



Protective effects of melatonin against fluoride-induced oxidative stress in rats at high altitude

PRINCE VIVEK¹, VIJAY K BHARTI², SAHIL KALIA³, ARUP GIRI⁴, KRISHNA KUMAR⁵ and BHUVNESH KUMAR⁶

Defence Institute of High Altitude Research, DRDO, Leh-Ladakh, Jammu and Kashmir 194 101 India

Received: 5 December 2017; Accepted: 6 July 2018

ABSTRACT

This study was conducted to evaluate the protective effects of melatonin against fluoride-induced oxidative stress-mediated haematological and biochemical changes in rat at high altitude. Adult male Wistar rats (6) were given the basal diet and drinking water *ad lib.* for first 7-days which was considered as the control period. Thereafter, they were exposed to NaF (@ 50 ppm) per-oral through drinking water for the next 14 days followed by melatonin treatment (@ 15 mg/kg BW, p.o.) for the next 14 days. The result showed induction of oxidative stress during NaF treatment alone, which caused significant increase in alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activity, and % inhibition of DPPH, MDA, and water intake. Whereas, total antioxidant capacity (FRAP) and body weight gain were significantly reduced during NaF exposure. Haemogram analysis indicated significant decrease in haemoglobin, packed cell volume, erythrocytes, lymphocytes, platelets count, whereas increase in MCH, monocytes, neutrophil, and eosinophil during NaF exposure. However, melatonin administration after 14 days of fluoride treatment resulted in significant amelioration of adverse changes occurred in different blood-biochemical parameters and also increased the total antioxidant status. Notably, the body weight gain improved during melatonin administration. These findings indicated induction of oxidative stress-mediated adverse change in haematology and biochemical parameters, and amelioration effect of oral dose of melatonin in rats under high altitude stress condition. Hence it can be concluded that melatonin acts as a potent antioxidant agent, which may be orally supplemented for amelioration of fluoride-mediated oxidative stress even under prevalent high altitude stress.

Key words: Antioxidant, Fluoride, Haematology, High altitude, Melatonin, Oxidative stress, Rats

Leh-Ladakh is a high altitude (HA) region situated at altitude varying from 10,000 to 18,500 feet from mean sea level, and has adverse environmental factors like hypobaric hypoxia, severe cold, high wind velocity and scorching solar radiation and extreme variation in environmental temperature range from +20 to –39°C (Bharti *et al.* 2017, Kalia *et al.* 2017). These environmental factors causes high altitude stress and which inevitably results in increased oxidative stress (West 2004, Miller *et al.* 2013). Increased level of fluoride in water in high altitude regions enhances the fluoride exposure dose and prevalence of fluorosis (Arjun *et al.* 2017). Earlier study reported that the prevalence of dental fluorosis often have dealt population living in areas of increased altitude and the fluoride level of water (Hou *et al.* 2014). Fan *et al.* (2016) reported positive correlation between altitude and skeletal fluorosis

with higher incidence of skeletal fluorosis at high altitude. Sodium fluoride (NaF) can exacerbate the additional effects of oxidative stress resulting in increased level of free radicals production which disrupts the efficiency of antioxidant system and causes the oxidative damage of macromolecules (Miller *et al.* 2013). The moderate level of fluoride intake leads to fluorosis, weakened antioxidant defence systems, and increased oxidative stress in rat liver and kidney (Bharti *et al.* 2011). Past study suggested that the alterations of acid-base balance in surviving people and animals at high altitude caused by hypoxia could decrease urinary excretion of fluoride and thus increase fluoride retention in the body (Whitford 1997). In addition, edema in liver and kidneys caused by hypoxia has been seen in people living at high altitude (Haijun *et al.* 2008). Therefore, human beings and livestock population existing under this adverse climatic condition of high altitude and simultaneously exposed to polluted water and air containing higher level of metal or toxic trace elements, including fluoride might have intense adverse impact on their health and performance. Hence, among various ameliorating strategies, supplementation of antioxidant seems to be an

Present address: ^{1,3,4}PhD Scholar (prince.v68 @gmail.com, arupsatadal@gmail.com, sahilkalia87@gmail.com), ²Scientist-D (vijaykbharti@rediffmail.com), ⁵Technical Officer (drkrishna02@gmail.com). ⁶Scientist-G (bhuvnesh_5aug @rediffmail.com), Defence Institute of Physiology and Allied Sciences (DIPAS), Timarpur, Delhi.

important step to decrease the elevated levels of free radicals production (Reiter *et al.* 2002) and so it could ameliorate the high altitude-induced oxidative stress. In this regard, the discovery of the pineal hormone melatonin heralded a new field of research in animal physiology. Various *in vivo* and *in vitro* studies revealed that melatonin have a powerful antioxidant potential through its free-radical scavenging actions on antioxidant enzyme systems and non-enzymatic antioxidants (Narayana *et al.* 2002, Vijayalaxmi *et al.* 2004, Reiter *et al.* 2005, Bharti and Srivastava 2009, Bharti *et al.* 2012). Hence, it could be beneficial in reduction or prevention of oxidative damage caused by toxicants and oxidative materials (Reiter *et al.* 2002, 2005). In spite of several reports advocating the protective role of melatonin in animal model, none of the studies investigated on single group of animals acting as control, fluoride treatment, as well as melatonin treatment, which could simulate clinical case for treatment. In addition, the ameliorative effect of oral dose of melatonin on blood-biochemical parameters is inconclusive and what is available in the literature is contradictory. Furthermore, there is no study that investigated the ameliorative effect of melatonin in high altitude stress coupled with fluoride exposed animal model. Therefore, the aim of present study was to evaluate the possible protective effects of oral dose of melatonin against fluoride-induced oxidative stress in rats under high altitude stress condition.

MATERIALS AND METHODS

This study was performed for 35 days (7-day control period, 14 day treatment I, 14 days treatment II) during winter at Defence Institute of High Altitude Research (DIHAR), Leh-Ladakh, a high altitude place situated at 3500 m above from mean sea level (MSL).

All the chemicals used were of AR grade. Double distilled water was used for the preparation of all solutions. While collecting blood samples, adequate human care were taken to minimize pain and discomfort to the animals. The collection of samples was carried out in accordance with the guidelines laid down by the CPCSEA laws and regulations. The design of this study was approved by the DIHAR/IAEC Committee.

Experimental animals: Male Wistar rats (6) of approximately three months of age, weighing 225.40 ± 0.93 g were kept under polycarbonate caging system and rice husk was used as the bedding material. All the animals were keenly examined for any abnormality or overt signs of ill health. The rats were housed with room temperature and relative humidity set at $21 \pm 2^\circ\text{C}$ and $50 \pm 10\%$, respectively and lighting was controlled to give 12 h light and 12 h darkness. One week of acclimatization was allowed before commencement of experimental trial and treatment.

Experimental diet: Ingredients for the experimental basal diet of the rats were supplied from ration store of the institute DIHAR, DRDO. The diet was prepared daily as per the ingredients level given in Table 1.

Experimental design: Single group of animals were used

Table 1. Ingredients used as rat feed

Ingredient	Percentage (%)	Amount/kg (g)
Milk	5	50
Fish meal	12	120
Soybean meal	20	200
Maize ground meal	55	550
Molasses	2.50	25
Soya oil	2.50	25
Salt	0.40	4
Ca-Phosphate	1.25	12.5
Ground limestone	0.50	5
Choline	0.15	1.5
Mineral mixture	0.15	1.5
Vit. Premixture	0.25	2.5
Niacin	0.30	3

for control, fluoride treatment as well as melatonin treatment to avoid effect of animal's on blood-biochemical changes on antioxidant therapy in clinically ailing individual. This study was conducted for 35 days, first 7 days was considered as control period, next 14 days was oral fluoride treatment period @ 50 ppm, and another 14 days all the animals were treated with melatonin till the end of 35th day of study. Appropriate oral dose of melatonin @ 15 mg/kg BW was optimized from earlier experiment as described by Tian *et al.* (2003), and was administered through basal diet after dissolving in 20% ethanol and thereafter evaporated before mixing to the diets. The F-level in drinking water was calculated and thereafter the required concentration @ 50 ppm NaF of F^- was made by addition of NaF daily to drinking water for 14 days. All the animals were given basal diet and drinking water *ad lib*. Food and water intake of animals were monitored every day.

Sample collection and preparation: Whole blood was collected aseptically in EDTA coated vacutainer vials under sterile conditions in the morning hours by the puncture of retro-orbital venous plexus. Plasma was separated from the collected whole blood at $3500 \times g$ for 10 min at 4°C and stored at -80°C for all future laboratory analysis. Prior to assay, plasma was thawed and centrifuged at $15000 \times g$ for 15 min to remove any insoluble matter present and only supernatant was used for evaluation of various parameters.

Analysis of haematological parameters: All the haematological parameters like Hb, RBC, basophil, neutrophil, eosinophil, monocytes, lymphocytes, platelets, MCH, MCV and PCV were estimated using Auto-Haematology Analyzer (BC-2800 Vet, Mindray) in freshly collected blood samples as per instrument protocols.

Analysis of blood biochemical parameters: Alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were estimated using commercially available kits as per the manufacturer's protocol (Span Diagnostics Pvt. Ltd) with the help of microplate reader (Spectra Max5e, Molecular Device).

Determination of oxidative stress and antioxidant parameters: Malondialdehyde (MDA) levels in plasma samples were measured by method of Beuge *et al.* (1978),

whereas the total antioxidant activity of plasma were measured by ferric reducing antioxidant power (FRAP) assay as per the method of Benzie and Strain (1996). The free radical scavenging activity of plasma was measured by DPPH assay as per Blais (1958).

Statistical analysis: With the help of Graph Pad Prism 5, data were analysed by one-way ANOVA method and level of significance considered at $P < 0.05$. All the results are expressed as mean $\pm$ SE.

RESULTS AND DISCUSSION

In present study, same animals were used as control, fluoride exposure and exposure to melatonin to understand the pathophysiology of body under concomitant exposure to pathological agents and response to protecting agent at high altitude. Parameters like feed and water intake, average body weight gain, haematological, biochemical and antioxidant parameters were investigated.

Changes in feed intake, water intake, and body weight: The rat exposed to fluoride showed a significant decrease in daily feed intake as compared to control period, however it increased significantly ($P < 0.05$) during melatonin administration. On the other hand, the water intake of the animals gradually increased significantly ($P < 0.05$) throughout the study period of 35 days. With respect to period of control days, a significant ($P < 0.05$) decrease in body weight was observed during fluoride exposure, which increased significantly ($P < 0.05$) during melatonin administration (Table 2).

So, present study revealed significant decrease in feed intake during fluoride exposure as compared to control day, thereafter significantly increased up to the normal level during melatonin administration. This might be due to alteration of the activities of various enzymes by the F ion, resulting in hyperglycemic effect and interruption of metabolic processes and antioxidative pathways. These results were in accordance with the studies done in past (Chlubek 2002, Kumar *et al.* 2007). These changes lead to reduced feed intake and loss in body weight. These metabolic enzymatic activities were normalized by administration of melatonin, hence improvement in feed intake was observed.

Table 2. Effect of melatonin on feed intake, water intake, and body weight of sodium fluoride exposed male rat at high altitude

Parameter	0–7 th day	14 th day	21 st day	28 th day	35 th day
Feed intake (g/day)	32.9 ± 0.00	30.9 ± 0.40	29.08 ± 0.50	36.3 ± 1.00	37.2 ^{abcd} ± 0.34
Water intake (ml/day)	6.01 ± 0.46	10.24 ^a ± 3.97	14.23 ^{ab} ± 2.54	16.82 ^{abc} ± 0.55	18.63 ^{abcd} ± 1.74
Body weight (g/animal)	225.40 ± 0.93	222.40 ^a ± 0.64	210.50 ^{abc} ± 1.08	228.40 ^{abc} ± 2.10	232.20 ^{abcd} ± 1.32

Values are presented as mean $\pm$ SEM; ^{abcd}Indicates significant difference ($P < 0.05$) within the same row in comparison with 7th, 14th, 21st, 28th and 35th days.

Water intake increased gradually during fluoride exposure and melatonin administration, which might be due to activation of thirst centre. Fluoride salt in water activates water intake for enhanced diuretic effect (Table 2). Other studies also observed significant increase in water consumption in animals exposed to the two higher doses of fluoride (50 and 100 ppm) in drinking water (Manocha *et al.* 1975, Tsunoda *et al.* 2005). Improvement of body weight gain during melatonin administration might be due to decrease in catabolic process and up-regulation of antioxidants which protect tissue damages.

Changes in haemogram: Haemoglobin (Hb), red blood cells (RBC), mean corpuscular haematocrit (MCH), mean corpuscular volume (MCV) and packed cell volume (PCV) were measured which indicated significant ($P < 0.05$) decrease in RBC, Hb and PCV during fluoride exposure and significant ($P < 0.05$) increase during administration of melatonin (Table 3). Also the significant ($P < 0.05$) increase of mean values of MCH and MCV during fluoride exposure decreased after melatonin administration. The leukogram indices like neutrophil, eosinophil and monocyte significantly ($P < 0.05$) increased during fluoride treatment and significantly decreased during melatonin administration (Table 3). The mean value of lymphocyte and platelet value significantly ($P < 0.05$) decreased during treatment of fluoride and simultaneously increased during administration of melatonin (Table 3).

Several studies about effect of NaF on haematological parameters in experimental animals reported similar trends (Eren *et al.* 2005, Karadeniz and Altintas 2008, Kant *et al.* 2009). There are no studies on sodium fluoride being taken as proven agent for inducing oxidative stress in rats under high altitude stress condition. Our studies showed significant decrease in erythrogram indices, i.e. RBC, Hb, and PCV during NaF exposure as compared to control days, which was in concord with study done in past (Giri *et al.* 2015). Sodium fluoride has the potential to cause destruction of haemopoietic tissue (Patel and Dhande 2000). The levels of the above parameters were significantly reversed during melatonin administration, which might be due to removal of free radicals by melatonin (Zaghloul and Gad 2014). These findings agreed with the previous studies on livestock animals as well as laboratory animals (Ahmed *et al.* 2011, Zaghloul and Gad 2014). The significant increase in the value of MCH and MCV during NaF exposure as compared to control days was in accordance to studies done in past (Giri *et al.* 2015) and the levels of the above parameters were significantly reversed during the administration of melatonin (Karimungi *et al.* 1996). Hence, decreasing trend of the mean values of erythrogram indices in rats might be due to rise of oxidative stress level and tissues damage by fluoride exposure at high altitude. These findings agreed with the previous studies in rat (Zaghloul and Gad 2014). These changes might be due to the fact that association of fluoride toxicity may increase the susceptibility to oxidative stress that may lead to a reduced capacity of rats to cope with stressors in this environment.

Table 3. Effect of melatonin on haemogram of sodium fluoride exposed male rat at high altitude

Parameter	0–7 th day	14 th day	21 st day	28 th day	35 th day
RBC ($\times 10^9/l$)	8.10 $\pm$ 0.10	7.66 $\pm$ 0.11	6.37 ^{ab} $\pm$ 0.13	7.47 ^{ac} $\pm$ 0.15	8.31 ^{bcd} $\pm$ 0.07
Hb (gm/dl)	15.03 $\pm$ 0.14	14.35 ^a $\pm$ 0.13	13.46 ^{ab} $\pm$ 0.15	14.49 ^{abc} $\pm$ 0.10	15.46 ^{bcd} $\pm$ 0.11
MCH (Pg/dl)	17.26 $\pm$ 0.26	18.48 ^a $\pm$ 0.15	19.35 ^{ab} $\pm$ 0.09	19.13 ^a $\pm$ 0.21	17.24 ^{bcd} $\pm$ 0.12
MCV (fl)	46.98 $\pm$ 0.74	47.76 $\pm$ 0.53	48.58 $\pm$ 0.26	47.54 $\pm$ 0.11	46.57 ^c $\pm$ 0.23
PCV (%)	50.86 $\pm$ 0.59	47.90 ^{ab} $\pm$ 0.12	46.20 ^{ab} $\pm$ 0.16	47.69 ^{ac} $\pm$ 0.23	49.57 ^{abcd} $\pm$ 0.11
Neutrophil (%)	3.43 $\pm$ 0.20	4.00 $\pm$ 0.00	5.14 ^{ab} $\pm$ 0.26	4.57 ^a $\pm$ 0.20	3.14 ^{bcd} $\pm$ 0.14
Eosinophil (%)	2.29 $\pm$ 0.42	4.43 ^a $\pm$ 0.20	4.14 ^a $\pm$ 0.26	3.43 $\pm$ 0.37	2.00 ^{bc} $\pm$ 0.58
Basophil (%)	2.29 $\pm$ 0.42	1.29 $\pm$ 0.18	1.43 $\pm$ 0.20	1.86 $\pm$ 0.26	2.57 $\pm$ 0.48
Lymphocyte (%)	94.00 $\pm$ 0.44	83.71 ^a $\pm$ 0.36	74.29 ^{ab} $\pm$ 0.68	87.43 ^{abc} $\pm$ 0.30	90.86 ^{abcd} $\pm$ 0.91
Monocyte (%)	1.57 $\pm$ 0.30	1.57 $\pm$ 0.20	3.14 ^{ab} $\pm$ 0.26	2.57 $\pm$ 0.30	2.43 $\pm$ 0.20
Platelet ($\times 10^3/\mu l$)	244.30 $\pm$ 2.30	227.10 ^a $\pm$ 0.40	224.90 ^a $\pm$ 0.51	236.10 ^{ab} $\pm$ 2.03	241.70 ^{bc} $\pm$ 0.42

Values are presented as mean $\pm$ SEM; ^{abcd}Indicates significant difference ($P < 0.05$) within the same row in comparison with 7th, 14th, 21st, 28th and 35th days.

Change in antioxidant and oxidative stress level: The MDA levels which are indicative of extent of lipid peroxidation rose significantly ($P < 0.05$) after fluoride exposure which were significantly reduced by melatonin supplementation and brought back to near control levels (Table 4). These results were in accordance with the studies done in the past (Błaszczuk *et al.* 2008). The MDA levels decreased significantly during administration of melatonin. It has been found that the melatonin is most effective in inhibiting lipid peroxidation process (Vural *et al.* 2001). These results confirmed the potent antioxidative properties of melatonin.

The total antioxidant capacity determined by FRAP assay decreased significantly ($P < 0.05$) during fluoride exposure and increased significantly ($P < 0.05$) during melatonin administration (Table 4). Similarly, the free radical scavenging activity determined by DPPH assay showed significant ($P < 0.05$) increase in scavenging activity by fluoride exposure which further decreased significantly after melatonin supplementation (Table 4). In one study, it was found that fluoride consumption decreases the total antioxidant capacity in colony-bred male albino rats (Vasant and Narasimhacharya 2012). Pohanka *et al.* (2010) found that FRAP value increased after treatment of melatonin in female rats.

Interestingly, Thangapandiyan and Miltonprabu (2013) reported insignificant increase in total antioxidant scavenging activity of plasma on fluoride exposure, which was decreased to significant level by melatonin supplementation. This may be attributed to the fact that with increase in levels of oxidative stress and increase in generation of free radicals by fluoride exposure, there was need of increased scavenging of the same, thereby leading to a rise in DPPH levels. All the above parameters indicate that melatonin supplementation could be instrumental in mitigating the oxidative stress induced by fluoride exposure in rats at high altitude.

Changes in liver function biomarkers: ALP, ALT and AST are biomarkers for clinical diagnosis of liver injury (Bulle *et al.* 1990). In present study, alkaline phosphatase

Table 4. Effect of fluoride and melatonin on antioxidant and oxidative stress level of male rats at high altitude

Parameter	0–7 th day	14 th day	21 st day	28 th day	35 th day
MDA (μ mole/ml)	7.40 $\pm$ 0.98	11.96 ^a $\pm$ 0.19	13.05 ^a $\pm$ 0.50	9.79 ^{ac} $\pm$ 0.32	8.17 ^{bc} $\pm$ 0.40
FRAP (μ mol/l)	216.00 $\pm$ 3.54	208.30 ^a $\pm$ 1.30	207.20 $\pm$ 1.99	209.70 $\pm$ 2.82	211.30 ^c $\pm$ 2.23
DPPH (% inhibition)	30.60 $\pm$ 0.51	38.84 ^a $\pm$ 1.41	41.74 ^a $\pm$ 0.67	40.05 ^a $\pm$ 0.65	31.82 ^{bcd} $\pm$ 0.79

Values are presented as mean $\pm$ SEM; ^{abcd}Indicates significant difference ($P < 0.05$) within the same row in comparison with 7th, 14th, 21st and 28th days. DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric reducing ability of plasma; MDA, Malondialdehyde.

(ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased significantly during fluoride exposure period, whereas their levels decreased significantly during melatonin administration (Table 5). These increased levels of ALP, ALT and AST during fluoride treatment can be attributed to the hepatic stress induced by fluoride. The exposure to sodium fluoride resulted in impairment in liver function as indicated by significant increase in the activity of AST, ALT and ALP (Wessam and Abdel-Wahab 2013). The biochemical alterations were dose dependent elevation ($P \leq 0.01$) in plasma enzyme activities of ALT, AST in rats (Giri *et al.* 2015).

Melatonin supplementation for 14 days was found to have an ameliorative effect on the levels on all these three enzymes, which might be due to scavenging activity of melatonin against the toxic metabolite produced during fluoride exposure. As per Sidhu *et al.* (2014), melatonin supplementation could be useful to decrease the hepatotoxicity by quenching oxidative stress in female wistar rats.

In conclusion, the present study observed protective effects of melatonin against sodium fluoride mediated oxidative stress-induced adverse changes in some haematological, biochemical and antioxidant related

Table 5. Change in liver function biomarkers on fluoride and melatonin administration in male rats at high altitude

Parameter	0–7 th day	14 th day	21 st day	28 th day	35 th day
ALP (IU/l)	3.62±0.33	9.16 ^a ±1.036	12.73 ^{ab} ±0.43	6.85 ^{abc} ±0.43	4.71 ^{bcd} ±0.43
ALT (IU/l)	79.27±0.54	91.35±1.26	94.67 ^a ±1.08	71.74 ^{bc} ±4.90	67.33 ^{bc} ±5.72
AST (IU/l)	65.42±2.33	93.41 ^a ±0.84	96.06 ^a ±0.75	83.74 ^{abc} ±1.21	74.85 ^{abcd} ±0.99

Values are presented as mean±SEM; ^{abcd}Indicates significant difference (P<0.05) within the same row in comparison with 7th, 14th, 21st and 28th days similarly. ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase

parameters in rat at high altitude. Sodium fluoride (@ 50 ppm, p.o.) caused adverse effect on feed and water intake, body weight, haemogram, plasma biochemical, and oxidative stress parameters in rats. Melatonin supplementation generally reversed the adverse changes brought by administration of sodium fluoride. This indicated protective effect of melatonin against oxidative stress conditions at high altitude. Our results prove that melatonin may be a potential agent in development of a nutraceutical diet for animals at high altitude for protection against fluoride induced stress in animal body. However, more detailed studies with respect to tissue specific effects of the melatonin need to be elucidated.

ACKNOWLEDGEMENT

This study was supported in the form of a Senior Research Fellowship (SRF) to the first author by CSIR, New Delhi and research facilities provided by Defence Institute of High Altitude Research (DIHAR), DRDO, India for conducting this study is duly acknowledged. We would like to thank all the technical staff of our animal facility and Dr Puneet Ranjan, Veterinarian (STA-B) for assistance throughout this study.

REFERENCES

- Ahmed H H, Essawy G S, Salem H A and Abdel Daim M A. 2005. Melatonin has a strong antioxidant activity and improves liver and kidney functions in broiler chicks. *Egyptian Journal of Basic and Applied Sciences* **4**: 77–92.
- Addya S, Chakravarti K, Das M, Ghosh N, Chakravarti D and Chatterjee G C. 1986. Effects of mercuric chloride antioxidant defense components of rat erythrocyte and plasma: influence of vitamin E supplementation. *Indian Journal of Experimental Biology* **24**: 468–70.
- Arjun L K, Shankar R G and Vakdevi V. 2017. Dental fluorosis, nutritional status, kidney damage, and thyroid function along with bone metabolic indicators in school-going children living in fluoride-affected hilly areas of Doda district, Jammu and Kashmir, India. *Environmental Monitoring and Assessment* **189**: 579.
- Akosu T J and Zoakah A I. 2008. Risk factors associated with dental fluorosis in Central Plateau state, Nigeria. *Community*

- Dentistry and Oral Epidemiology* **36**(2): 144–48.
- Bakonyi T and Radak Z. 2004. High altitude and free radicals. *Journal of Sports Science and Medicine* **3**: 64–69.
- Bharti V K and Srivastava R S. 2011. Effect of pineal proteins and melatonin on certain biochemical parameters of rats exposed to high-fluoride drinking water. *Fluoride* **44**: 30–36.
- Bharti V K and Srivastava R S. 2009. Protective role of buffalo pineal proteins on arsenic-induced oxidative stress in blood and kidney of rats. *Health* **1**: 167–72.
- Bharti V K, Srivastava R S and Anand A K. 2012. Effect of melatonin and buffalo epiphyseal proteins on vital organs tissues proteins. *Indian Journal of Animal Sciences* **82**: 176–79.
- Buege J A and Aust S D. 1978. Microsomal lipid peroxidation. *Methods in Enzymology* **52**: 302–10.
- Benzie Iris F F and Strain J J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry* **239**: 70–76.
- Blios M S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature* **26**: 1199–1200.
- Blaszczak I, Grucka-Mamczar E, Kasprczyk S and Birkner E. 2008. Influence of fluoride on rat kidney antioxidant system: effects of methionine and vitamin E. *Biological Trace Element Research* **121**: 51–59.
- Bulle F, Mavier P, Zafrani E S, Preaux A M, Lescs M C and Siegrist S. 1990. Mechanism of gamma-glutamyl transpeptidase release in serum during intrahepatic and extrahepatic cholestasis in the rat: a histochemical, biochemical and molecular approach. *Hepatology* **11**: 545–50.
- Chlubek D. 2003. Fluoride and oxidative stress [editorial review]. *Fluoride* **36**: 217–28.
- Chawla S L, Yadav R, Shah D and Rao M V. 2008. Protective action of melatonin against fluoride-induced hepatotoxicity in adult female mice. *Fluoride* **41**: 44–51.
- Eren E, Ozturk M and Mumcu E F. 2005. Fluorosis and its haematological effects. *Toxicology and Industrial Health* **21**: 255–58.
- Giri D K, Ghosh R C, Kashyap D K, Dewangan G and Maiti S K. 2015. Haemato-biochemical alterations in subacute oral toxicity of sodium fluoride in wistar rats. *Journal of Animal Research* **5**: 595–98.
- Haijun Y, Jianghua C and Xizhou Z *et al.* 2008. Ultrasonographic analysis of the liver, spleen and kidney in young emigrants to high altitude areas. *Shanghai Medical Imaging* **17**: 149–52.
- Hou R, Mi Y, Xu Q, Wu F, Ma Y, Xue P and Yang W. 2014. Oral health survey and oral health questionnaire for high school students in Tibet, China. *Head and Face Medicine* **10**: 17.
- Kalia S, Bharti V K, Gogoi D, Giri Arup and Kumar B. 2017. Studies on the growth performance of different broiler strains at high altitude and evaluation of probiotic effect on their survivability. *Scientific Reports*. #Article ID 46074.
- Karadeniz A and Altintas L. 2008. Effects of panax ginseng on fluoride-induced haematological pattern changes in mice. *Fluoride* **41**: 67–71.
- Kant V, Verma P K and Pankaj N K. 2009. Haematological profile of sub acute oral toxicity of fluoride and ameliorative efficacy of aluminium sulphate in goats. *Toxicology International* **16**: 31–35.
- Karimungi M G and Joshi B N. 1996. Diurnal sensitivity in melatonin-induced hematological changes in the male albino rat. *Biological Signals* **5**: 283–90.
- Kumar A, Tripathi N and Tripathi M. 2007. Fluoride-induced biochemical changes in fresh water catfish (*Clarias batrachus*

- L.). *Fluoride* **40**: 37–41.
- Manocha S L, Warner H and Olkowski Z L. 1975. Cytochemical response of kidney, liver, and nervous system of fluoride ions in drinking water. *Histochemical Journal* **7**: 343–55.
- Miller L E, McGinnis G R, Kliszczewicz B, Slivka D, Hailes W, Cuddy J, Dumke C, Ruby B and Quindry J C. 2013. Blood oxidative-stress markers during a high-altitude trek. *International Journal of Sport Nutrition and Exercise Metabolism* **23**: 65–72.
- Narayana K D, Souza U J and Rao K P. 2002. Effect of ribavirin on epididymal spermcount in rat. *Indian Journal of Physiology and Pharmacology* **46**: 97–101.
- Pohanka M, Sobotka J, Jilkova M and Stetina R. 2010. Oxidative stress after sulfur mustard intoxication and its reduction by melatonin: efficacy of antioxidant therapy during serious intoxication. *Drug and Chemical Toxicology* **34**: 85–91.
- Plateau State, Nigeria. Community Dentistry and Oral Epidemiology. **36**: 144–148.
- Patil G P and Dhande R R. 2000. Effect of HgCl and CdCl on haematobiochemical parameters of the fresh water fish, *Channa punctatus*. *Journal of Ecotoxicology and Environmental Monitoring* **10**: 177–81.
- Park K. 2011. Passmore, p 577. *Textbook of Preventive and Social Medicine*. 21 Edn. Banarasisdas Bhanot Publishers, Jabalpur, India.
- Reiter R J, Tan D X and Maldonado M D. 2005. Melatonin as an antioxidant: physiology versus pharmacology. *Journal of Pineal Research* **39**: 215–26.
- Reiter R J, Tan D X and Sainz R M. 2002. Melatonin: reducing the toxicity and increasing the efficacy of drugs. *Journal of Pharmacy and Pharmacology* **54**: 1299–21.
- Susheela A K. 2001. Fluorosis Indian scienario: A treatise on fluorosis. Fluorosis Research and Rural Development Foundation, New Delhi, India.
- Saravanan S, Kalyani C, Vijayarani M, Jayakodi P, Felix A, Nagarajan S, Arunmozhi P and Krishnan V. 2008. Prevalence of dental fluorosis among primary school children in rural areas of Chidambaram Taluk, Cuddalore District, Tamil Nadu, India. *Indian Journal of Community Medicine* **33**: 146–50.
- Sidhu I P S, Bhatti J S and Bhatti G K. 2014. Modulatory action of melatonin against chlorpyrifos induced hepatotoxicity in Wistar rats. *Asian Journal of Multidisciplinary Studies* **2**(9): 123–31.
- Tian Y M, Zhang G Y and Dai Y R. 2003. Melatonin rejuvenates degenerated thymus and redresses peripheral immune functions in aged mice. *Immunology Letters* **88**: 101–104.
- Tsunoda M, Aizawa Y, Nakana K, Liu Y, Horuchi T, Hai K and Tsunoda H. 2005. Changes in fluoride levels in the liver, kidney and brain and in neurotransmitters of mice after sub acute administration of fluoride. *Fluoride* **38**: 284–92.
- Thangapandiyan S and Miltonprabu S. 2013. An *in vivo* and *in vitro* studies on the antioxidant property of epigallocatechin gallate on sodium fluoride induced toxicity in rats. *International Journal of Phytopharmacology* **4**: 245–54.
- Vijayalaxmi Russel J, Reiter Dun-Xiantan, Terence S, Herman and Charles R. 2004. Melatonin as a radioprotective agent: A review. *International Journal of Radiation Oncology Biology Physics* **59**: 639–53.
- Vural H, Sabuncu T, Oktay Arslan S and Aksoy N. 2001. Melatonin inhibits lipid peroxidation and stimulates the antioxidant status of diabetic rats. *Journal of Pineal Research* **31**: 193–98.
- Vasant R A and Narasimhacharya A V. 2012. Ameliorative effect of tamarind leaf on fluoride-induced metabolic alterations. *Environmental Health and Preventive Medicine* **17**: 484–93.
- Wessam M and Abdel-Wahab. 2013. Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats. *Journal of Basic and Applied Zoology* **66**: 263–70.
- West J B. 2004. The physiologic basis of high-altitude diseases. *Annals of Internal Medicine* **141**: 789–800.
- Whitford G M. 1997. Determinants and mechanisms of enamel fluorosis. *Ciba Foundation Symposium* **205**: 226–41.
- Zaghloul D M and Gad S B. 2014. Melatonin restores renal functions and histology in aged rats. *Research Opinions in Animal and Veterinary Sciences* **4**: 249–57.
- Zhipeng Fan, Yanhui Gao, Wei Wang, Hongqiang Gong, Min Guo, Shengcheng Zhao, Xuehui Liu, Bing Yu and Dianjun Sun. 2016. Prevalence of brick tea-type fluorosis in the Tibet autonomous region. *Journal of Epidemiology* **26**: 57–63.