEL alone resulted in significant ($p < 0.05$) increase in plasma $Na^+$, ALP, and cholesterol. These findings clearly indicated that BPP and MEL had significant ameliorative effects on adverse F-induced changes in certain biochemical parameters in rats. Since they also exhibited no evidence of deleterious alterations in biochemical profiles, BPP and MEL can be considered suitable for testing their beneficial effects on F toxicity in humans.

Keywords: Alkaline phosphatase; Buffalo pineal proteins; Fluoride in rats; Glucose; Melatonin; Plasma biochemical parameters.

## INTRODUCTION

Widely present in the environment, especially when it contaminates drinking water, fluoride (F) has many harmful effects.[1] As a potent protoplasmic poison, it is toxic to living cells, generating reactive free radicals and causing destructive biochemical alterations including oxidative stress in a variety of animal species.[2-4] In animals, various changes occur after chronic administration of F including inhibition of pineal function, membrane-bound ion transport, neurotransmission, and adverse changes in enzymatic activities and balance of blood electrolytes.[1,5,6] The mammalian pineal gland secretes biologically active proteins, peptides, and enzymes that have many physiological roles.[7] Numerous biochemical and behavioral functions of organisms are controlled by the pineal gland through melatonin (MEL) and pineal protein secretions.[8-10] Pineal gland functions include its ability to directly neutralize a number of toxic agents and stimulate antioxidative enzymes.[11] The pineal gland also has the ability to reduce F-induced

[a]Neurophysiology Laboratory, Division of Physiology & Climatology, Indian Veterinary Research Institute, Izatnagar – 243 122, UP, India. For correspondence: Dr Vijay K Bharti, Scientist, Nutrition and Toxicology Laboratory, DIHAR (Defence Institute of High Altitude Research), and DRDO (Defence Research and Development Organization), Leh–194101, Jammu and Kashmir, India. E-mail: vijaykbharti@rediffmail.com.

31  Research report
Fluoride 44(1)30–36
January-March 2011

Effect of pineal proteins and melatonin on biochemical parameters
of F-exposed rats
Bharti, Srivastava  31

oxidative stress and adverse biochemical changes via secretion of MEL in several species.[4,6,12,13]

Recently, we found that buffalo pineal proteins (BPP) and MEL are able to ameliorate arsenic and F-induced oxidative stress in rats.[6,10,14,15] However, the effects of BPP and MEL in ameliorating F-induced biochemical changes remained to be determined, and here we report the results of our investigation of their effects on plasma biochemical parameters in rats.

## MATERIALS AND METHODS

All the procedures conducted on the experimental animals were duly approved by the Institutional Animal Ethics Committee and the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Chemicals: The chemicals used in the study were of analytical grade from Loba Chemie (Mumbai, India), and diagnostic Kits were procured from Span Diagnostic Ltd. Melatonin was purchased from Sigma Chemical Co., St. Louis, USA.

*Experimental design:* The present study was carried out on 36 adult female Wistar rats weighing 123–142 g that were procured from the Laboratory Animal Resource Section of the Institute. Details of treatments including dosages and route of administration are presented in Table 1. Other aspects of the experimental design are similar to those in our previous studies,[6,10,14,15]

**Table 1.** Distribution of experimental female rats at the start of 28-day treatments

(n=6; Values are means±S.E)

| Group | Body weight (g) | Treatment | Dosage | Route of administration |
|---|---|---|---|---|
| Control | 141.66[c] ±3.57 | Drinking water + Normal saline | Ad libitum | Oral Intraperitoneal |
| BPP | 130.00[b] ±3.41 | Buffalo pineal proteins (BPP) | 100 µg/kg bw | Intraperitoneal |
| MEL | 135.00[b,c] ±5.92 | Melatonin | 10 mg/kg bw | Intraperitoneal |
| F | 123.33[a] ±1.05 | NaF + Normal saline | 150 ppm F | Oral with drinking water Intraperitoneal |
| F+BPP | 142.17[c] ±5.66 | NaF + Buffalo pineal | 150 ppm F | Oral with drinking water Intraperitoneal |
| F+MEL | 137.50[b,c] ±4.79 | NaF + Melatonin | 150 ppm F 10 mg/kg bw | Oral with drinking water Intraperitoneal |

[a,b,c]Values in the same column bearing no common superscripts differ significantly (p<0.05).

32 | Research report
Fluoride 44(1)30–36
January-March 2011
Effect of pineal proteins and melatonin on biochemical parameters
of F-exposed rats
Bharti, Srivastava | 32

*Sample collection and analysis:* Daily observations were taken for behavioral changes, clinical signs of toxicity, and mortality, if any, throughout the experimental period. Blood samples were collected by cardiac puncture under ether anesthesia at the end of experiment (28 days). The plasma was then separated from the blood and stored at $-20^\circ$C for biochemical analysis.

Blood glucose, total serum protein, creatinine, cholesterol, urea, blood urea nitrogen (BUN), serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP): These parameters were estimated using Span Diagnostics Ltd kits with a semi-auto analyzer (ERBA CHEM-5 Plus).

Plasma sodium ($Na^+$) and potassium ($K^+$): Plasma $Na^+$ and $K^+$ concentration were estimated simultaneously using a Flame photometer (Mediflame 127, Systronics) as described by Oser.[16]

*Statistical Analysis:* Differences between groups were analyzed statistically by one-way ANOVA, and the differences between the means of groups were separated by least significant difference (LSD) test. Values with $p < 0.05$ were regarded as significant. A computer program (SPSS 10.01, SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

### RESULTS

During the 28-day investigation no symptoms of acute toxicity (salivation, diarrhea, tremors, muscle spasm, shock) or any abnormal behaviors in the rats were observed, and none of the animals in any group died. As seen in Table 2, high levels of plasma glucose were observed in the F+BPP and F+MEL-treated rats as compared to the control, F, BPP, and MEL-administered animals. Except for a small elevation in the F-treated group, there was no significant variation in the glucose level among the control, BPP, and MEL groups. Also in Table 2, plasma $K^+$ and $Na^+$ concentrations were significantly higher ($p < 0.05$) in F-treated animals as compared to the other groups. However, no significant differences were found in plasma $K^+$ and $Na^+$ between the BPP and MEL groups.

As seen in Table 3, higher and lower plasma protein levels were found in the F+BPP and F+MEL-treated rats, respectively, compared to the other groups. Table 3, also shows that F treatment caused significant elevation in the levels of creatinine, urea, BUN, cholesterol, which were significantly reduced in the F+BPP and F+MEL groups. Likewise, as seen in Table 2, F greatly increased ALP, SGOT, and SGPT activities, which were significantly decreased in the F+BPP and F+MEL groups but not all to the levels of the Control, BPP, and MEL groups. Administration of BPP and MEL in F-treated rats caused significant ($p < 0.05$) reduction in adverse changes of $Na^+$, $K^+$, creatinine, urea, BUN, cholesterol, SGPT, SGOT, and ALP level (Tables 2 and 3).

33    Research report
Fluoride 44(1)30–36
January-March 2011

Effect of pineal proteins and melatonin on biochemical parameters
of F-exposed rats    33
Bharti, Srivastava

**Table 2.** Effect on certain plasma biochemical parameters in female rats exposed to 28-day
treatments (n=6; Values are means±S.E)

| Group | Parameter | | | | | |
|---|---|---|---|---|---|---|
| | Glucose (mg/dL) | Na$^+$ (mEq/L) | K$^+$ (mEq/L) | ALP (IU/L) | SGPT (IU/L) | SGOT (IU/L) |
| Control | 99.39[a] ±4.07 | 120.67[a] ±2.22 | 33.83[b,c] ±2.61 | 25.39[a] ±0.53 | 15.30[a] ±0.48 | 14.34[a,b] ±0.87 |
| BPP | 95.14[a] ±3.26 | 128.83[b] ±3.09 | 36.80[c] ±4.71 | 27.81[a] ±1.61 | 16.76[a] ±0.64 | 12.04[a] ±1.72 |
| MEL | 97.29[a] ±3.62 | 134.50[c] ±1.82 | 33.50[bc] ±1.12 | 37.11[c] ±2.72 | 16.10[a] ±1.32 | 13.21[a] ±1.13 |
| F | 106.36[b] ±5.97 | 147.00[d] ±3.48 | 51.17[d] ±3.40 | 47.95[d] ±1.03 | 24.45[c] ±1.13 | 20.90[c] ±1.19 |
| F+BPP | 128.22[b] ±4.19 | 134.17[c] ±0.94 | 31.83[a,b] ±2.10 | 34.10[c] ±1.62 | 19.21[b] ±0.94 | 13.32[a] ±0.76 |
| F+MEL | 133.28[b] ±1.84 | 131.67[b] ±0.88 | 28.50[a] ±0.88 | 31.31[b] ±0.96 | 20.06[b] ±0.84 | 16.22[b] ±1.00 |

[a,b,c,d]Values in the same column bearing no common superscripts differ significantly (p<0.05).

**Table 3.** Effect on additional plasma biochemical parameters in female rats exposed to the
28-day treatments. (n=6; Values are means±S.E)

| Group | Parameter | | | | |
|---|---|---|---|---|---|
| | Total protein (g/dL) | Creatinine (mg/dL) | Urea (mg/dL) | BUN (mg/dL) | Cholesterol (mg/dL) |
| Control | 8.01[a] ±0.73 | 1.16[a] ±0.08 | 15.90[a] ±1.88 | 8.00[a] ±0.78 | 26.7[a] ±1.73 |
| BPP | 8.70[a] ±0.57 | 1.21[a,b] ±0.05 | 13.96[a] ±0.67 | 7.03[a] ±0.19 | 48.66[c] ±3.07 |
| MEL | 8.37[a] ±0.90 | 1.24[a,b] ±0.05 | 15.74[a] ±1.41 | 7.38[a] ±0.63 | 46.31[c] ±2.01 |
| F | 8.39[a] ±0.62 | 2.07[d] ±0.05 | 19.90[b] ±0.99 | 9.58[b] ±0.47 | 58.58[d] ±3.45 |
| F+BPP | 9.12[b] ±0.37 | 1.39[c] ±0.05 | 14.90[a] ±1.29 | 6.90[a] ±0.60 | 37.93[b] ±3.39 |
| F+MEL | 7.37[a] ±0.47 | 1.28[b] ±0.03 | 13.51[a] ±0.86 | 7.08[a] ±0.47 | 38.79[b] ±4.76 |

[a,b,c,d]Values in the same column bearing no common superscripts differ significantly (p<0.05).

34    Research report
      Fluoride 44(1)30–36
      January-March 2011

Effect of pineal proteins and melatonin on biochemical parameters    34
of F-exposed rats
Bharti, Srivastava

As seen in Table 3, higher and lower plasma protein levels were found in the F+BPP and F+MEL-treated rats, respectively, compared to the other groups. Table 3, also shows that F treatment caused significant elevation in the levels of creatinine, urea, BUN, cholesterol, which were significantly reduced in the F+BPP and F+MEL groups. Likewise, as seen in Table 2, F greatly increased ALP, SGOT, and SGPT activities, which were significantly decreased in the F+BPP and F+MEL groups but not all to the levels of the Control, BPP, and MEL groups. Administration of BPP and MEL in F-treated rats caused significant (p<0.05) reduction in adverse changes of $Na^+$, $K^+$, creatinine, urea, BUN, cholesterol, SGPT, SGOT, and ALP level (Tables 2 and 3).

## DISCUSSION

The significant increase in the level of serum glucose in the F, F+BPP, and F+MEL groups suggests that administration of melatonin or pineal proteins (PP) in F intoxication may prove beneficial. Elevation of blood glucose in animals to combat oxidative stress helps meet increased energy demands.[17] The literature concerning the effect of MEL and PP on glucose metabolism is scarce. However, Bojkova et al.[18] have reported a significant increase in plasma glucose by long-term administration of MEL to 48-hr fasting rats of both sexes. Grucka-Mamczar et al.[19] found a 12% increase in serum glucose in 4.5 month-old male Sprague-Dawley rats after receiving 4.9 mg F/kg bw/day for 50 days.

As found here, F, BPP, and MEL alone did not induce any significant changes in plasma protein levels. Thus it appears that administration of relatively high levels of F for short periods has little effect on plasma proteins in female rats. These findings are supported by Ogeturk et al.,[20] who reported insignificant change in plasma proteins level in rats treated with MEL. However, PP treatment brought an increase in plasma protein in goats and guinea pigs.[10,21] This stimulatory effect of PP on total plasma proteins might involve production of higher globulin levels. Part of these discrepancies between different studies may be attributed to the species variation.

Alkaline phosphatase (ALP) is a key marker enzyme of F toxicosis and bone pathology.[22] Therefore, increased activity of plasma ALP in our study may be due to the effect of F intoxication on bone and other connective tissues. Insult of F to bone may increase osteoclastic activity which initiates repair response, and therefore elevated ALP activity is observed in NaF-treated rats.[22-25] These results are consistent with previous studies in rats,[22] humans,[26] and buffalo calves.[27]

An increased ALP activity on MEL administration was contradictory to the effect produced by BPP alone, as the later did not alter ALP activity with respect to control animals. It remains elusive at present as to how MEL triggers ALP activity in rats. However, BPP and MEL with F did not cause increased activity of plasma ALP. These findings might be explained by removal of F from the cell, so no adverse effect was observed on F+MEL or F+BPP administration in rats.[7,14,15]

As is well known, F has diverse actions on variety of cellular and physiological functions including inhibition of a variety of enzymes and the production of

35  Research report                 Effect of pineal proteins and melatonin on biochemical parameters   35
    Fluoride 44(1)30–36                                                    of F-exposed rats
    January-March 2011                                                     Bharti, Srivastava

hypocalcaemia and hyperkalemia.[24,28] We did not estimate calcium; however, in the present study hyperkalemia and hypernatremia were recorded in the F-treated rats. Therefore, our findings support the earlier observations of Varner et al.[28] and Verma and Guna Sherlin[29] that high F causes hyperkalemia and hypernatremia, and causes imbalance in blood electrolyte homeostasis. Our study also showed that MEL and BPP countered the effect of high F on plasma $Na^+$ and $K^+$ in F-treated animals. These findings indicate that MEL and PP are capable of improving the serum $Na^+$ level to some extant but not at an abnormally high level, and therefore may prove beneficial in fluid-electrolyte imbalance conditions.

The liver and renal system, appears to be at higher risk of F toxicity than most other soft tissue organs.[1] Increased serum levels of urea, BUN, creatinine, and cholesterol in the present study may be due to renal and liver dysfunction in rats exposed to high F.[23,27,30,31] Liver dysfunction in the present study was further confirmed by increased SGPT and SGOT activity in F-treated rats. The increase in cholesterol level may be accompanied by a decrease in cholesterol catabolism and/or inhibited lipoprotein activity.[31] These adverse changes were significantly counteracted by BPP and MEL administration in F-treated rats. Because of their free radical scavenging ability, PP and MEL can have an important role in ameliorating F toxicity.[4,7,11,14,15]

It is noteworthy that BPP and MEL, when administered separately, resulted in an increase in the plasma cholesterol level as compared to the control group. These changes might be due to the stimulation of lipolysis by PP and MEL. Some studies have reported a similar lipidemic-stimulating activity of MEL.[32] However, the mechanism underlying this hypercholesterolemic effect of PP and MEL will need further investigation.

Nevertheless, the present study showed that buffalo pineal proteins (BPP) and melatonin do not induce any appreciable toxic effects on the plasma biochemical parameters we investigated. They should therefore be safe for therapeutic trials as agents to counter or alleviate F intoxication. However, these results require further investigation.

## CONCLUSIONS

Our findings clearly indicate the important neuroendocrine role of the pineal gland in protection of fluoride-induced adverse biochemical changes in rats. This study has also shown the ameliorative effects of pineal proteins and melatonin against high fluoride intake. It further indicates that PP and MEL can be tested for their beneficial effects, since they have no adverse effects on normal plasma biochemical profiles.

## ACKNOWLEDGEMENTS

36  Research report
    Fluoride 44(1)30–36
    January-March 2011

    Effect of pineal proteins and melatonin on biochemical parameters
    of F-exposed rats
    Bharti, Srivastava    36

# REFERENCES

1   Committee on Fluoride in Drinking Water, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council. Fluoride in drinking water: a scientific review of EPA's standards. Washington, DC: National Research Council, The National Academic Press; 2006. p.1-12.
2   Rzeuski R, Chlubek D, Machoy Z. Interactions between fluoride and biological free radical reactions. Fluoride 1998;31:43-5.
3   Guo XY, Sun GF, Sun YC. Oxidative stress from fluoride-induced hepatotoxicity in rats. Fluoride 2003;36:25-9.
4   Chawla SL, Yadav R, Shah D, Rao MV. Protective action of melatonin against fluoride-induced hepatotoxicity in adult female mice. Fluoride 2008;41:44-51.
5   Vani ML, Reddy KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Fluoride 2000;33:17-6.
6   Bharti VK, Srivastava RS. Fluoride-induced oxidative stress in rat's brain and its amelioration by buffalo (*Bubalus bubalis*) pineal proteins and melatonin. Biol Trace Elem Res 2009;130:131-40. (see abstract on page 45 in this issue of *Fluoride*).
7   Blask DE, Vaughan MK, Reiter RJ. In: The pineal gland. Relkin R, editor. Amsterdam: Elsevier; 1983. p.201-24.
8   Romijn HJ. The pineal: a tranquillizing organ? Life Sci 1978;23:2257-74.
9   Sejian V. Studies on pineal-adrenal relationship in goats (*Capra hircus*) under thermal stress [PhD thesis]. Izatnagar, India: Indian Veterinary Research Institute; 2006.
10  Bharti VK, Srivastava RS. Pineal proteins upregulate specific antioxidant defense systems in the brain. Oxid Med Cell Longev 2009;2:88-92.
11  Reiter RJ, Melchiorri D, Sewerynek E, Poeggler B. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 1995;23:43-50.
12  Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocrin Rev 1991;12:151-80.
13  Sejian V, Srivastava RS. Effects of melatonin on adrenal cortical functions of Indian goats under thermal stress. Vet Med Int 2010; 2010:348919. Epub 2009 Dec 6. DOI:10.4061/2010/348919. PMID: 20445781.
14  Bharti VK, Srivastava RS. Protective role of buffalo pineal proteins on arsenic-induced oxidative stress in blood and kidney of rats. Health 2009;1:167-2.
15  Bharti VK, Srivastava RS. Effect of pineal proteins at different dose level on fluoride-induced changes in plasma biochemicals and blood antioxidants enzymes in rats. Biol Trace Elem Res 2010. DOI: 10.1007/s12011-010-8733-y. (see abstract on page 48 in this issue of *Fluoride*).
16  Oser BL. Hawk's physiological chemistry. 14 ed. New York: McGraw-Hill Book Co.;1965.
17  Menon V, Ram M, Dorn J, Armstrong D, Muti P, Freudenheim JL, et al. Oxidative stress and glucose levels in a population-based sample. Diabetic Medi 2004;21:1346-52.
18  Bojková B, Marková M, Ahlersová E, Ahlers I, Adámeková E, Kubatka P, et al. Metabolic effects of prolonged melatonin administration and short-term fasting in laboratory rats. Acta Veterinary Brno 2006;75:21-32.
19  Grucka-Mamczar E, Birkner E, Zalejska-Fiolka J, Machoy Z, Kasperczyk S, Błaszczyka I. Influence of extended exposure to sodium fluoride and caffeine on the activity of carbohydrate metabolism enzymes in rat blood serum and liver. Fluoride 2007;40(1):62-6.
20  Ogeturk M, Kus I, Kavakli A, Zararsiz I, Ilhan N, Sarsilmaz M. Effects of melatonin on carbon tetrachloride-induced changes in rat serum. J Physiol Biochem 2004;60:205-10.
21  Ramasamy M. Studies on bubaline pineal proteins/peptides below 20 kDa and their immunopotentiation in guinea pigs [PhD thesis]. Izatnagar, India: Indian Veterinary Research Institute; 2006.
22  Shanthakumari D, Srinivasalu S, Subramanian S. Effects of fluoride intoxication on lipid peroxidation and antioxidant status in experimental rats. Toxicology 2004;204:219-28.
23  Bharti VK. Fluorosis: a serious health problem of domestic animals. Livestock Int 2006;10:17-20.
24  Bharti VK, Gupta M, Lall D, Kapoor V. Ameliorative effects of boron on serum profile in buffalo (*Bubalus bubalis*) fed high fluoride ration. Trop Anim Health Prod 2008;40:111-6.
25  Farley SM, Wergedal JE, Smith LC, Lundy MW, Farley J, Baylink DJ. Fluoride therapy for osteoporosis: characterization of the skeletal response by serial measurements of serum alkaline phosphatase activity. Metabolism 1987;36:211-8.
26  Teotia SPS, Teotia M. Endemic fluoride: bone and teeth update. Indian J Environ Toxicol 1991;1:1-16.
27  Bharti VK, Gupta M, Lall D, Kapoor V. Effects of boron on haemogram and biochemical profile of urine in buffalo calves fed a high fluoride ration. Fluoride 2007;40(4):238-43.
28  Varner JA, Jensen KF, Horvath W, Isaacson RL. Chronic administration of aluminum-fluoride or sodium-fluoride to rats in drinking water: alterations in neuronal and cerebrovascular integrity. Brain Res 1998;784:284-98.
29  Verma RJ, Guna Sherlin D M. Sodium fluoride-induced hypoproteinemia and hypoglycemia in parental and F (1)-generation rats and amelioration by vitamins. Food Chem Toxicol 2002;40:1781-8.
30  Birkner E, Grucka-Mamczar E, Zwirska-Korczala K, Zalejska-Fiolka J, Stawiarska-Pieta B, Kasperczyk S, et al. Influence of sodium fluoride and caffeine on the kidney function and free-radical processes in that organ in adult rats. Biol Trace Elem Res 2006;109:35-47.
31  Gupta M, Mazumder UK, Das S. Effect of alkaloidal extract from *Clerodendron colebrookianum* on hematological parameters and hepatorenal functions on mice. Indian J Physiol Pharmacol 1994;27:83-91.
32  Lena PJ, Subramanian P. Effects of melatonin on the levels of antioxidants and lipid peroxidation products in rats treated with ammonium acetate. Pharmazie 2004;59:636-9.