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Alleviation of Fluoride Toxicity by Melatonin in Reproductive Function of Male Rat

KEYWORDS: Fluoride, Melatonin, Energy metabolic, Alleviation.

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ABSTRACT *The therapeutic effects of melatonin (MEL) (10 mg/kg body weight) supplementation on reproductive functions of fluoride-treated (5 and 10 mg/kg body weight) male rats were investigated. Sodium fluoride treatment resulted in a decrease in almost all parameters studied. Treatment brought about alterations in energy metabolic indices like, protein, succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) levels and other biochemical parameters such as phosphatases (ACPase; ALPase). There changes were related to reduction in testicular function. Thus, there fluoretic effects are ameliorated by supplementation of Melatonin which supports their antioxidant properties. The significance of these data will be discussed.*

INTRODUCTION

The sources of fluoride (F-) are drinking water, air, food, industrial exposure, drugs, cosmetics, dental products etc. The high fluoride in different food products were reported in tea (4.97 ppm), canned fish (4.57 ppm), shellfish (3.36 ppm) and cooked wheat cereal (1.02 ppm).[1] Fluoride (F) has strong affinity to combine chemically with others to form compounds called "fluoride'. Examples of fluorides include sodium fluoride and calcium fluoride. As a potent protoplasmic poison, it is toxic to living cells, generating reactive free radicals and causing destructive biochemical alterations including energy metabolism and 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.[5-8] Numerous biochemical and behavioral functions of organisms are controlled by the pineal gland through melatonin (MEL) secretions.[9-11] MEL have affinity to directly neutralize a number of toxic agents and stimulate antioxidative enzymes.[12] The pineal gland also has the ability to reduce F-induced oxidative stress and adverse biochemical changes via secretion of MEL in several species.[4,6,7]

Recently, we found that MEL is able to mitigate F- induced oxidative metabolism in rats. However, the toxic effects of F- on reproductive system of rats and the influence of melatonin on various aspects of testicular function after F- ingestion.

MATERIALS AND METHODS

Adult male Wistar rats (Rattus norvegicus) weighing 250-300gm 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*. The animals were divided into five different groups (see protocol in Table 1) and caged separately. Details of treatments including dosages and route of administration are presented in Table 1.

After 60 days, the rats were fasted overnight and sacrificed under mild ether anesthesia. The weight of the testis were recorded, and used for various biochemical parameters. The testis were dissected out carefully, blotted free of blood, weighed to the nearest milligram, and used for the estimation of Total Protein, succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) levels and phosphatases (ACPase; ALPase) by using the method of Lowry et al.,[13] Beatty et al.,[14] Quinn and White,[15] Bessey et al.,[16] respectively.

Table 1. Experimental Protocol

Group	Treatment and dose	Duration (days)	Day of autopsy
I	Untreated control	-	Sacrificed with treated
II	MEL alone (10 mg/kg bwt, i.v)	60	61st
II	NaF (Low dose (LD) : 5mg/kg bwt)	60	61st
IV	NaF (High dose (HD) : 10mg/kg bwt)	60	61st
V	NaF treated (High dose) + Melatonin (10 mg/kg bwt, i.v)	60	61st

Statistical Analysis: Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) with Turkey'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

All biochemical parameters had manifested alterations after fluoride treatment in all the groups of rats. In NaF treated groups (LD; HD), a significant ($p<0.01$; $p<0.001$) decrement showed in the energy metabolic parameters including total proteins, SDH, and ATPase. Whereas, acid and alkaline phosphatase (ACPase; ALPase) also showed a significant ($p<0.1$; $p<0.001$) reduction in rat testis. These variations were remarkably alleviated in all the parameters following co-supplementation of MEL. Treatment of melatonin alone revealed no changes as compared to control. (Tables 2).

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Table2. Biochemical parameters in testis of control and experimental groups

Parameters	Control	MEL	NaF LD	NaF HD	NaF +MEL
Total Proteins[A]	14.49 ±0.10	14.96 ±0.28[NS]	11.68 ±0.24**	8.26 ±0.04+	13.07 ±0.35[NS]
SDH[B]	31.09 ±0.34	31.67 ±0.39[NS]	25.83 ±0.55**	17.82 ±0.96+	28.38 ±1.39[NS]
ATPase[C]	3.46 ±0.08	3.52 ±0.09[NS]	2.67 ±0.14**	1.94 ±0.16+	3.19 ±0.11[NS]
ACPase[D]	2.29 ±0.04	2.31 ±0.04[NS]	1.91 ±0.01**	1.58 ±0.11+	2.06 ±0.04[NS]
ALPase[D]	2.14 ±0.05	2.16 ±0.04[NS]	1.61 ±0.11**	1.11 ±0.08+	2.03 ±0.10[NS]

Values are Mean ± S.E.,

NS = Non Significant, **=p<0.01, += p<0.001

(Groups II to V compared with control group I),

A= mg/100mg tissue weight, B= µg formazan formed/15min/mg protein, C= µmoles of inorganic phosphate released/30min/mg protein, D= µ moles of p-nitro phenol released/30 min/mg protein

DISCUSSION

The various biochemical parameters studied at the end of all treatments. Also the effects of sodium fluoride on energy metabolism, levels of total protein, activity of succinate dehydrogenase (SDH), adenosine triphosphatase, (ATPase) and other parameter like phosphatases (ACPase; ALPase) were studied in rat testis.

In the reproductive system, the total proteins, SDH and ATPase contents run parallel to the growth of the reproductive organs and are androgen sensitive in male individuals. The reduction in proteins, SDH and ATPase contents of fluoride treated animals support the view that fluoride inhibits oxidative metabolism. Fluoride is known to affects the protein synthesis in rats, which is mainly due to impairment of peptide chain initiation.[17]

The concentration of proteins, SDH, ATPase was declined significantly in testis following NaF treatment for 60 days in the present study. Several studies show a decline in proteins, SDH and ATPase content of various soft tissues and serum of rats, mice, rabbits and guinea pigs treated with sodium fluoride at different dose and duration.[18-32]

To support our data, the level of proteins in adrenal gland exhibited a significant decline in the NaF treated rabbits.[33] Rao *et al*.[34] observed that administration of sodium fluoride at dose of 10 mg/kg body weight for 30 days resulted in reduced total proteins levels, phosphorylase and SDH enzyme activities followed by an increase in glycogen levels in the gastrocnemius muscle. Whereas, Mathur[35] and Miao *et al*.[36] also observed, a decremental trend in SDH, ATPase intensity was quite evident treatment of sodium fluoride from control level.

Phosphatases are significantly associated with many functions at the cellular level. Acid phosphatase (ACPase) activity is associated with the activity of lysosome. It is involved in a number of activities such as phagocytosis, autolysis, cellular differentiation, keratinization, fat absorption in intestine, and dissolution of tissue components.[37] Alkaline phosphatase has ubiquitous distribution in all tissues of the body especially in cell membrane where it is associated with the transport of metabolite across the membrane. It is highly sensitive to different heavy metals and its inhibition leads to disturbances to the cellular functions.[38]

The phosphatases activity was decreased, compared to their respective control in response to NaF feeding for 60 days to rat in present study. These decreased in enzyme activities may be correlated to excessive formation of free radicals and lipid peroxidation.

From our laboratory and other researchers have obtained similar results regarding phosphatases activity. The activities of ACPase and ALPase significantly declined in the kidney of NaF treated mice as compared to control.[28,29] Chinoy and Shah[39] and Chinoy *et al*.[40] were reported that marked decline in enzyme activity of acid phosphatase in kidney (5 mg/kg body weight) and in ventral prostate (10 mg/kg body weight) of NaF treated mice for 30 days. Miao *et al*.[34] also documented that the reduction in alkaline phosphatase activity in the mid gut of silkworms.

Consequently, melatonin treatment along with NaF diminished the toxic effects of fluoride in testis, since all the above-mentioned biochemical parameters were not noticeably distorted as compared to the controls, confirming the anti-oxidative properties of melatonin.

The results confirmed that F induced testicular dysfunction is mediated by increase of oxidative stress affecting its internal environment in rats. But, the supplementation of melatonin along with NaF mitigated the NaF generated testicular dysfunction in rats. Consequently, the pineal hormone melatonin is advantageous to trounce F induced reproductive toxicity in male rats.

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