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L-ascorbic Acid Supplementation Ameliorates Sodium Fluoride Induced Alteration of Cardiac Autonomic Functions in Hypoxic Rats

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ABSTRACT

Introduction: Long term exposure to fluoride is toxic to bones and organs like heart, liver, lung and kidney. Fluoride toxicities induced by oxygen sensing cell signal pathways, hence low oxygen microenvironment and fluoride exposure might have similar toxic manifestation in physiological system and a possible protective mechanism by antioxidant like l-ascorbic acid supplementation.

Aim: To investigate the supplementation of l-ascorbic acid on hypoxia or sodium fluoride (NaF) alone or in combination in cardiovascular electrophysiology of male albino rats.

Materials &Method: Male albino rats were divided into 8 groups (n= 6/group), group I(control), group II (l-ascorbic acid,50 mg/100g. b.wt), group III (hypoxia, 10%O_2), group IV (NaF ;20 mg/kg b.wt/day ; ip), group V (NaF + hypoxia, 10% O_2), group VI (l–ascorbic acid + hypoxia, 10% O_2), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia, 10% O_2). The treatments were carried for 21 days. Gravimetry, electrophysiological parameters like noninvasive blood pressure (NIBP), mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), LF (low frequency), HF (high frequency) and LF/HF ratio were measured. Histopathological evaluations were done to identify changes in myocardial tissue (ventricle).

Results: Electrophysiological evaluation showed significant alteration in MAP in rats treated with hypoxia (group III), NaF (group IV) and NaF with hypoxia (group V). In case of l-ascorbic acid supplementation in group VI, VII and VIII showed remarkable improvement of altered MAP levels. Alteration in heart rate, LF, HF, LF/HF ratio and histopathology in myocardial tissue(ventricle) in rats treated with hypoxia, NaF and hypoxia with NaF indicate cardiac autonomic dysfunctions, but simultaneous supplementation with l-ascorbic acid were found to be beneficial against fluoride and hypoxia induced alteration of cardiovascular functions.

Conclusion: The study indicates a protective role of l-ascorbic acid on cardiovascular pathophysiology in rats treated with NaF, hypoxia and in combination of both.

Keywords: *sodium fluoride, hypoxia, heart rate, sympathovagal balance, left ventricular myocardium*

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Introduction

Long term exposure to fluoride is toxic to bones and organs like heart, liver, lung, spleen and kidney[1]. Fluoride induces oxidative stress through low oxygen cell signal transduction pathways like hypoxia. Fluorosis is irreversible but preventable by appropriate timely intervention. Antioxidants and antioxidant rich food supplementation acts as antidote in the fluorosis management and fluoride intoxication [2,3.] It is important

to know how low oxygen microenvironment influences cardiovascular autonomic malfunctions in presence of fluoride and a possible protective mechanism by antioxidant like l-ascorbic acid supplementation.

Hence the present study was designed to assess the possible protective role of l-ascorbic acid, a potent antioxidant on NaF and hypoxia or in combination on cardiovascular pathophysiology in male albino rats.

Materials and Method

Healthy adult male albino Wistar rats weighing about 150 -180 grams were procured from animal house. Acclimatized for one week to the laboratory conditions at 21-25^{o}C and fed with laboratory stock diet and water *ad libitum.*

Experimental Groups: Male albino rats were divided into 8 groups (n= 6 in each group), group I (control), group II (l-ascorbic acid,50 mg/100g. b.wt), group III (hypoxia, 10%O_2), group IV (NaF ; 20 mg/kg b. wt/ day ; ip), group V (NaF + Hypoxia, 10% O_2), group VI (l-ascorbic acid + hypoxia, 10% O_2), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia, 10% O_2). The interventions were carried for 21 days.

NaF was dissolved in distilled water and injected intra peritoneally at a dose of 20 mg/kg body weight/day for 21 days[4]. L-ascorbic acid was administered orally by using force feeding needle with syringe for 21 days (50mg/100g body weight).

Exposure of Rats to Hypoxia: Rats in cage were kept in acrylic chamber and given mixture of 10% oxygen and 90% nitrogen to induce chronic normobaric hypoxia for 21 days.Soda lime granules were used to absorb carbon dioxide. Temperature was maintained at 22-27^{o}C [5].

Gravimetry: Animals of all groups were weighed on the starting day of protocol and on the 21st day i.e. on the day of sacrifice using digital weighing balance. Percentage of body weight gain was determined. Heart was weighed after sacrifices at the end of experiment and further cardio somatic index was determined by using the following formula:

$$\text{Cardio somatic index} = \frac{\text{Weight of heart} \times 100}{\text{Body weight}}$$

Electrophysiology: Animals were anaesthetized with an intra peritoneal injection of Ketamine (60 mg/kg b.wt) with Xylazine (6 mg/kg b.wt) after the intervention period of 21 days. Heart rate variability (HRV) analysis were done by using Kubois software version 3.0.2. Heart rate (bpm), MAP Respiratory rate, LF (low frequency), HF (high frequency) and LF/HF ratio for finding of sympathovagal balance were recorded by using Biopac MP45 instrument attached to PC with BSL 4.1 software.

Histopathology Procedure: Animals of all groups were sacrificed by cervical dislocation after electrophysiological analysis at the end of 21 days. The heart was carefully collected, isolated immediately and fixed in freshly prepared 10 % formalin for 24 hours. All the fixed tissues were embedded in paraffin and thin sections were taken. Staining was done with hematoxylin and eosin. Histopathological evaluations were done to identify changes in myocardial tissue (ventricle) for treatment groups and compared with control[5]. CPCSEA guidelines were carefully followed during experiments.

Statistical Analysis: SPSS software version 16.0 was used. One-way ANOVA followed by "Tukey" test were done to find out intergroup significant differences. All values were represented as mean ± SD. $p \leq 0.05$ considered as statistically significant.

Results

Table-1 shows significant decrease in % of body weight gain, heart weight and cardio somatic index of rats exposed to hypoxia (group III), NaF (group IV), hypoxia and NaF (group V) as compared to control (group I) at the end of 21st day. However simultaneous treatment with l-ascorbic acid showed greater % of body weight gain, heart weight and cardio somatic index in group VI (l-ascorbic acid + hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia).

Table 1: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes in gravimetry. Group I(control), group II(l-ascorbic acid), group III(hypoxia), group IV(NaF), group V (hypoxia + NaF), group VI (l-ascorbic acid +hypoxia), group VII(l- ascorbic acid +NaF) and group VIII (l-ascorbic acid +hypoxia + NaF), values expressed as Mean ± SD, $p \leq 0.05$ is significant, values with different superscripts a, b, c, d, e are significantly different from each other

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	P Value
Initial body weight (gm) (1st day)	158.9 ± 5.31[a]	155.33 ± 6.6[a]	155.53 ± 1.75[a]	155.33 ± 6.11[a]	155.93 ± 2.6[a]	154.47 ± 4.01[a]	156.53 ± 4.6[a]	155.67 ± 5.4[a]	0.9693
Final body weight (gm) (21st day)	208.33 ± 3.1[a]	214.33 ± 3.7[a]	180.67 ± 7.5[b]	169.67 ± 8.6[c]	163.03 ± 3.2[c]	195.33 ± 5.5[d]	193 ± 2.6[d]	186.67 ± 2.8[e]	<0.0001*
Percent of body weight gain	31.17 ± 3.2[a]	38.11 ± 4.9[a]	16.14 ± 3.6[b]	9.19 ± 1.3[c]	4.55 ± 1.6[d]	25.93 ± 1.4[e]	23.33 ± 2.1[e]	20.62 ± 2.4[e]	<0.0001*
Heart weight (gm)	0.78 ± 0.03[a]	0.83 ± 0.03[a]	0.52 ± 0.02[b]	0.49 ± 0.03[b]	0.38 ± 0.02[d]	0.65 ± 0.01[c]	0.61 ± 0.01[c]	0.61 ± 0.01[c]	<0.0001*
Cardio-somatic index	0.37 ± 0.02[a]	0.39 ± 0.02[a]	0.29 ± 0.02[b]	0.29 ± 0.01[b]	0.23 ± 0.02[d]	0.33 ± 0.01[c]	0.32 ± 0.01[c]	0.33 ± 0.01[c]	<0.0001*

Fig-1 shows significant increase of MAP in group III (hypoxia) rats whereas in case of group IV (NaF) and group V (NaF + hypoxia) rats showed decrease of MAP as compared to their respective controls. However simultaneous supplementation with l-ascorbic acid in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats showed significant improvements of MAP.

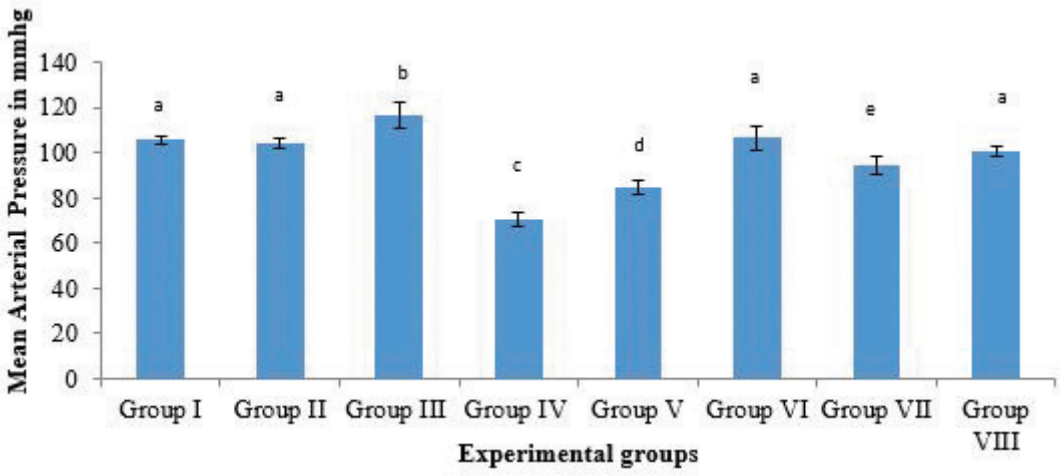

Fig. 1: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes on mean arterial pressure in all groups. Group I(control), group II(l-ascorbic acid), group III(hypoxia), group IV(NaF), group V (hypoxia + NaF), group VI (l-ascorbic acid +hypoxia), group VII(l- ascorbic acid + NaF) and group VIII (l-ascorbic acid +hypoxia + NaF), values are expressed as Mean ± SD, $p \leq 0.05$ is significant, values with different superscripts a, b, c, d, e, f are significantly different from each other.

Table-2 shows significant increase of heart rate in group III (hypoxia) rats whereas in case of group IV (NaF) and group V (NaF + hypoxia) rats showed decrease of heart rate as compared to their respective controls. However simultaneous supplementation with l- ascorbic acid in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats showed significant improvements in all the parameters. HF power band of HRV analysis showed a significant increase in group IV (NaF) and group V (NaF + hypoxia) rats

as compared to group I (control). In case of LF power band, group IV (NaF) and group V (NaF + hypoxia) showed significant decrease as compared to group I (control). In case of group III (hypoxia) a decrease in HF and increase in LF power band were noticed. However simultaneous treatment with l-ascorbic acid supplementation in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group-VIII (l-ascorbic acid + NaF + hypoxia) showed significant improvements in LF and HF power band.

Table 2: Effect of L-ascorbic acid supplementation on sodium fluoride and hypoxia induced changes on heart rate, LF (sympathetic activity) and HF (parasympathetic activity) in all groups. Group I(control), group II(l-ascorbic acid), group III(hypoxia), group IV(NaF), group V (hypoxia + NaF), group VI (l-ascorbic acid +hypoxia), group VII(l- ascorbic acid +NaF) and group VIII (l-ascorbic acid +hypoxia + NaF),values are expressed as Mean ± SD, p ≤ 0.05 is significant, values with different superscripts a, b, c, d,e, f are significantly different from each other. LF- low frequency; HF, high frequency ; n.u., power in band; heart rate (bpm, beats/minute)

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	P value
Respiratory rate (cycles/ min)	18.33 ± 0.58^{a}	16.67 ± 1.53^{a}	26.67 ± 2.52^{b}	13.67 ± 1.53^{c}	15.33 ± 1.53^{a}	18.33 ± 1.53^{a}	16 ± 1^{a}	15.67 ± 0.58^{a}	<0.0001*
Heart Rate (bpm)	313 ± 8^{a}	308 ± 9^{a}	360 ± 16^{b}	156 ± 10^{c}	211 ± 4^{d}	311 ± 1^{a}	256 ± 1^{e}	227 ± 5^{f}	<0.0001*
LF Power (n.u)	51 ± 0.6^{a}	49 ± 1.16^{a}	60 ± 5.01^{b}	33 ± 2.6^{c}	36 ± 1.2^{c}	55 ± 1.9^{d}	47 ± 1.4^{a}	53 ± 1.0^{a}	<0.0001*
HF power (n.u)	48 ± 0.64^{a}	49 ± 1.05^{a}	39 ± 4.83^{b}	65 ± 2.9^{c}	62 ± 1.9^{d}	44 ± 1.8^{e}	52 ± 1.4^{a}	45 ± 0.8^{e}	<0.0001*

Fig-2 shows significant increase of LF/HF ratio in group III (hypoxia) rats whereas in case of group IV (NaF) and group V (NaF + hypoxia) rats showed decrease of LF/HF ratio as compared to their respective controls. L-ascorbic acid supplemented in group VI (l-ascorbic acid + hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats showed significant improvements in LF/HF ratio.

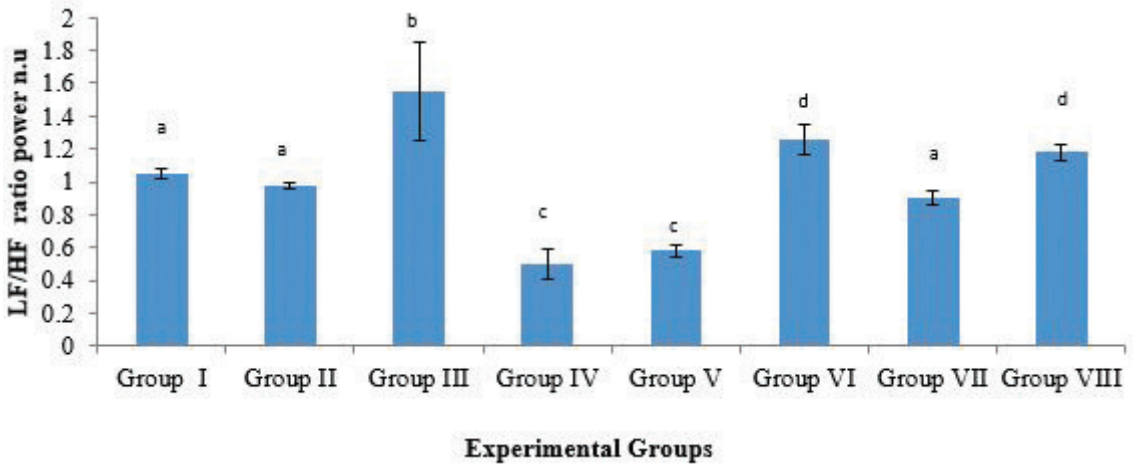

Fig. 2: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes on LF/ HF ratio in all groups, Group I (control), group II (l-ascorbic acid), group III (hypoxia), group IV (NaF), group V (hypoxia + NaF), group VI (l-ascorbic acid +Hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid +Hypoxia + NaF), values are expressed as Mean ± SD,values with different superscripts a,b,c,d and e are significantly different from each other.

Fig-3 (a-h) shows H&E stained sections of left ventricular myocardium. Group I (control) and group II (l-ascorbic acid) showed normal left ventricular myocardium (Table/Fig- 5a&b). Group III (hypoxia) showed mild hypertrophy (Table/Fig-5c), in group IV (NaF) left ventricular myocardium appeared fibrotic (Table/Fig-5d) and group V (hypoxia + NaF) showed focal degeneration in left ventricle (Table/Fig- 5e) whereas in case of l- ascorbic acid supplementation left ventricular myocardium appeared normal in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats (Table/Fig-5f-h).

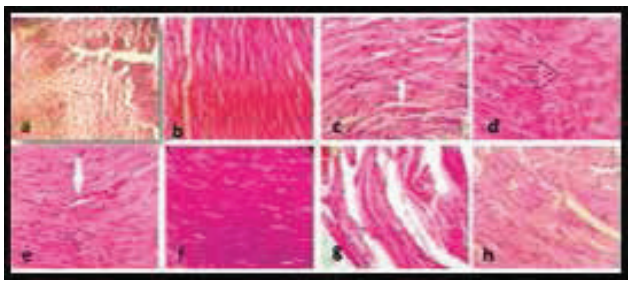

Fig. 3: Histopathology of left ventricular myocardium in all groups of rats, (a) group I-control (40x), (b) group II- supplemented with l-ascorbic acid (40x), (c) group III- hypoxia (40x), (d) group IV- NaF (40x), (e)group V-l-ascorbic acid and hypoxia(40x),(f) group VI- l-ascorbic acid and NaF (40x), (g)group VII -hypoxia and NaF (40x) and (h) group VIII - l-ascorbic acid and hypoxia with NaF (40x)

Discussion

In this study protective role of l-ascorbic acid on cardiac autonomic functions were studied in hypoxia, sodium fluoride and in combination of hypoxia and sodium fluoride in experimental rats through evaluation of HRV, blood pressure and cardiac autonomic functions along with histopathology of cardiac tissues.

Results of our study are indicative of decrease in body weight and % of body weight gain, heart weight and cardio somatic index in hypoxic and NaF administered rats or in combination of both. This decrease in body wt (%) and cardio somatic index may be due to fluoride induced decrease of food and water consumption with altered growth rate [6]. Supplementation with l-ascorbic acid showed % of body weight gain, heart weight gain and increased cardio somatic index due to cardio-protective actions of l-ascorbic acid in hypoxia and NaF induced alteration of growth rate [7].

In our study hypoxia induced alterations of sympathovagal balance in experimental rats are indicative of cardiac autonomic dysfunctions with possible sympathetic dominance [8]. Our results on supplementation of l-ascorbic acid in cardiovascular electrophysiology of hypoxic rats corroborated with the findings of Kane et al [9].

Hypoxia alters the neuronal effector pathways controlling the heartbeat. Oxidant tone and nitric oxide within the medulla alter sympathetic and vagal outflow. L-ascorbic acid increases parasympathetic outflow at the level of nucleus ambiguous.

The present study also showed parasympathetic dominance in NaF treated rats which clearly indicates altered autonomic functions. These observations on NaF treatment in present study was found to be contrary to other previous study [10]. Decrease in heart rate, MAP in case of NaF treated rats in present study may be due to vasodepressor action of NaF by combined depression of the vasomotor center of the brain and of vascular smooth muscle [11].

Improvement of all the electrophysiological parameters and ventricular histopathology in l-ascorbic acid supplemented hypoxia exposed or NaF treated or in combination of hypoxia exposure and NaF treatment indicate protective effects of l-ascorbic acid on cardiovascular pathophysiology. This could be due to potential antioxidant property of l-ascorbic acid against low oxygen microenvironment due to fluoride exposure *in vivo* in experimental rats.

Conclusion

Observations from the present study indicate amelioratic effects of l- ascorbic acid on cardiac autonomic functions in rats exposed to fluoride and hypoxia or in combination of both hypoxia and fluoride.

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Conflict of Interest: None declared.

Ethical Clearance: The entire protocol for experiment was approved by Institutional Animal Ethical Committee bearing approval no (LCP/PG. Col/IAEC/Oct-2015/66).

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