



Melatonin Protection on Fluoride Induced Neurotoxicity in the Male Rat

KEYWORDS

Brain, Melatonin, Oxidative stress, Rats

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ABSTRACT This study was conducted to investigate effects of sodium fluoride (NaF) on brain (cerebral hemisphere) of rat and its possible amelioration by melatonin. Adult male Wistar rats (*Rattus norvegicus*) were administered sodium fluoride (NaF) at the doses of 5 and 10 mg/kg body weight and melatonin 10 mg/kg body weight for 60 days. Gravitometric and biochemical indices were done of brain and also studied. The increased lipid peroxidation (LPO) indicated an oxidative stress and the elevated fluoride levels in brain tissue could be the result of the inhibition of antioxidant enzymes (SOD, CAT) in the brain region of rats fed with fluoride. These changes were correlated with accumulation of fluoride in the region. However, these effects were ameliorated by co-supplementation of melatonin along with NaF in rats, because of its antioxidant cascade phenomenon.

INTRODUCTION

The presence of fluorine in ground water is mainly a natural phenomenon and influenced by local and regional conditions.¹ Intake of high levels of fluoride is known to cause structural deformities, altered activities of enzymes, and metabolic lesions in the brain of experimental animals. Fluorosis exhibits neurological problems such as a tingling sensation in the fingers and toes, nervousness and depression. In the advanced stages of fluorosis, neurological manifestations such as paralysis of the limbs, vertigo, spasticity in the extremities, and impaired mental acuity, are observed in human beings.² Sharma et al.³ reported that inhabitants of certain villages in Sanganeer Tehsil, Jaipur, India were found to be suffering from various neurological disorders due to high levels of F⁻ in the ground water; the main neurological manifestations observed were headache, insomnia, lethargy, depression, polyuria and polydipsia. Effects of NaF on the expression of intracellular Ca²⁺ fluxes, apoptosis and the antagonism of taurine in murine neuron were reported.^{4,5} Melatonin is a methoxyindole synthesized within the pineal gland. The hormone has strong antioxidant action neurodegenerative disorders.⁶ Therefore, to test this hypothesis, the present study was designed to investigate the role of melatonin administration in fluoride induced neurotoxicity in rats.

MATERIALS AND METHODS

Animals: Mature male Wistar rats (*Rattus norvegicus*) weighing between 200 and 300gm were procured from Zydus-Cadila Health Care, Ahmedabad under the Animal Maintenance and Registration No. 167/PO/c/99/CPCSEA from the Ministry of Social Justice and Empowerment, Government of India. The animals were housed under standard temperature (24±1°C) at a 12-hr dark/light cycle. They were fed standard rodent food (Pranav Agro Industries, Vadodara, India) and water *ad libitum*.

Experimental protocol: After 15 days adaptation period, the animals were divided into five different groups (Table 1) of 15 each and caged separately. Based on our earlier studies,⁷ the following doses were given to rats for 60 days.

After 60 days, the rats were fasted overnight and sacrificed under mild ether anesthesia. The weight of cerebral hemi-

sphere was recorded, and the tissue was utilized for the estimation of different biochemical parameters.

Table 1. Experimental Protocol

Group	Treatment and daily dose (15 rats in each group)	Duration (days)	Day of autopsy
I	Untreated (control)	-	Sacrificed with treated
II	Melatonin alone (10 mg/kg b.w, i.p)	60	61 st
III	Sodium fluoride (Low dose: 5 mg/kg b.w, orally)	60	61 st
IV	Sodium fluoride (High dose: 10 mg/kg b.w, orally)	60	61 st
V	NaF treated (High dose: 10 mg/kg b.w) + Melatonin (10 mg/kg b.w, i.p.)	60	61 st

Biochemical Analysis: Brain (cerebral hemisphere) tissue, lipid peroxidation (by increased malondialdehyde [MDA] concentration), superoxide dismutase (SOD, E.C.1.1.15.11), and catalase (CAT, E.C.1.11.1.6), were determined according to standard methods.⁸⁻¹⁰

Statistical Analysis: Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's significant difference post hoc test was used to compare differences among groups. Data were analyzed statistically by Graph Pad Prism 5.0 statistical software. P values <0.05 were considered significant.

RESULTS

Body and organ weights: The weights of body ($p < 0.05$; $p < 0.001$) and brain of the rats treated with NaF (Group III, IV) were decreased significantly ($p < 0.05$; $p < 0.01$) as compared to the control animals (Group I). But melatonin alone (Group II) and combined groups (V) did not show any significant changes compared to the (Group I) controls (Figure 1, 2).

Antioxidant indices: All antioxidant enzyme activities, SOD, CAT declined in the NaF-treated groups (III, IV) in brain region (CH). Contrarily, NaF treatment also produced markedly ($p < 0.01$; $p < 0.001$) elevated levels of lipid peroxidation in brain (CH) tissue as compared to the control groups. Administration of melatonin along with NaF-treated (Group V) rats showed no much significant differences in anti-oxidant indices as compared to control animals. Melatonin alone treated group has revealed no changes (Figures 3 to 5).

Fluoride levels in the brain region:

Fluoride levels in the CH of sodium fluoride (LD and HD) treated rats, registered a highly significant ($p < 0.001$) increase as compared to control groups. However, the fluoride levels were restored to almost normal levels on administration of melatonin to the NaF ingested groups for 60 days, whereas, melatonin alone group did not show any change in F- Levels (Figure 6).

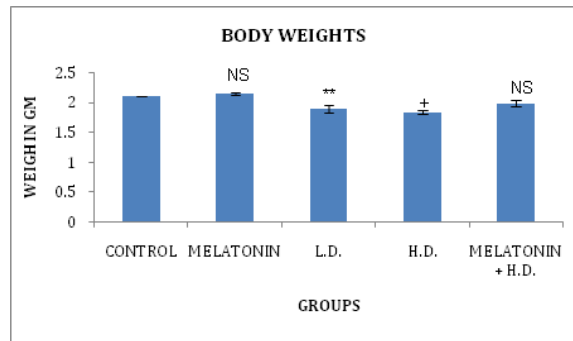


Figure 1. Body weights of control, melatonin, and NaF treated (L.D. and H.D.) groups.

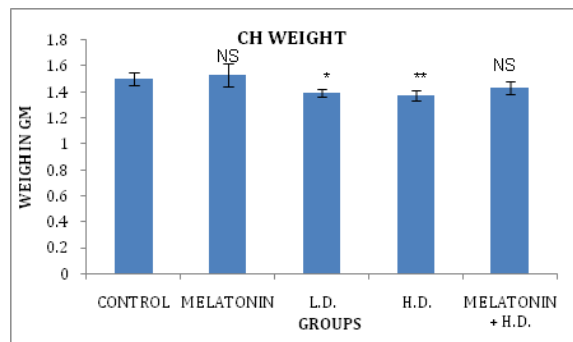


Figure 2. Organ weights of control, melatonin, and NaF treated (L.D. and H.D.) groups

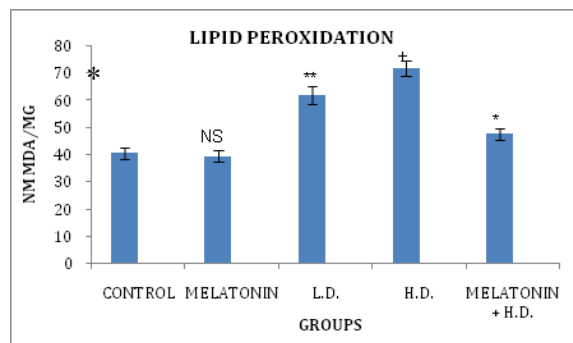


Figure 3. Malondialdehyde levels in CH of control, melatonin, and NaF treated (L.D. and H.D.) groups.

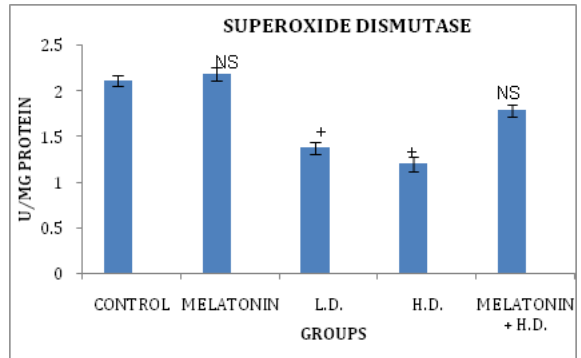


Figure 4. Activity of SOD in CH of control, melatonin, and NaF treated (L.D. and H.D.) groups.

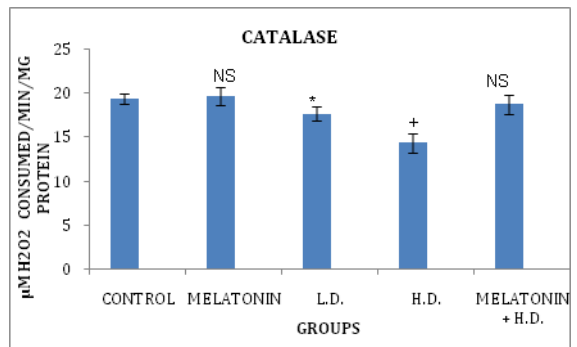


Figure 5. Activity of catalase in CH of control, melatonin, and NaF treated (L.D. and H.D.) groups.

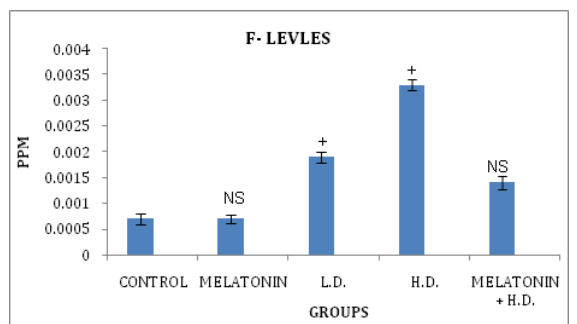


Figure 6. Fluoride levels in CH of control, melatonin, and NaF treated (L.D. and H.D.) groups.

For all above figures, values are Mean ± S.E. * $P < 0.05$, + $P < 0.001$ NS = Non Significant.

DISCUSSION

The current study was commenced to investigate the toxic effects of NaF *in vivo* in brain (cerebral hemisphere) of adult male Wistar rats (*Rattus norvegicus*). In this study, NaF treatment brought about a reduction in body and CH weights of rats, which could be ascribed to very low food consumption, altered protein energy metabolism and electrolyte imbalance.¹¹ Another recent study from our laboratory on reproductive and other organs also supports present findings of gravimetric data.^{12,13} In accordance with our records, the body and brain weights and somatic index of brain of animal decreased significantly in NaF treated group.¹⁴

In the present study, an increased LPO levels and decreased SOD and CAT activities in brain region by NaF treatment are in agreement with others.^{5,15} This might be a result of either over production or accumulation of ROS, resulting from the loss of antioxidants and indicating damage to membrane un-

saturated fatty acids of brain cells. Fluoride induced free radical toxicity has been reported recently in the brain of male rat.^{16,17} Bharti and Srivastava¹⁸ found that fluoride become toxic at high dose and increased oxidative stress in the brain affecting behavioral changes in rats. Our results further supported that region of brain might have differential effects to F⁻ exposure.

Fluoride levels were estimated in CH region of the rat brain. Fluoride accumulation in brain (cerebral hemisphere) also supported its neurotoxicity. Fluoride is known to pass blood-brain barrier for its accumulation in brain, which supported our data. Chawla and Rao¹⁹ reported high levels of fluoride in the brain of mouse in support of the investigation. In this study, the elevated fluoride levels in the CH region of brain in NaF treated rats revealed an increased neurotoxicity and altered internal milieu of brain affecting its functions.

However, supplementation of melatonin to fluoride ingested rats mitigated these effects due to its antioxidant proper-

ties. The mechanism of the ameliorative role of melatonin in mitigating fluoride toxicity in mice, rats, and rabbits has been presented earlier.¹⁹⁻²¹ Melatonin is known to inhibit the production of reactive oxygen species (ROS) like superoxide free radicals, H₂O₂ and nitrite radical generation by activated macrophages, which play an important role in inflammation. Electron donation by melatonin is not only an aspect of direct radical scavenging, additionally represents the basis for the formation of protective metabolites like N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK). Studies on physiological levels of this indole have been proved to be beneficial against oxidative stress in support of our data.²²

Thus, we concluded that melatonin could attribute to its protective action against the fluoride induced neurotoxicity, as observed in the present study. Supplementation of melatonin might thus be beneficial for the mitigation of fluorotic effects in people exposed to F⁻ in endemic areas.

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