Effects of black tea in mitigation of sodium fluoride potency to suppress motor activity and coordination in laboratory rats

Heba S. El-lethey, Mervat M. Kamel*

Department of Animal Hygiene and Management, Faculty of veterinary Medicine, Cairo University, Cairo, Egypt
mevy58@yahoo.com

Abstract: The present study was designed to assess the potential impact of Na-F alone or in conjugation with black tea on motor function and coordination performance in laboratory rats. An array of behavioural motor tasks, viz., open field, plank walking and rod walking tests were employed in our study in order to evaluate animals' motor heath. Body weight gain as a performance criterion was also monitored. Eighty weanling 32-days old Wistar male rats randomly allotted to four groups of 20 animals each, were administered Na-F at 100 ppm and 2% black tea for a period of twelve weeks in a factorial pattern to constitute 4 experimental treatments. Black tea significantly improved Na-F-induced marked losses in body weight gains of rats. In the open field test, Na-F-treated rats displayed no significant changes in the levels of motor activities (horizontal locomotion) compared to control. However, fluorotic animals performed poorly in all studied motor-coordination tests. Administration of black tea to Na-F-exposed rats also significantly enhanced their motor performance and coordination ability during psychomotor testing. Concerning animals' walking pattern, high incidence of shaky movements with unsteady gait was markedly observed in Na-F-intoxicated rats, as compared to control, confirms lacking of muscle tone and coordination. Our findings illustrate that black tea affords a profound protection against fluoride intoxication-provoked harmful effects on motor health as signified by inhibited motor activities accompanied by poor coordination proficiency in laboratory rats, and hearten to recommend for simultaneous supplementation of black tea to Na-F-jeopardized individuals in order to help mitigate fluorosis-inflicted hazards.
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1. Introduction

Fluoride is omnipresent in our environment and has been added to drinking water supplies for cariostatic purposes as a prophylactic agent in dental caries, with a recommended dose between 0.7 and 1.2 mg/litre (Leverett et al., 1997). Further fluoride sources, other than drinkable water, are drinks, tooth pastes, mouth rinses, dietary supplements and foods in general.

Fluorosis caused by excess intake of fluoride is a slow, progressive degenerative disorder that represents a significant adverse impact on public health and well-being in many parts of the world (Susheela, 1999). Long term ingestion of high levels of fluoride results in various pathological alterations in overall organs and tissues predominantly the bones, teeth, the structure and function of skeletal muscles, brain and spinal cord (Shashi et al., 1992; 1994; Mullenix et al., 1995; Vani and Reddy, 2000).

More than 90% of the total body burden of fluoride is retained in bones and teeth, because of its profound affinity for calcified tissues, where most of the remaining portion is distributed in highly vascularized soft tissues and blood (Hardman et al., 2005; Fawell et al., 2006). Therefore, the most obvious early toxic effects of fluoride in humans are skeletal fluorosis, leading to a variable degree of combined locomotor disability and neurological impairment (Reddy, 2009). Individuals affected with skeletal fluorosis revealed joints pain in limbs, numbing, cramping and tingling of extremities accompanied with back pain with difficulties during walking, aggravated by activity (Shashi et al., 2008). Since animal studies and human clinical trials indicated that fluoride can reduce bone strength even before skeletal fluorosis is present, a heavy and tired feeling in the legs with frequent falling, or "a foot-slapping gait" is also a commonly noticeable manifestation (Mousny et al., 2006).

In advanced stages, "crippling skeletal fluorosis" characterized by damage of musculoskeletal and nervous systems is then observed. The later disorder results in mal shaping of bones, muscles wasting and arthritic pain with restricted joints motion. Neurological complications of skeletal fluorosis, namely paralysis of limbs, vertigo, spasticity in extremities, arise primarily from mechanical compression of the spinal cord and nerve

roots from sclerosed vertebral column and ossified ligaments (Fisher et al., 1989; Reddy, 2009). Alarm was then given about an increased fluoride toxicity-inflicted risk on physical activity and motor health.

In addition to the effect on hard tissues, fluoride also manifests its toxicity on soft tissues, where it is known to cross the cell membranes and to enter soft tissues, impairing its function (Sharma and Chinoy, 1998; Vani and Reddy, 2000). Fluoride-increased generation of free radicals, lipid peroxidation and depleted antioxidant defense systems shifting the oxidant/antioxidant balance towards oxidative stress are proposed to mediate the toxic effects of fluoride on soft tissues (Shivarajashankara et al., 2001; 2002a; Trivedi et al., 2008; Kaur et al., 2009).

Amongst soft tissues, muscles and brain have been reported to retain the ingested fluoride which may in turn interfere with their physiological functions. Despite the fact that muscles were more affected than brain, probably due to the protective role of the blood brain barrier, there is a paucity of studies on the effect of fluoride intoxication on motor function and coordination performance in rats (Vani and Reddy, 2000).

Intact cerebellum has been reported primarily to be indispensable for successful coordination of voluntary motor function and to make an important contribution to control of muscle tone, equilibrium, gait and posture (Morton and Bastian, 2004; Thach and Bastian, 2004; Konarski et al., 2005; Koros et al., 2007; Baldacara et al., 2008). Cerebellar cortex has been evidenced to be particularly susceptible to sodium fluoride-induced oxidative stress and could contribute to the development of neurodegenerative diseases (Saad El-Dien et al., 2010). Moreover, recent findings from our preceding complementary study, where fluoride toxicity-enhanced oxidative stress and induced neurodegenerative lesions in the cerebellum of rats, have prompted us to further investigate the toxic potential of fluoride to disrupt motor-coordination function as an index for the health of musclo-skeletal system (Kamel et al., 2010).

On the positive side, however, the link between fluoride and oxidative stress may help efforts to mitigate the symptoms among individuals suffering from fluoride toxicity. Therefore, nutritional intervention with antioxidant-rich substances is the ultimate goal as antidotes for combating with the health complaints arising from fluorosis (Chinoy and Memon, 2001; Chinoy and Patel, 2001; Susheela and Bhatnagar, 2002).

Most of the health benefits of different types of tea are attributed to their antioxidant content where it is rich in polyphenolic compounds, collectively known as the tea flavonoids (du Toit et al., 2001). Tea flavanoids are known to exhibit antioxidative property being comparable to that of fruits and vegetables, thus the high, yet varying levels of anti-oxidants in teas might mitigate the deleterious toxic effects of fluoride.

Tea is most commonly consumed in its black form (Das et al., 2005). Since the fermentation process used to make black tea converts the green tea catechins into other compounds, theaflavins and thearubingins, it has been assumed that the health benefits of black tea are lesser than that of green one. However, recent studies indicate otherwise, where the latter compounds provide the health benefits of black tea which were originally attributed solely to green tea (Leung et al., 2001; Unno and Hoshino, 2007).

Our earlier experiment revealed a profound neuroprotective effect of black tea against Na-F-induced deleterious effects in brain tissues of rats, namely in the hippocampus and cerebellum, as reflected by learning deficits and confirmed by detrimental pathological lesions (Kamel et al., 2010). In view of this, the present study was considered necessary in order to further explore the toxic potency of fluoride intoxication to impair motor-coordination abilities and also to evaluate the possible role of black tea to counteract these predictable negative impact triggered by Na-F in laboratory rats. In addition, rats' body performance in the form of weight gains was also explored in the current study.

2. Material and Methods

2.1. Animals and housing:

Animal experimentation was performed in accordance with guidelines released by Cairo University Policy on Animal Care and Use, with the International regulations, as adopted and promulgated by Faculty of Veterinary Medicine. According to the above guidelines, all efforts were made to minimize the number of animals and their suffering.

Eighty weanling 32-days old Wistar male rats, weighing approximately 45g were obtained from the Unit for Laboratory Animals at Faculty of Veterinary Medicine, Cairo University and used in our study. They were housed in standard polypropylene cages with stainless steel wire lids, bedded with wood shavings. Animals were maintained on a 12-h light/dark cycle at a constant room temperature of 20-22°c and 60% humidity with free access to feed (standard commercially available pellets for laboratory rodents) and water throughout the course of the present study.

2.2. Experimental design:

All males were randomly distributed into four groups having 20 animals each, divided on 2 replicates. The route chosen in this study for exposure was via drinking water to mimic human exposure. Animals were administered our treatments, throughout the study till its completion at 115 days of age, in a 2 x 2 factorial design as follows:

Group (1) control (C), n=20: Weanling pups were administered plain water.

Group (2) Na-F group (F), n=20: Weanling pups were exposed to *ad libitum* supply of Na-F alone (Sigma Chemical Company) in drinking distilled water at 100 ppm on a mg/kg/day basis of 10.77 Na-F (Chioca et al., 2008).

Group (3) black tea group (T), n=20: Weanling pups were exposed to *ad libitum* supply of 2% black tea alone in drinking water (Trivedi et al., 2006). Twenty grams of black tea solids (Lipton Yellow label, Unilever Limited, India) and 1000 ml boiled drinking water were used to produce a 2% tea solution.

Group (4) ameliorated group (Na-F+T), n=20: Weanling pups were exposed to *ad libitum* supply of 100 ppm Na-F in combination with 2% black tea solution.

2.3. General observations in rats:

During experimental period, clinical signs and general appearances that included awareness, motor activity, and posture were checked daily.

2.4. Body weight gain:

Initial weight of all pups were recorded on postnatal day 32, and then all rats per group were individually weighed weekly afterwards throughout the study up to 115 days of age. Body weight gain was calculated as the difference between final and baseline weight. Mortalities were recorded as it occurred.

2.5. Behavioural tests:

All behavioural testing were conducted by the same personnel throughout the study, started at 90 days and ended at 115 days of animals' age.

2.5.1. Open field test

The open-field exposure is commonly used as a measurement of locomotor activity and can also serve as good preliminary test to determine motor deficits (Kelly, 1993; Chioca et al., 2008). The test was performed in a square wooden arena measured (90 x 90 x 25 cm). The wood of the apparatus is covered with a plastic laminate (formica), which prevents absorption of fluids (urine of rats). The floor was divided by black lines into 36 small squares (15 x 15 cm). All testing was conducted between 09:00 and 15:00 h. All treatment groups were tested at the same day in a random array. Rats were gently placed into a corner of the arena and allowed to explore it freely for 3 minutes.

Levels of animals' locomotory activity were determined by measuring changes in ambulation (horizontal locomotion). Ambulation is assessed in relation to lines drawn on the floor (the number of squares crossed). A crossed square was defined as the rat placing its two forepaws in the next square and moving forward (Chioca et al., 2008). The number of units crossed in the open field was used as a primary index of locomotor activity. Hand operated counters and stop watches were used to score the behaviour of animals.

After the 3 minutes test session, the rat was returned to its home cage and the open field was cleaned with 70% ethyl alcohol, to remove olfactory cues and permitted to dry between tests.

2.5.2. Psychomotor testing

Animals of the four treatments were examined with two different motor tests; plank walking and rod walking. All tests were conducted between 09:00 and 11:00 h for all treatment groups and only once on each animal.

2.5.2.1. Plank walking:

Balance and coordination were measured by exposing the rats to one trial on each of two horizontal planks (narrow = 1.5 cm and wide = 4.0 cm), each of 100 cm long and placed 34 cm high above a table-top. Distances traveled as well as number of turns on the planks were recorded (Shukitt-Hale et al., 1998).

2.5.2.2. Rod walking:

The ability of rats to balance on a stationary horizontal rod measures psychomotor coordination. Animals were placed in the center of a rod (100 cm long, 2.6 cm in diameter and positioned 23 cm high above a table-top), parallel to it, and their latency to fall off the rod onto a cushion placed below , with a time ceiling of 60 s, was recorded (Shukitt-Hale et al., 1998).

2.6. Statistical analysis

Statistical analysis was performed on all parameters by means of analyses of variance (ANOVA) to judge the influence of supplementation of Na-F and black tea to rats using the general linear models procedure in SPSS® statistical software (SPSS, 2006). After confirmation of significant effects in the overall ANOVA, data for different treatment groups was compared using post hoc Tukey HSD test. For all tests, significance was set at $p<0.05$. Data are presented as mean ± SEM.

3. Results

3.1. Body weight gain:

As seen in Table 1, oral administration of Na-F as compared with control group caused a their counterparts in control group. Nevertheless, the intensity of diminution in gains was significantly different between animals in Na-F and those in T group. Moreover, administration of black tea along with Na-F significantly improved Na-F-induced losses in weight gains. Comparable averages of gains were recorded in the ameliorated and tea groups.

3.2. Open field test:

There were no significant differences in horizontal activity (numbers of line crossings) between the animals treated and control group as shown in table 2.

3.3. Psychomotor testing:

Performance in psychmotor tests was demonstrated in Table 3. In the plank walking test, total distance traveled on the narrow plank as well as numbers of turns were significantly lower in Na-F-significant reduction in body weight gains ($F_{(1, 36)}$ = 18.56; p = 0.00). Likewise, rats administered black tea solution alone showed lowered weight gains than treated rats compared to control rats ($F_{(1, 36)}$ = 11.27; p = 0.00) and ($F_{(1, 36)}$ = 13.94; p = 0.00), respectively. For the wide plank walk, rats administered Na-F showed no improvement, where their measurements were still significantly lower compared to counterparts administered no Na-F ($F_{(1, 36)}$ = 8.31; p = 0.01) and ($F_{(1, 36)}$ = 46.25; p = 0.00), respectively. Administration of black tea solution to Na-F-treated rats significantly improved performance in the motor task to a similar level observed in the control and tea groups.

On the rod walk, less latencies to fall were significantly detected in Na-F group compared to all other treatments ($F_{(1, 36)}$ = 28.60; p = 0.00). Black tea significantly extended time required to fall when administered to Na-F-exposed rats, to the extent shown in the control and tea groups.

Table 1. Effect of Na-F and its amelioration by black tea on body weight gain (BWG) of rats.

	Experimental Groups			
	(C) Group	**(Na-F) Group**	**(T) Group**	**(Na-F+T) Group**
BWG (g)				
Day 32-115	**180±4.36[a]**	**91.6±3.82[b]**	**137.4±3.41[c]**	**134.2±3.09[c]**
Day 32-115 (change from control%)	**0**	**-49.11**	**-23.67**	**-25.44**

(C) Group: Animals received plain water without any treatment and served as a control.
(Na-F) Group: Animals received 100 ppm Na-F.
(Na-F+T) Group: Animals received 100 ppm Na-F + 2% black tea solution.
(T) Group: Animals received 2% black tea solution alone.
[a-c]Values within row with unlike superscripts differ significantly (p<0.05), according to ANOVA. Values represent mean±SEM of 10 animals per treatment.

Table 2. Effect of Na-F and its amelioration by black tea on locomotor activities in open field test in rats.

	Experimental Groups			
	(C) Group	**(Na-F) Group**	**(T) Group**	**(Na-F+T) Group**
Ambulation (Horizontal locomotion)	**50.3±3.93[a]**	**55.8±1.26[a]**	**46.9±4.44[a]**	**51.8±3.14[a]**

(C) Group: Animals received plain water without any treatment and served as a control.
(Na-F) Group: Animals received 100 ppm Na–F.
(Na-F+T) Group: Animals received 100 ppm Na-F + 2% black tea solution.
(T) Group: Animals received 2% black tea solution alone.
[a-c]Values within row with unlike superscripts differ significantly (p<0.05), according to ANOVA. Values represent mean±SEM of 10 animals per treatment.

Table 3. Effect of Na-F and its amelioration by black tea on performance in psychomotor tests in rats.

	Experimental Groups			
	(C) Group	(Na-F) Group	(T) Group	(Na-F+T) Group
1) Plank walking:				
a. Narrow plank				
Distance traveled (cm)	**64.00±3.79[a]**	**33.00±5.49[b]**	**67.50±8.93[a]**	**57.50±5.02[a]**
No. of turns	**2.40±0.27[a]**	**0.60±0.22[b]**	**2.60±0.50[a]**	**2.00±0.21[a]**
b. Wide plank				
Distance traveled (cm)	**102.50±17.67[a]**	**37.00±6.88[b]**	**125.00±19.99[a]**	**99.00±15.80[a]**
No. of turns	**3.50±0.17[a]**	**1.10±0.18[b]**	**3.60±0.22[a]**	**2.90±0.31[a]**
2) Rod walking:				
Latency to fall (s)	**40.40±2.74[a]**	**14.80±1.36[b]**	**45.80±4.34[a]**	**32.80±4.90[a]**

(C) Group: Animals received plain water without any treatment and served as a control.
(Na-F) Group: Animals received 100 ppm Na–F.
(Na-F+T) Group: Animals received 100 ppm Na-F + 2% black tea solution.
(T) Group: Animals received 2% black tea solution alone.
[a-c]Values within row with unlike superscripts differ significantly ($p<0.05$), according to ANOVA. Values represent mean±SEM of 10 animals per treatment.

4. Discussions

Unlike previous studies that showed no effects of fluoride on body weight (Chioca et al. 2008; Pereira et al., 2009), the current impairment in weight gains of rats affected by fluoride is consistent with our earlier findings (El-lethey et al., 2010). Correspondingly, numerous studies have reported a drop in body growth of Na-F-intoxicated rats and mice (Vani and Reddy, 2000; Ekambaram and Paul, 2001; Trabelsi et al., 2001; Wang et al., 2004; Pushpalatha et al., 2005; Basha et al., 2010; Madhusudhan et al., 2010). Although feed and water consumption was not recorded here, the earlier reduction in feed intake as a result of Na-F-induced atrophic gastritis and poor gastrointestinal absorption (Das et al., 1994), with decreased water intake (Ross and Daston, 1995), might justify the current retardation in weight gains. Furthermore, the dental lesions observed in former studies in Na-F-treated rats (Shupe et al., 1984; Ekambaram and Paul, 2003), where the incisors became white and chalk-like with broken tips might impair the ability of animals to masticate food prior to swallowing, and therefore contribute to a reduced feed intake with a consequent decrease in body weight gain. Suppressed appetite and disturbed nutrient digestibility that can eventually lead to excessive breakdown of cellular macromolecules might also cause weight loss (Madhusudhan et al., 2010).

In agreement with our results, several studies revealed a positive effect of tea on body weight reduction (Chantre and Lairon, 2002; Nagao et al., 2005; Chan et al., 2006). Investigations have proved the potential capacity of tea to inhibit lipogenic enzymes; gastric lipase and in a lower extent also the pancreatic lipase (Juhel et al., 2000). Moreover, tea extracts interfere with fat emulsification process, which occurs before enzymes act, and is indispensable for lipid intestinal absorption (Chantre and Lairon, 2002). The efficacy of tea extract to help with weight loss by speeding up fat oxidation process has been also evidenced (Dulloo et al., 1999). Other studies indicated the role of tea polyphenols to stimulate thermogenesis of brown fat; a thermogenic or heat-producing type of adipose tissue, resulting in increased energy expenditure (Han, et al., l999; Nagao, et al., 2005; St-Onge, 2005). This thermogenic property of tea could reside primarily in a synergistic interaction between polyphenols and caffeine (Dullo, et al., 1999; Dullo, et al., 2000). Caffeine and theanine have been reported to strengthen the polyphenols effects on body weight control and fat accumulation (Zheng et al., 2004). In addition, black tea contains theaflavins, which compare equally to green tea catechins as antioxidants, and was evidenced to have a unique lipid-lowering function via inhibiting a key enzyme in the pathway of cholesterol synthesis (Ishikawa et al., 1997; Leung et al., 2001). Lastly, administration of tea might support weight loss by relatively sustaining satiety and suppressing appetite (Reinbach et al., 2009).

Here, the Na-F-induced suppressive effect on animals' weight gains was significantly stronger than that resulted from black tea. Simultaneous administration of black tea with Na-F successfully alleviated the marked drop in weights observed with fluoride alone to the same extent noticed with tea group. This observation could be of value to signify the potential impact of tea to counteract Na-F-induced harmful effects, generating a state like that provoked by tea alone.

Since the behavior of both humans and animals is the product of what occurs in the nervous system, behavioral analysis is an essential assay of neural function (Whishaw et al., 1999). The present study included monitoring motor activities as an example for motivated behaviour; the most common predictor of CNS dysfunction (Mullenix and Kernan, 1989).

Open field activity monitoring provides a non-invasive method for an accurate and comprehensive assessment of the motor activities of rats. Therefore, it is an ideal method for assessing the degree of locomotor impairment as well as to evaluate the efficacy of elements affecting muscle function and locomotion (Raben et al., 1998; Nagaraju et al., 2000). The number of line crossing is usually used as a measure of locomotor activity, ataxia and other gait disturbances, with high frequencies of this behaviour indicating increased locomotion activities (Eisenhaver and Murphy, 1998). In this study and as a trial to dissociate between "general activity" and "exploration", ambulation was only related to horizontal locomotion (amount of distance traveled) than vertical activity which is more sensitive to anxiety state of the individual (Lapin et al., 1995; Brown et al., 1999).

Although some investigations revealed altered locomotor behaviour after treatment with Na-F in rats (Mullenix et al., 1995; Paul et al., 1998; Ekambaram and Paul, 2001; Niu et al., 2008), a failure of Na-F to impair locomotor activities in open field test was noted in the present study. These results were in line with animal data reported earlier (Bera et al., 2007; Chioca et al., 2008). The Na-F-induced inconsistent effects on motor activity across different studies could be imputed to a variety of factors, including the somewhat larger group sizes in the positive studies and its use of mixed sex groups (*vs.* only males) in the present research as well as the variation in Na-F dose, duration of supplementation and the behavioural motor testing implemented . However, this major disparity in the literature highlights the need for further investigation and urges to replicate observational studies to evaluate the reproducibility of the effect.

A considerable amount of caffeine is consumed daily among individuals who drink tea. Caffeine content is higher in fermented than non-fermented teas, showing values of 3.86% in black tea versus 2.04% in green one (Komes et al., 2009). Administration of caffeine has been evidenced to have favorable effects on locomotion in rats (Haleem, 1994). In addition, theophylline; another alkaloid present in tea, is also accountable for enhanced locomotor ability (Haider et al., 1998). In the current study, supplementation with black tea had no influence on rats' locomotor activity in the open field test. Similarly, Haider et al. (1998) reported no changes in open field activities in tea-treated rats as measured by numbers of crossed squares, however home cage activity was reported to be increased. Lack of consistency of the effect of tea on home cage and open field activities might be attributable to stress effect of novelty on exposure to open field to an extent that suppress locomotor enhancing effects of tea stimulants. Data reported in our previous article (Kamel et al., 2010), confirmed this justification, where tea-treated rats exhibited higher levels of anxiety upon exposure to open field test as revealed in enhanced rearing activity. Also, tolerance development to caffeine-induced locomotion promoting effect might be experienced in the current study, where partial tolerence has been reported to occur following administration of high doses of caffeine for about a week (Haleem, 1994).

Motor performance deficits include slowing of movement, decreases in balance and muscle strength as well as coordination difficulty (Joseph et al., 1983; Diggles-Buckles, 1993; Kluger et al., 1997; Seidler et al., 2002). Tasks requiring coordinated control of motor and reflexive responses, such as the length of time an animal can traverse/balance on a wooden rod or plank are among tests which attempt to assess motor incapacities (Dean et al., 1981; Joseph and Lippa, 1986; Ingram et al., 1994). Our study revealed a poor performance of fluoride-intoxicated rats in motor-coordination tests that rely on balance and coordination. The present results are comparable to those from other study with mice, where inability to perform in motor-coordination tests increased with higher fluoride concentration in drinking water (Bhatnagar et al., 2002). Previous study has shown a shortening of rotarod endurance time in Na-F-treated rats (Ekambaram and Paul, 2001). However, Paul et al. (1998) indicated no change in the motor-coordination of rats after treating with Na-F. Since a defect in motivated locomotor behavior may lead to suppression of eating, this behavioral impairment may in part account for the current depression in weight gains reported earlier as a consequence for decreased feed intake.

A large body of evidences might explain the motor deficits currently observed with Na-F. Decline in antioxidant defense mechanisms have been postulated as a causative factor in decrements of motor function (Shukitt-Hale, 1999). So, increased vulnerability to effects of oxidative stress with inability to cope is thought to be contributing factor to the motor deficits experienced here with fluoride exposure; a prooxidant element. Free radicals-induced oxidative stress causing damage to muscular tissue is thought to be involved in the process of fatigue; or the inability to generate power as well as muscle soreness (Dekkers et al., 1996).

Motor impairment can be also attributed to disruptions in neuronal functioning. The current lessening in motor performance was concomitant with marked neuronal dysfunction reported in the earlier complementary part of our study, as a result of increased levels of oxidative stress (Kamel et al., 2010). Such increase in free radicals in neuronal cell bodies could be correlated with loss of neurons in synaptic structures in neuromuscular junctions. The disturbed gait observed here in fluorotic rats confirms the dysfunction of neurotransmission caused by fluoride intake (Bhatnagar et al., 2006). Further proof derived from a fluoride study with rabbits, where the observed neurotoxic changes in brain suggested a direct action of fluoride upon the nerve tissue which was responsible for central nervous system problems such as tremors, seizures, and paralysis indicating brain dysfunction (Shashi, 2003).

In conditions with a high level of fluoride present, skeletal muscle necrosis might occur as a result of impairment of energy metabolism via destroyed stability of mitochondrial membrane with decreased activities of mitochondrial enzymes (Pang et al., 1996). Vani and Reddy (2000) also displayed an affection of both brain and muscles with fluorosis with inhibition of some enzymes associated with free radical metabolism, energy production and transport as well as synaptic transmission in mice.

Moreover, deficits in motor performance are thought to be the results of cerebellum disorders, where cerebellum is known to be crucial for functions related to movement, gait, posture, and balance (Ivry et al., 1988; Bickford et al., 1992; Bickford, 1993; Joyal et al., 1996; Konarski et al., 2005). As such, one of the most important signs of cerebellar damage is walking ataxia (Morton and Bastian, 2004). Our former study (Kamel et al., 2010), supported this notion displaying marked neurological alterations in cerebellum of Na-F-administered rats. Evidence continues to come demonstrating that fluoride may disrupt cerebellum, where ingested fluoride was retained by the cerebellum, of rats and mice, interfering with its physiology and inducing neurotoxicity, cell damage, and even cell death (Trabelsi et al., 2001; Shivarajashankara, 2002b; Trivedi et al., 2007; Bouaziz et al., 2010).

It is well established that mammalian spinal cord contains the neural circuitry required to generate a variety of rhythmic behaviors, including locomotion (Grillner, 1981). Hence, the indications of spinal cord involvement in fluorosis might also answer for the motor deficits observed here (Mrabet et al., 1995).

Locomotion, a cholinergically driven mechanism, is the most important acetylcholine-mediated behavior, where it involves by far the greatest number of cholinergic neurons (Day et al., 1991; Mitsushima et al., 1998; De Parada et al., 2002). The involvement of acetylcholine (ACh) in locomotion includes not only neuromuscular transmission, but also nerve-nerve transmission. It was shown that functional activity of cholinergic system; ACh-acetylcholinestrase (AChE) was lower in hypokinetic rats (Abzalov et al., 1997). Moreover, AChE deficit has been reported to leads to marked neuromuscular alterations in hind limb muscle functioning and a prominent symptom was the lack of resistance to fatigue (Mouisel et al., 2006). Since, a depletion of AChE activities in Na-F-treated rats was documented in our previous investigation (Kamel et al., 2010), this modulation of the central cholinergic mechanism probably accounts for inhibited motor activities seen in Na-F-exposed animals (Heiland and Greenfield, 1999).

Last of all, suppression of spontaneous motor activity suggests that fluoride has, by a central action, inhibited motivation of these animals to exhibit locomotor behavior (Paul et al., 1998).

Previous research showed that, although some flavonoids-rich diets were effective in reversing neuronal deficits, only a few enhanced motor performance (Joseph et al., 1999; Galli et al., 2002; Shukitt-Hale et al., 2005; 2006; 2008). Such findings point that it might be more difficult to reverse motor deficits than decline in cognitive function, where enhancement of motor behaviors may require recruitment of additional signaling pathway and may involve peripheral mediation (Shukitt-Hale et al., 2006). Although several studies have started to show a significant effect of tea in forestalling cognitive decline, to date, little is known about the effects of black tea on psychomotor function. Day-long consumption of black tea has been shown to improve aspects of psychomotor performance (Hindmarch et al., 2000). Also, prominent improvement in motor deficits was shown in rats receiving black tea extract (Chaturvedi et al., 2006). Compatible results derived from the current research which, as far as we can determine, is the first study to demonstrate a

profound restorative effect of black tea on Na-F-induced motor impairment in rats during psychomotor testing.

Research suggests that flavonoids may exert their beneficial effects either through their ability to lower oxidative stress and inflammation or directly by altering the signaling involved in neuronal communication, calcium buffering ability, neuroprotective stress shock proteins, plasticity, and stress signaling pathways. These interventions, in turn, may exert protection against motor dysfunction (Shukitt-Hale et al., 2008). Furthermore, the potential effects of tea consumption on the skeleton were reported where tea was associated with benefits on bone density (Hegarty et al., 2000; Wu et al., 2002; Chen et al., 2003; Devine et al., 2007). This beneficial influence was proposed to be mediated via a potent stimulatory effect of tea-derived flavonoids and lignans on osteoblast function (Cabrera et al., 2006; Whelan et al., 2006). It appears that the rejuvenating effects of tea on psychomotor performance was not entirely due to caffeine per se; where other intrinsic biologically active ingredients appear to be responsible for the beverage's inverse association with motor deficits. Black tea has been credited with capacity to modulate motor function via presence of theaflavins which have a potentiating effect on the contractile mechanism of mammalian skeletal muscle (Basu et al., 2005). The potential application of theaflavins for improving physical performance and recovery from high intensity exercise has been also evidenced (Arent et al., 2010).

In conclusion and as a trial to correlate the two complementary parts of our study, we find that fluoride intoxication-induced marked neurodegenerative changes in the brain of rats might form the neural basis for impaired motor function of the body. In view of the fact that humans incorporate fluoride into the skeleton about 18 times more readily than rats (Turner et al., 1992), understanding of the problem of fluoride conveying an increased risk for motor deficits, can further alert to be more vigilant and to consider preventive measures more seriously as the best approach to tackle this public menace. The use of black tea is a promising avenue that could help bring about substantial reduction of the fluoride-induced enormous healthcare costs in individuals.

References

1. Abzalov, R., Nigmatullina, R., Khuramshin, I. (1997): Acetylcholine content and tissue cholinesterase activity in rats with different motor activity. Bulletin Exp Biol Med, 124(6): 1177-1179.
2. Arent, S., Senso, M., Golem, D., McKeever, K. (2010): The effects of theaflavin-enriched black tea extract on muscle soreness, oxidative stress, inflammation, and endocrine responses to acute anaerobic interval training: a randomized, double-blind, crossover study. J Int Soc Sp Nutr, 7: 11-21.
3. Baldacara, L., Borgio, J., de Lacerda, A., Jackowski, A. (2008): Cerebellum and psychiatric disorders. Rev Bras Psiquiatr, 30(3): 281-289.
4. Basha, P., Rai, P., Begum, S. (2010): Evaluation of fluoride-induced oxidative stress in rat brain: a multigeneration study. Biol Trace Element Res. (Epub ahead of print).
5. Basu, S., Chaudhuri, T., Chauhan, S., Das Gupta, A., Chaudhury, L., Vedasiromoni, J. (2005): The theaflavin fraction is responsible for the facilitatory effect of black tea at the skeletal myoneural junction. Life Sci, 76: 3081-3088.
6. Bera, I., Sabatini, R., Auteri, P., Flace^, P., Sisto, G., Montagnani, M., Potenza, M., Marasciulo, F., Carratu, M., Coluccia, A., Borracci, P., Tarullo, A., Cagiano, R. (2007): Neurofunctional effects of developmental sodium fluoride exposure in rats. Eur Rev Med Pharmacol Sci, 11: 211-224.
7. Bhatnagar, M., Rao, P., Saxena, A., Bhatnagar, R., Meena, P., Barbar, S., Chouhan, A., Vimal, S. (2006): Biochemical changes in brain and other tissues of young adult female mice from fluoride in their drinking water. Fluoride, 39(4): 280-284.
8. Bhatnagar M., Rao, P., Sushma, J., Bhatnagar, R. (2002): Neurotoxicity of fluoride: neurodegeneration in hippocampus of female mice. Indian J Exp Biol, 40: 546-554.
9. Bickford, P. (1993): Motor learning deficits in aged rats are correlated with loss of cerebellar noradrenergic function. Brain Res, 620(1): 133-138.
10. Bickford, P., Heron, C., Young, D., Gerhardt, G., De La Garza, R. (1992): Impaired acquisition of novel locomotor tasks in aged and norepinephrine-depleted F344 rats. Neurobiol. Aging, 13: 475-481.
11. Bouaziz, H., Ben Ammar, I., Essefi, M., Croute, F., Zeghal, N. (2010): Fluoride-induced brain damages in suckling mice. Pesticide Biochem Physiol, 96(1): 24-29.
12. Brown, R., Corey, S., Moore, A. (1999): Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behav Gen 29(4): 263-271.
13. Cabrera, C., Artacho, R., Gimenez, R. (2006): Beneficial effects of green tea-a review. J Am Coll Nutr, 25: 79-99.
14. Chan, C., Koo, M., Ng, E. (2006): Modulation of Chinese green tea on weight and hormonal and biochemical profiles in obese patients with polycystic ovary syndrome-a randomized placebo-controlled trial. J Soc Gynecol Investig, 13: 63-68.
15. Chantre, P., Lairon, D. (2002): Recent findings of green tea extract AR25 (exolise) and its activity for the treatment of obesity. Phytomed, 9: 3-8.

16. Chaturvedi, R., Shukla, S., Seth, K., Chauhan, S., Sinha, C., Shukla, Agrawal, A. (2006): Neuroprotective and neurorescue effect of black tea extract in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Neurobiol Dis, 22(2): 421-434.
17. Chen, Z., Pettinger, M., Ritenbaugh, C., LaCroix, A., Robbins, J., Caan, B., Barad, D., Hakim, I. (2003): Habitual tea consumption and risk of osteoporosis: a prospective study in the women's health initiative observational cohort. Am J Epidemiol, 158: 772-781.
18. Chinoy, N., Memon, M. (2001): Beneficial effects of some vitamins and calcium on gastrocnemius muscle and liver of male mice. Fluoride**,** 34: 21-33.
19. Chinoy, N., and Patel, J. (2001): Effects of sodium fluoride and aluminium chloride on ovary and uterus of mice and their reversal by some antidotes. Fluoride, 34: 9–20.
20. Chioca, L., Raupp, I., Da Cunha, C., Losso, E., Andreatini, R. (2008): Subchronic fluoride intake induces impairment in habituation and active avoidance tasks in rats. Eur J Pharmacol, 579(1-3): 196-201.
21. Das, D., Mukherjee, S., Mukherjee, M., Das, A., and Mitra, C. (2005): Aqueous extract of black tea (*Camellia sinensis*) prevents chronic ethanol toxicity. Curr Sci, 88(6): 952-961.
22. Das, T., Susheela, A., Gupta, I., Dasrathy, S., Tandon, R. (1994): Toxic effects of chronic fluoride ingestion on the upper gastro-intestinal tract. J Clin Gastroenterol, 18: 194-199.
23. Day, J., Damsma, G., Fibiger, H. (1991): Cholinergic activity in the rat hippocampus, cortex and striatum correlates with locomotor activity: An in vivo microdialysis study. Pharmacol Biochem Behav, 38(4): 723-729.
24. De Parada M., Parada, M., Rada, P., Hernandez, L., Hoebel, B. (2002): Dopamine-acetylcholine interaction in the rat lateral hypothalamus in the control of locomotion. Pharmacol Biochem Behav, 66(2): 227-234.
25. Dean, R., Scozzafava, J., Goas, J., Regan, B., Beer, B., Bartus, R. (1981): Age-related differences in behavior across the life span of the C57BL/6J mouse. Exp. Aging Res, **7**: 427-451.
26. Dekkers, J., van Doornen, L., and Kemper, H. (1996): The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Med, 21: 213-238.
27. Devine, A., Hodgson, J., Dick, I., Prince, R. (2007): Tea drinking is associated with benefits on bone density in older women. Am J Clin Nutr, 86(4): 1243-1247.
28. Diggles-Buckles, V. Age-related slowing. In: Stelmach, G., Homberg, V. (eds.), Sensorimotor impairmrnt in the elderly. Kluwer Academic, Norwell, MA, 1993, pp. 73-87.
29. Du Toit, R., Volsteedt, Y., Apostolides, Z. (2001): Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. Toxicol, 166(1-2): 63-69.
30. Dulloo, A., Duret, C., Rohrer, D. (1999): Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24 hour energy expenditure and fat oxidation in humans. Am J Clin Nutr; 70(6): 1040-1045
31. Dulloo, A., Seydoux, J., Girardier, L. (2000): Green tea and thermogenesis: Interactions between catechin-polyphenols, caffeine and sympathetic activity. Int J Obes Relat Metab Disord, 24: 252-258.
32. Eisenhaver, L., Murphy, M. Drug therapy and Physical assessment. In: Pharmacotherapeutics and advanced Nursing practice. NY: Mc Graw Hill, 1998, pp. 1-2.
33. Ekambaram, P., Paul,V. (2001): Calcium preventing locomotor behavioral and dental toxicities of fluoride by decreasing serum fluoride level in rats. Environ Toxicol Pharmacol, 9: 141-146.
34. Ekambaram, P., Paul, V. (2003): Effect of vitamin D on chronic behavioural and dental toxicities of sodium fluoride in rats. Fluoride, 36(3): 189-197.
35. El-lethey, H., Kamel, M., Shaheed, I. (2010): Neurobehavioral toxicity produced by sodium fluoride in drinking water of laboratory rats. J Am Sci, 6(5): 54-63.
36. Fawell, J., Bailey K., Chilton, J., Dahi, E., Fewtrell, L., Magara, Y. (eds.), WHO, Fluoride in Drinking-Water by Publisher IWA publishing, London, UK, 2006.
37. Fisher, R., Medcalf, T., Henderson, M., (1989). Endemic fluorosis with spinal cord compression. A case report and review. Arch Intern Med, 149(3): 697-700.
38. Galli, R., Shukitt-Hale, B., Bielinski, D., Andres-Lacueva, C., Joseph, J. (2002): Dietary supplementation with fruit polyphenolics ameliorates age-related deficits in behavior and neuronal markers of inflammation and oxidative stress. Soc Neurosci, Abstr, 28: 294.
39. Grillner, S. Control of locomotion in bipeds, tetrapods, and fish. In: Hand book of Physiology. The Nervous System. Motor Control. Bethesda, MD:Am Physiol Soc, 1981, sect. 1, vol. II, pp. 1179-1236.
40. Haider, S., Yasmeen, A., Parveen, T., Haleem, D. (1998): Neurochemical and behavioural effects of long term intake of tea. Pak J Pharm Sci, 11(2): 55-60.
41. Haleem, D., Yasmeen, A., Parveen T., Zafar A. (1994): Enhancement of hepatic tryptophan pyrrolase activity and decrease of open field locomotion following single and repeated administration of high doses of caffeine in rats. Life Sci. 54: 297-304.

42. Han, L., Takaku, T., Li, J. (1999): Anti-obesity action of oolong tea. Int J Obes Relat Metab Disord, 23: 98-105.
43. Hardman, J., Limbird, L., Gilman, A. (eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th ed., Mcgraw Hill, New York, 2005, pp. 1735-1739.
44. Hegarty, V., May, H., Khaw, K. (2000): Tea drinking and bone mineral density in older women. Am J Clin Nutr, 71: 1003-1007.
45. Heiland, B., Greenfield, S. (1999): Rat Locomotion and Release of Acetylcholinesterase. Pharmacol Biochem Beh, 62(1): 81-87.
46. Hindmarch, I., Rigney, U., Stanley, N., Quinlan, P., Rycroft, J., Lane, J. (2000): "A naturalistic investigation of the effects of day-long consumption of tea, coffee and water on alertness, sleep onset and sleep quality". Psychopharmacol, 149(3): 203-216.
47. Ingram, D., Jucker, M., Spangler, E. Behavioral manifestations of aging. In: Mohr, U., Cungworth, D., Capen, C. (eds.), Pathobiology of the Aging Rat. ILSI Press, Washington, DC, 1994, vol. 2, pp. 149-170.
48. Ishikawa, T., Suzukawa, M., Ito, T., Yoshida, H., Ayaori, M., Nishiwaki, M., Yonemura, A., Hara, Y., Nakamura, H. (1997): Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. Am J of Clin Nutr, 66: 261-266.
49. Ivry, R., Keele , S., Diener, H. (1988): Dissociation of the lateral and medial cerebellum in movement timing and movement execution. Exp Brain Res, 73: 167-180.
50. Joseph, J., Bartus, R., Clody, D., Morgan, D., Finch, C., Beer, B., Sesack, S. (1983): Psychomotor performance in the senescent rodent: Reduction of deficits via striatal dopamine receptor up-regulation. Neurobiol Aging, 4: 313-319.
51. Joseph, J., Lippa, A. (1986): Reduction of motor behavioral deficits in senescent animals via chronic prolactin administration-II. Non-stereotypic behaviors. Neurobiol Aging, **7**: 37-40.
52. Joseph, J., Shukitt-Hale, B., Denisova, N., Bielinski, D., Martin, A., McEwen, J. (1999): Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with diets supplemented with blueberry, spinach or strawberry dietary supplementation. J Neurosci, 19: 8114 -8121.
53. Joyal, C., Meyer, C., Jacquart, G., Mahler, P., Caston, J., Lalonde, R. (1996): Effect of midline and lateral cerebellar lesions on motor coordination and spatial orientation. Brain Res., 739(1-2): 1-11.
54. Juhel, C., Armand, M., Pafumi, Y., Rosier, C., Vandermander, J., Larson, D. (2000): Green tea extract (AR25) inhibits lipolysis of triglycerides in gastric and duodenal medium *in vitro*. J Nutr Biochem 11: 45–51.
55. Kamel, M., El-lethey, H., Shaheed, I., Kamel, G. (2010): Black tea forestalls sodium fluoride-induced neurobehavioral toxicity in laboratory rats. J. Am. Sci., 6(12): 1655-1673.
56. Kaur T, Bijarnia R., Nehru B. (2009): Effect of concurrent chronic exposure of fluoride and aluminum on rat brain. Drug Chem Toxicol, 32(3): 215-221.
57. Kelly, A. (1993): Locomotor activity and exploration. In: Sahgal, A. (Ed.), Behavioural Neuroscience vol. II: a Practical Approach. Oxford University Press, Oxford, pp. 1-21.
58. Kluger, A., Gianutsos, J., Golomb, J., Ferris, S., George, A., Frannssen, E., Reisberg, B. (1997): Patterns of motor impairment in normal aging, mild cognitive decline, and early Alzheimer's disease. J Gerontol, 52: 28-39.
59. Komes, D., Horzic, D., Belscak, A., Kovacevic, G., Boljak, A. (2009): Determination of caffeine contents in tea and mate tea by using different methods. Czech J Food Sci, 27: 69.
60. Konarski, J., McIntyre, R., Grupp, L., Kennedy, S. (2005): Is the cerebellum relevant in the circuitry of neuropsychiatric disorders? J Psychiatry Neurosci, 30(3): 178-186.
61. Koros, C., Papalexi, E., Anastasopoulos, D., Kittas, C., Kitraki, E. (2007): Effects of AraC treatment on motor coordination and cerebellar cytoarchitecture in the adult rat. A possible protective role of NAC. Neurotoxicol., 28(1):83-92.
62. Lapin, I., Khaunina, R., Mirzaev, S. (1995): Vertical motor activity of mice is slowed by lower doses of psychotropic drugs than horizontal. Bull Eksp Biol Med, 120: 385-387.
63. Leung, L., Su, Y., Chen, R., Zhang, Z., Huang, Y., Chen, Z. (2001): Theaflavins in black tea and catechins in green tea are equally effective antioxidants. J. Nutr. 131: 2248-2251.
64. Leverett, D., Adair, S., Vaughan, B., Proskin, H., Moss, M. (1997): Randomized clinical trial of the effect of prenatal fluoride supplements in preventing dental caries. Caries Res, 31: 174-179.
65. Madhusudhan, N., Basha, P., Rai, P., Ahmed, F., Prasad, G. (2010): Effect of maternal fluoride exposure on developing CNS of rats: Protective role of Aloe vera, Curuma longa and Ocimum sanctum. Ind J Exp Biol, 48: 830-836.
66. Mitsushima, D., Yamanoi, C., Kimura, F. (1998): Restriction of environmental space attenuates locomotor activity and hippocampal acetylcholine release in male rats**.** Brain Res, 805(1-2): 207-212.
67. Morton, S., Bastian, A. (2004): Cerebellar Control of Balance and Locomotion. Neurosci, 10(3): 247-259.
68. Mouisel, E., Blondet, B., Escourrou, P., Chatonnet, A., Molgo´, J., Ferry, A. (2006):

Outcome of acetylcholinesterase deficiency for neuromuscular functioning. Neurosci Res, 55: 389-396.
69. Mousny, M., Banse, X., Wise, L., Everett, E., Hancock, R., Vieth, R., Devogelaer, J., Grynpas, M. (2006): The genetic influence on bone susceptibility to fluoride. Fluoride, 39(4): 331-335.
70. Mrabet, A., Fredj, M., Ammou, S., Tounsi, H., Haddad, A. (1995): Spinal cord compression in bone fluorosis: Apropos of 4 cases. Rev Med Int, 16: 533-535.
71. Mullenix, P., Denbesten, P., Schunior, A., Kernan, W. (1995): Neurotoxicity of sodium fluoride in rats. Neurotoxicol Teratol, 17(2): 169-177.
72. Mullenix, P., Kernan, W. (1989): Extension of the analysis of the time structure of behavioral acts. Intern J Neuroscience, 44(3-4): 251-262.
73. Nagao, T., Komine, Y., Soga, S., Meguro, S., Hase, T., Tanaka, Y., Tokimitsu, I. (2005): Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. Am J Clin Nutr, 81: 122-129.
74. Nagaraju, K., Raben, N., Loeffler, L., Parker, T., Rochon, P. (2000): Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. Proc Nat Acad Sci USA, 97: 9209-9214.
75. Niu, R., Sun, Z., Wang, J., Cheng, Z., Wang, J. (2008): Effects of fluoride and lead on locomotor behaviour and expression of Nissl body in brain of adult rats. Fluoride 41: 276-282.
76. Pang,Y., Guo, Y., Zhu, P. Fu, K., Sunb, Y., Tangb, R. (1996): The effects of fluoride, alone and in combination with selenium, on the morphology and histochemistry of skeletal muscle. Fluoride, 29(2): 59-62.
77. Paul V., Ekambaram, P., Jayakumar, A. (1998). Effects of sodium fluoride on locomotor behavior and a few biochemical parameters in rats. Environmental Toxicology and Pharmacology 6: 187–191.
78. Pereira, M., Dombrowski, P., Losso, E., Chioca, L., Da Cunha, C., Andreatini, R. (2009): Memory impairment induced by sodium fluoride is associated with changes in brain monoamine levels. Neurotox Res, (Epub).
79. Pushpalatha, T., Srinivas, M., Reddy, P. (2005): Exposure to high fluoride concentration in drinking water will affect spermatogenesis and steroidogenesis in male albino rats. Biometals, 18: 207-212.
80. Raben, N., Nagaraju, K., Lee, E., Kessler, P., Byrne, B. (1998): Targeted disruption of the acid alpha-glucosidase gene in mice causes an illness with critical features of both infantile and adult human glycogen storage disease type II. J Biol Chem, 273: 19086-19092.
81. Reddy, D. (2009): Neurology of endemic skeletal fluorosis. Neurol India, 57(1): 7-12.
82. Reinbach, H., Smeets, A., Martinussen, T., Møller, P., Westerterp-Plantenga, M. (2009): Effects of capsaicin, green tea and CH-19 sweet pepper on appetite and energy intake in humans in negative and positive energy balance. Clin Nutr., 28: 260-265.
83. Ross, J., Daston, G. (1995): Neurotoxicity of sodium fluoride in rats. Neurotoxicol Teratol, 17: 685-688.
84. Saad El-Dien, H., El Gamal, D., Mubarak, H., Saleh, S. (2010): Effect of fluoride on rat cerebellar cortex: Light and electron microscopic studies. Egypt. J. Histol., 33(2): 245-256.
85. Seidler, R., Alberts, J., Stelmach, G. (2002): Changes in multi-joint performance with age. Motor control, 6(1): 19-31.
86. Sharma A., Chinoy, N. (1998): Role of free radicals in fluoride-induced toxicity in liver and kidney of mice and its reversal. Fluoride, 31: S26.
87. Shashi A. (2003): Histopathological investigation of fluoride-induced neurotoxicity in rabbits. Fluoride, 36: 95-105.
88. *Shashi, A., Kumar, M., Bhardwaj, M. (2008): Incidence of skeletal deformities in endemic fluorosis. Tropical Doctor, 38(4): 231-233.*
89. Shashi, A., Singh, J., Thapar, S. (1992): Protein degradation in skeletal muscle of rabbit during experimental fluorosis. Fluoride, 25(3): 155-158.
90. Shashi, A., Singh, J., Thapar S. (1994): Effect of long-term administration of fluoride on levels of proteins, free amino acids and RNA in rabbit brain. Fluoride, 27: 155-159.
91. Shivarajashankara, Y., Shivashankara, A., Rao, S., Bhatc, P. (2001): Oxidative stress in children with endemic skeletal fluorosis. Fluoride, 34(2): 103-107.
92. Shivarajashankara, Y., Shivashankara, A., Bhatc, P., Rao, S., Raod, S. (2002a): Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. Fluoride, 35(3): 197-203.
93. Shivarajashankara,Y., Shivashankara, A., Bhat, P., Rao, S., Raod, S. (2002b): Histological changes in the brain of young fluoride-intoxicated rats. Fluoride, 35(1): 12-21.
94. Shukitt-Hale, B. (1999): The effects of aging and oxidative stress on psychomotor and cognitive behavior. Age, 22(1): 9-17.
95. Shukitt-Hale, B., Carey, A., Simon, L., Mark, D., Joseph, J. (2006): Effects of Concord grape juice on cognitive and motor deficits in aging. Nutr, 22: 295-302.
96. Shukitt-Hale, B., Galli, R., Meterko, V., Carey, A., Bielinski, D., McGuie, T. (2005): Dietary supplementation with fruit polyphenolics ameliorates age-related deficits in behavior and

neuronal markers of inflammation and oxidative stress. Age, 27:49-57.
97. Shukitt-Hale, B., Lau, F., Joseph, J. (2008): Berry fruit supplementation and the aging brain. J Agric Food Chem, 56(3): 636-641.
98. Shukitt-Hale, B., Mouzakis, G., Joseph, J. (1998): Psychomotor and spatial memory performance in aging male Fischer 344 rats. Exp Gerontol, 33: 615-624.
99. Shupe, J., Olson, A., Peterson, H., Low, J. (1984): Fluoride toxicosis in wild ungulates. J Am Vet Assoc, 185: 1295-1300.
100. SPSS (2006): SPSS for Windows 14.0.
101. St-Onge, M. (2005): Dietary fats, teas, dairy, and nuts: potential functional foods for weight control? Am J Clin Nutr, 81: 7-15.
102. Susheela A. (1999): Fluorosis management programme in India. Curr Sci, 77 (10): 1050-1056.
103. Susheela, A., Bhatnagar M. (2002): Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. Mol Cell Biochem, 234-235(1): 335-340.
104. Thach, W., Bastian, A. (2004): Role of the cerebellum in the control and adaptation of gait in health and disease. Prog Brain Res, 143: 353-366.
105. Trabelsi, M., Guermazi, F., Zeghal, N. (2001): Effect of fluoride on thyroid function and cerebellar development in mice. Fluoride, 34(3): 165-173.
106. Trivedi, M., Verma, R., Chinoy, N. (2006): Amelioration by black tea of changes induced by sodium fluoride in protein content of liver and kidney in mice. Fluoride 39(4): 269-273.
107. Trivedi, M., Verma, R., Chinoy, N. (2007): Amelioration by black tea of sodium fluoride-induced changes in protein content of cerebral hemisphere, cerebellum and medulla oblongata in brain region of mice. Acta Pol Pharm, 64(3): 221-225.
108. Trivedi, M., Verma, R., Chinoy, N. (2008): Amelioration by black tea of sodium fluoride-induced effects on DNA, RNA and protein contents of liver and kidney and on serum transaminases activities in Swiss albino mice, Fluoride, 41(1): 61-66.
109. Turner, C., Akhten, M., Heaney, R. (1992): The effects of fluoridated water on bone strength. J Orthop Res, 10: 581-587.
110. Unno, K., Hoshino, M. (2007): Brain senescence and neuroprotective dietary components. Cent Nerv Sys Ag Med Chem**, 7**: 109-114.
111. Vani, M., Reddy, K. (2000): Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Fluoride, 33(1): 17-26.
112. Wang, J., Ge, Y., Ning, H., Wang, S. (2004): Effects of high fluoride and low iodine on biochemical indexes of the brain and learning memory of offspring rats. Fluoride, 37: 201-208.
113. Whelan, A., Jurgens, T., Bowles, S. (2006): Natural health products in the prevention and treatment of osteoporosis: systematic review of randomized controlled trials. Ann Pharmacother, 40: 836-849.
114. Whishaw, I., Haun, F., Kolb, B. Analysis of Behavior in Laboratory Rodents. In: Windhorst, U., Johansson, H. (eds.), Modern techniques in neuroscience research (with CD-ROM for Windows and Macintosh, Springer Lab. Manuals). Secaucus, New Jersey, USA: Springer Verlag; 1999. Chapter 44: 1243-1276, pp. 1243-1244.
115. Wu, C., Yang, Y., Yao, W., Lu, F., Wu, J., Chang, C. (2002): Epidemiological evidence of increased bone mineral density in habitual tea drinkers. Arch Intern Med, 162: 1001-1006.
116. Zheng, G., Sayama, K., Okubo, T., Junefa, L., Oguni, I. (2004): Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine in mice. In vivo, 18: 55-62.

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