SUMMARY: Given peritoneally over a 30-day period, melatonin (10 mg/kg bw/day) effectively countered toxic effects of orally administered sodium fluoride (NaF, 10 mg/kg bw/day) in the liver of adult female albino mice. Compared to NaF alone, the combined treatment prevented the NaF-induced decrease in body and liver weight as well as the decreased liver enzyme activity of succinate dehydrogenase (SDH), acid phosphatase (ACP), alkaline phosphatase (ALP), and the level of total liver protein. The NaF-induced increase in the activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in the liver was also significantly diminished by melatonin. When the mice were given melatonin without exposure to NaF, no significant changes occurred in the above indices compared to controls. Thus melatonin was found to exert a significant protective action against fluoride-induced hepatotoxicity in mice.

Keywords: Fluoride hepatotoxicity; Liver enzymes; Melatonin and fluoride; Mice and melatonin; Melatonin protective effects.

INTRODUCTION

Fluorosis is an endemic public health problem in 23 nations around the world including India, where it is endemic in 17 out of 32 States and Union territories, with Gujarat being the most heavily impacted state. In recent years, soft tissue involvement in fluorosis has attracted increasing attention with convincing evidence from fluorosis patients and animal studies demonstrating damage/involvement of soft/tissue/organ systems. Previous work at our institution and elsewhere has revealed that fluoride affects the structure and function of liver of different animal models.

Melatonin (N-Acetyl-5-methoxytryptamine) is a secretory product of the pineal gland as well as other select organs. Among its many beneficial effects, melatonin has been shown to be highly effective in reducing oxidative stress at many levels. Mechanisms of the protective action of melatonin against oxidative stress involve direct free radical scavenging activity and indirect antioxidant reactions. Under conditions of high oxidative stress in vivo, melatonin has proved superior to vitamins C and E in reducing oxidative damage.

With these reports in mind, we have investigated the possible protective effect of melatonin on fluoride-induced hepatotoxicity in mice.

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, 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±2°C with exposure to 10–12 hr of daylight at a relative humidity of 30–70%. They were fed a standard mouse chow (National Institute of Occupational Health (NIOH), Ahmedabad) and were given water (0.6–1.0 ppm F) ad libitum.

**Exposure:** The mice were divided into four groups of 10 each with a 30-day treatment period for each group. Group I served as control; Group II mice were injected intraperitoneally with melatonin (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. Group IV mice were pretreated intraperitoneally with melatonin (10 mg/kg bw/day), and after 30 min NaF (10 mg/kg bw/day) was administered orally.

At the end of the 30-day treatments, the mice were weighed on an animal weighing balance (Ohaus, USA) and sacrificed by cervical dislocation. The liver was dissected out carefully, blotted free of blood, weighed to the nearest milligram, and used for the estimation of protein, succinate dehydrogenase (SDH) (E.C.1.3.99.1), acid phosphatase (ACP) (E.C.3.1.3.2.), and alkaline phosphatase (ALP) (E.C.3.1.3.1.).

For estimation of two serum transaminases, blood was collected by cardiac puncture, and the serum was separated and used. Activities of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) (E.C. 2.6.1.2.) were assayed by the method of Reitman and Frankel.

**Statistical analysis:** For all biochemical parameters, a minimum of 6–8 replicates were performed. The data were analyzed statistically using Student’s t test and Analysis of Variance (ANOVA). A significance level of $P<0.05$ was accepted.

**RESULTS**

**Body and organ weights:** As shown in Figures 1 and 2, the body and liver weights of the mice treated with NaF (Group III) decreased significantly ($p<0.001$) compared to the Group I controls and the mice administered melatonin alone (Group II) or with NaF (Group IV).

**Protein levels:** A significant decline was observed in the levels of protein following NaF exposure (Figure 3).

**SDH, ACP, and ALP:** The activities of SDH, ACP, and ALP decreased significantly ($p<0.001$) in the liver of the NaF-treated mice as compared to the control group (Figures 4-6).

**SGOT and SGPT:** The activities of SGOT and SGPT were significantly ($p<0.001$) elevated following NaF treatment as compared to the control group (Figure 7).
As with the body and liver weights, the above-mentioned parameters were essentially unchanged in the Group II mice treated with melatonin alone. Similarly, pretreatment with melatonin of the Group IV NaF-melatonin-treated mice revealed no significant changes in these indices compared to the controls in Group I.

**Figure 1.** Body weights of control and treated groups. Data are mean ± SEM of 10 animals in each group, p<0.001 when compared to control group (MLT=melatonin).

**Figure 2.** Liver weights of control and treated groups. Data are mean ± SEM of 10 animals in each group, p<0.001 when compared to control group (MLT=melatonin).
**Figure 3.** Total proteins in liver of control and treated groups. Data are mean ± SEM of 10 animals in each group, p<0.001 when compared to control group (MLT=melatonin).

**Figure 4.** Activity of SDH in liver of control and treated groups. Data are mean ± SEM of 10 animals in each group, p<0.001 when compared to control group (MLT=melatonin).
Figure 5. Activity of ACP in liver of control and treated groups. Data are mean ± SEM of 10 animals in each group, p<0.001 when compared to control group (MLT=melatonin).

Figure 6. Activity of ALP in liver of control and treated groups. Data are mean ± SEM of 10 animals in each group, p<0.001 when compared to control group (MLT=melatonin).
DISCUSSION

The significant reduction observed in the body and liver weight of the NaF-treated mice in this study is consistent with earlier results, attributed to decreased food intake and reduction in protein levels. Those results further demonstrated a significant decline in the total protein levels in the liver after NaF exposure, a reduction that could be due to impaired protein synthesis known to be caused by fluoride. In support of our findings, Nair et al. have documented reduced protein levels in hepatic tissue.

The decreased SDH activity in the liver resulting from NaF treatment also corroborates earlier findings. SDH is a mitochondrial enzyme involved in oxidative metabolism. Any change in the structure and function of mitochondria would be expected to alter its metabolism. Fluoride has been reported to cause changes in structure and function of mitochondria leading to decreased oxidative metabolism. The reduction we observed in the activity of ALP is in agreement with the findings of Bogin et al. Since this enzyme was inhibited by F, it is likely that the membrane permeability was affected. ACP activity also showed a marked decline in the liver of the NaF treated mice, in agreement with earlier data on different tissues in rats and mice. The alterations in ALP and ACP activity point to diminished hepatic function, supported by the increased activity levels of SGOT and SGPT and therefore liver damage and release of hepatocellular enzymes. Similar changes in transaminases from F intoxication have also been reported earlier by other workers.

Melatonin has been documented as a direct free radical scavenger and an indirect antioxidant. A wide range of doses of melatonin has been tested in

![Figure 7. Activities of SGPT and SGOT in liver of control and treated groups. Data are mean ± SEM of 10 animals in each group, p<0.001 when compared to control group (MLT=melatonin).](chart)
different animal species. Dosages of melatonin that have been used in vivo are 250 mg/kg body wt in mice, 10 mg/kg body wt in rats, 800 mg/kg in mice, rabbits, cats and dogs. These studies have not identified any side effects of melatonin. In a randomized double-blind clinical trial in healthy adult male subjects, oral administration of melatonin (10 mg/day for 28 days) resulted in no observable adverse side effects. In general, there appear to be no serious bad effects of melatonin.

In the present study a marked ameliorative effect of melatonin toward F-induced hepatotoxic effects of F in mice was clearly evident, since most of the biochemical indices were comparable to control levels. In this connection, melatonin and its metabolites are known to be potent antioxidants. The metabolites that are produced during the scavenging actions of melatonin, i.e., cyclic 3-hydroxymelatonin (cyclic 3-OHM), N1-acetyl-N2-formyl-5-methoxykynuramine (AMFK), and N1-acetyl-5-methoxykynuramine (AMF), also seem to be efficient scavengers. Thus the second and third generation metabolites of melatonin may also contribute to the ability of the parent molecule to protect against oxidative stress. Because of this, rather than scavenging a single radical, melatonin via an antioxidant cascade may neutralize toxic effects and metabolic damage caused by wide range of toxicants.

In conclusion, the present study has shown that melatonin ameliorates F-induced hepatotoxicity in mice as seen in its ability to maintain normal critical antioxidant enzyme levels in the liver.

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