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Effect of melatonin and buffalo epiphyseal proteins on vital organs tissues proteins contents in rats exposed to arsenic and fluoride

VIJAY K BHARTI[1], R S SRIVASTAVA[2] and AJAY K ANAND[3]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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Heart, liver, lungs, kidney, and brain are more sensitive organs in which various metabolic pathways take place (Manna *et al.* 2007). High arsenic and fluoride intake causes reduction in weights of reproductive organs and concentration of protein in ovary, uterus, and liver in female rats (Chiony and Memon 2001, Shah and Chinoy 2004, Verma *et al.* 2004, Wang *et al.* 2004, Sharma *et al.* 2007). Arsenic and fluoride has been reported for causing abnormal changes in tissues of various vital organs through oxidative stress pathways and affects fat and carbohydrate balance, and protein equilibrium (Guan *et al.* 1998, Flora 1999, Birkner *et al.* 2000, Wang *et al.* 2000, Biswas *et al.* 2000, Bharti and Srivastava 2009a). These metabolic interferences may cause extensive tissue damage leading to decreased organs weight, tissues protein contents and death from multi-system organ failure (Chiony and Memon 2001, Mondal *et al.* 2006). Cells possess antioxidative defense mechanisms to protect against free radical-mediated damage in toxicity (Reiter *et al.* 2002). Therefore, hypothesis of this study was that use of antioxidants as a protective agent may prove effective against toxicity-induced adverse changes in organ's protein content. Cerebral epiphysis (pineal glands) secrets melatonin and various proteins that have different physiological functions including antioxidant function (Reiter *et al.* 2004, Bharti and Srivastava 2009b,c). With keeping this view, present experiment was conducted to know the effect of melatonin (MEL) and buffalo epiphyseal proteins (BEP) on tissues proteins contents of vital organs, viz. heart, brain, liver, and kidney of female rats exposed to arsenic (As) and fluoride (F) treatments.

The present study was designed to study the effect of buffalo epiphyseal proteins, melatonin, fluoride, and arsenic alone or combination on proteins concentration of different vital organs of female rats. All the investigative procedures conducted on the experimental animals were duly approved by the Institutional Animal Ethics Committee (IAEC) for the purpose of control and supervision of experiments on animals.

Experimental design: Adult female Wistar rats (48),123–218 g BW, were procured from the Indian Veterinary Research Institute, Izatnagar. Rats were housed in polypropylene cages in a light/dark (LD) cycle of 12 h in a pathogen-free, temperature and humidity-controlled environment (set at 21±2°C and relative humidity at 50±10%, respectively). After 1 week acclimatization period, they were weighed and randomly assigned to 8 groups (Table 1) so as to give approximately equal initial group mean body weights (Bharti and Srivastava 2009b). Buffalo (*Bubalus bubalis*) epiphyseal proteins (BEP) was used as agent since we have previously published with this reagent. Appropriate dose of melatonin (MEL, 10 mg/kg BW) and BEP (100 µg/kg BW) were optimized from our previous study and thereafter they were dissolved in suitable vehicle before administration (Bharti and Srivastava 2011). Fluoride (F) and arsenic (As) level in drinking water was calculated and thereafter-required concentration of F (150 ppm) and As (100 ppm) was made by addition of sodium fluoride and sodium arsenite respectively, daily for 28 days (Table 1). All the animals had free access to standard laboratory animal diet and clean water. The animals were checked daily for the health and husbandry conditions.

Sample collection: All the animals were under continuous observations for their behavioural changes, clinical signs of toxicity, and mortality, if any. The rats were euthanized-using ether, at the end of experiments (28 day) and brain, heart, liver, and kidney were collected. Immediately, all the organs were cleaned, rinsed in chilled saline, blotted and weighed.

Present address: [1]Scientist C (vijaykbharti@rediffmail.com), Nutrition and Toxicology Laboratory, DIHAR, DRDO, Ministry of Defence, C/o 56 APO, Leh Ladakh 194 101, India.

[2]Formerly Principal Scientist (srivarsvet@yahoo.co.in), Neurophysiology Laboratory, Division of Physiology and Climatology.

[3]Ph.D. Scholar (ajaykanand.2011@rediffmail.com), Department of Science (Zoology), B N M University, Laloo Nagar, Madhepura, Bihar 852 113, India.

Table 1. Distribution of experimental rats to different treatments

Groups	Body weight (g)	Treatments	Dose	Route of administration
Control	141.67 ^{b}±3.57	Drinking water+ normal saline	*Ad lib.*	Oral Intra peritoneal
BEP	131.00 ^{b}±3.42	Buffalo epiphyseal proteins (BEP)	100 µg/kg BW	Intra peritoneal
MEL	135.00 ^{b}±5.92	Melatonin (MEL)	10 mg/kg BW	Intra peritoneal
F	123.33 ^{a}±1.05	Fluoride (F) +normal saline	150 ppm F	Oral with drinking water, Intra peritoneal
F+BEP	142.17 ^{b}±5.66	F + BEP	150 ppm F100 µg/kg BW	Oral with drinking water, Intra peritoneal
F+MEL	137.50 ^{b}±4.79	F +MEL	150 ppm F10 mg/kg BW	Oral with drinking water, Intra peritoneal
As	218.33 ^{c}±11.53	Arsenic (As)+normal saline	100 ppm As	Oral with drinking water, Intra peritoneal
As+ BEP	145.00 ^{b}±6.58	As + BEP	100 ppm As100 µg/kg BW	Oral with drinking water, Intra peritoneal

Values (n,6; means±SE) in the same column bearing superscript ^{a,b,c}vary significantly (P<0.05).

Thereafter, 200 mg of sample was weighed and homogenized in 2 ml of ice-cold saline with IKA homogenizer, under ice-cold condition. All the organs homogenate were collected and centrifuged for 10 min at 3000 rpm at 4°C. Thereafter, cell free supernatant were collected and used for estimation of total proteins concentration.

Analytical procedures: Cell free extract of tissue homogenates from different organs (heart, liver, kidney, and brain) were taken for analysis of vital organs proteins concentration with the help of double beam UV-VIS spectrophotometer (Lowry *et al.* 1951). Protein concentration was calculated as protein mg/g of tissue.

Statistical Analysis: Differences between groups were statistically analyzed by one-way ANOVA, and the differences between the means of groups were separated by least significant difference (LSD) test. All data were presented as mean±standard error. Values of P< 0.05 were regarded as significant. A computer program (SPSS 10.01) was used for statistical analysis (Bharti and Srivastava 2009a).

The reduction in weight of vital organs and tissues protein contents in toxicities and chronic illness condition is very common. None of the animals died during entire experiments and no abnormal behaviour as well as clinical signs of toxicity were seen in any group of rats.

Effect of buffalo epiphyseal proteins and melatonin alone: Any adverse change in brain, cardiac, renal, and hepatic tissues protein level in BEP and MEL-treated animals were not recorded. In contrast to BEP treatment, MEL caused higher cardiac protein concentration than control animals (Table 2). However, BEP treatment caused significant (P<0.05) elevation of protein content in brain than control animals. These findings clearly indicated that MEL and BEP has no toxic effect on vital organs protein content rather beneficial effect.

Effect of buffalo epiphyseal proteins in fluoride and arsenic-treated animals: F and As caused significant (P<0.05) decrease in brain (only by As), cardiac, hepatic, and renal tissues protein contents. Interestingly, F had higher adverse effect than As on renal tissues protein content. These findings indicated the potential vulnerability of different vital organs in arsenic and fluoride toxicity. This reduction of organs tissues protein content might be the result of F- and As-induced oxidative stress and toxicities and damages to the tissues (Shanthakumari *et al.* 2004, Mittal and Flora 2006, Sharma *et al.* 2007). The cellular macromolecules, in particular DNA, proteins and lipids, are natural targets of oxidation (Reiter *et al.* 2004). Since, in spite of numerous biological defense systems, oxidative stress promotes cellular injury that modifies proteins and other biomolecules which leads to tissue damage (Reiter *et al.* 2004, Halliwell 2006). Therefore, reduction of organ's protein content may be due to amplified activities of catabolic enzymes in organs that cause enhanced tissue proteolysis and reduction of organs weight and tissues protein content (Halliwell 2006, Nassr-Allah and Abdel-Hameid 2009).

Chinoy and Memon (2001) reported declined protein level in liver and gastrocnemius muscle of male mice following NaF exposure for 30 days. Sharma *et al.* (2007) also documented reduced weights of ovary, uterus, vagina, adrenal gland and concentration of protein in ovary, uterus, adrenal and liver of fluoride-exposed female rats. Verma *et al.* (2004) recorded significant reductions in body weight, protein, and glycogen contents in the liver and kidney of As-treated mice.

Interestingly, BEP treatment significantly (P<0.05) reduced the adverse effect of F and As in all tissues (Table 2). This protection of BEP might be due to their antioxidant properties (Bharti and Srivastava 2009a, b, c, 2011). Pal and Chatterjee (2006) also reported beneficial effect of antioxidants (melatonin) on arsenic-induced oxidative stress in liver and kidney of rats. Our earlier findings also supported the protective function of BEP and MEL in As and F-induced oxidative stress in rats (Bharti and Srivastava 2009a, c, 2011).

Effect of melatonin in fluoride-treated animals: Interestingly, F+MEL treatment significantly (P<0.05) ameliorated the depression in brain, cardiac, hepatic, and renal protein content caused by F treatment (Table 2). These beneficial effects may be due to antioxidants function of MEL in female rats (Bharti and Srivastava 2009a, b, c, 2011). However, MEL administration with F has shown better effect than F+BEP treatment on hepatic and renal tissues content (Table 2).

Table 2. Effect of different treatments on proteins concentration (mg/g tissues) of brain, heart, liver and kidney of female rats

Groups	Brain	Heart	Liver	Kidney
Control	20.63^{b} ±1.32	28.60^{b} ±1.77	63.82^{c} ±2.28	59.33^{cd} ±1.63
BEP	25.88^{c} ±0.98	26.26^{b} ±1.24	60.61^{c} ±0.91	57.82^{cd} ±1.54
MEL	24.77^{c} ±0.68	33.29^{c} ±2.38	61.23^{c} ±1.32	60.27^{d} ±0.83
F	20.14^{b} ±1.11	21.95^{a} ±0.51	44.85^{a} ±2.61	40.99^{a} ±0.95
F+BEP	26.78^{c} ±0.43	31.99^{c} ±0.86	52.89^{b} ±1.07	46.75^{b} ±0.23
F+MEL	28.41^{c} ±1.52	35.03^{c} ±1.75	65.14^{c} ±0.91	58.66^{cd} ±1.16
As	17.16^{a} ±0.96	22.75^{a} ±1.25	47.82^{a} ±1.06	45.45^{b} ±0.40
As+ BEP	23.44^{c} ±0.76	35.76^{c} ±2.03	63.89^{c} ±0.60	55.98^{c} ±1.36

Values (n,6; means±SE) in the same column bearing superscript $^{a, b, c, d}$ vary significantly (P<0.05).

SUMMARY

Present experiment was conducted to know the effect of melatonin (MEL) and buffalo epiphyseal proteins (BEP) on tissues proteins contents of vital organs of female rats exposed to arsenic (As) and fluoride (F) treatments. As (100 ppm), F (150 ppm) through drinking water and MEL (10 mg/kg BW) and BEP (100 µg/kg BW) through intraperitoneally were administered daily, for 28 days. Fluoride and As treatment caused significant reduction in protein concentration in brain (only by As), cardiac, hepatic, and renal tissues. Melatonin and BEP alone did not cause any adverse effect on brain, cardiac, renal, and hepatic tissues protein level. However, protein depression caused by F and As was significantly (P<0.05) reduced by MEL and BEP treatment. Hence, this study clearly indicates the beneficial effect of MEL and BEP on ill effects of As and F on vital organs proteins content. Therefore, study could probably enlighten the use of antioxidants as an ameliorative agents to prevent different pollutants and metal toxicity-induced organs dysfunction in animals.

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