

Potential Health Impact of Black Tea against Na-F-Induced Alterations in Territorial Aggression, Sexual Behaviour and Fertility of Male Rats

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Abstract: In an extension of previous work on sodium fluoride (Na-F) toxicity, the ameliorative effect of black tea on Na-F-induced behavioural and reproductive toxicity was evaluated in male rats in terms of territorial aggressive behaviour, sex behaviour, along with fertility indices. Oral administration of 100 ppm Na-F and 2% black tea to eighty weanling 32-days old male Wistar rats, randomly allotted into 4 groups of 20, were performed daily for 14-weeks treatment period in a 2 x 2 factorial manner. A marked suppression in all parameters of territorial aggression was seen in adult male Na-F-treated rats. This suppression was significantly alleviated when black tea was concurrently administered. Ingestion of black tea alone significantly improved territorial aggression responses, namely lateralization and boxing bouts. The ingested Na-F also suppress sexual behaviour in adult male rats expressed by a prolongation of first mount, intromission and ejaculation latencies, decrease in numbers of mounts, intromissions and ejaculations along with increased post-ejaculatory intervals. A profound ameliorative effect was noted for all abolished male sex behaviour when black tea solution was supplemented to Na-exposed rats. Black tea exhibited an aphrodisiac tendency when solitary administered to male rats, as reflected in significant shortening of mount and intromission latencies as well as increase in mount and intromission frequencies. This aphrodisiac activity was not associated with influence on ejaculation-related parameters. Furthermore, an obvious impairment in all fertility indices was detected in Na-F-treated males as displayed by reduced numbers of impregnations, implantations and viable fetuses accompanied by increased resorptions. This observed diminution in fertility was significantly mitigated by black tea. Similarly, the lessening effect of Na-F on relative weights of male sex organs was noticeably improved when black tea was given. Our histopathological investigations revealed severe degenerative changes in testes, seminal vesicles and prostate gland. Combined administration of black tea with Na-F resulted in marked amelioration of the pathological alterations. Our study denotes a powerful mitigative effect of black tea in combating behavioural and reproductive toxicity triggered by Na-F as signified by harmed aggressive and sexual behaviour together with abolished fertility in adult male rats. Our findings also evidently point toward the aphrodisiac property of black tea which might be of help in certain forms of sexual dysfunction in male individuals. [Heba S. El-Iethy¹, Mervat M. Kamel^{1*} and Iman B. Shaheed² Potential Health Impact of Black Tea against Na-F-Induced Alterations in Territorial Aggression, Sexual Behaviour and Fertility of Male Rats. Life Science Journal. 2011;8(2):828-839] (ISSN: 1097-8135). <http://www.lifesciencesite.com>.

Key words: Sodium fluoride, black tea, territorial aggression, sex behaviour, fertility, Wistar rats.

1. Introduction

Major sources of individuals' exposure to fluorides are the diet (food, water, beverages) and fluoridated dentifrices (toothpastes and other preparations for cleaning teeth). Exposure to fluoride has been reported to interfere with the functional status of several tissues and organs, causing toxic hazards, namely of reproductive effects (Al-Hiyasat et al., 2000; Dhar and Bhatnagar, 2009). Epidemiological studies have shown that there is an association of decreasing total fertility rate with increasing fluoride levels in drinking water (Freni, 1994). Additionally, previous hazard identification studies in male rodents have evidenced reproductive toxicity of sodium fluoride in concentrations higher than the permissible level (Narayana and Chinoy, 1994; Elbetieha et al., 2000; Chinoy and Sharma, 2000). However, so far these studies have focused mainly on structural and functional defects in

spermatozoa (Kumar and Susheela, 1994; Chinoy and Sharma, 1998), a decrease in sperm count (Ghosh et al., 2002; Pushpalatha et al., 2005), disturbances in the levels of reproductive hormones (Ortiz-Perez et al., 2003), alterations in the epididymis and accessory reproductive glands (Chinoy and Sequeira, 1989; Kumar and Susheela, 1994; Tiwari and Pande, 2009) and interference with fertility (Chinoy and Sequeira, 1992; Elbetieha et al., 2000). In contrast to reproductive toxicity of fluoride, less is known about its effect on sexual behaviour, an androgen dependent behaviour. It is noteworthy that suppression of endogenous testosterone secretion has been reported following fluoride treatments (Huang et al., 2007; Reddy et al., 2007). Thus, the possibility cannot be excluded that fluoride might eliminate the expression of male sex behaviour. Also, androgens have long been recognized as modulators of aggression in male rats (Blanco et al., 1997). Many studies of androgens

effects on aggression have focused on inter-male aggression, a pattern of aggressive behavior that is dependent on presence of androgens (Christie and Barfield, 1979). Inter-male aggression can be measured by assessing the quality and quantity of aggressive acts displayed by a 'resident' male towards a strange or 'intruder' male.

Fluorosis, being an untreatable disease, can only be mitigated through prevention and control. Nutritional supplementation has to be practiced for combating with the health complaints arising due to fluorosis. Natural antioxidants with free radical-scavenging activity such as tea flavonoids have received much attention as potential, non-toxic treatments for oxidative stress-related pathological conditions (Serafini et al., 1996; Leung et al., 2001; Satoh et al., 2005; Trivedi et al., 2006; Ojo et al., 2007). Although the sexual stimulant activities of black tea have been advocated, animal studies addressing the effects of black tea exposure on sexual competence, are very limited in number and further research are essential in order to scientifically test and validate this conjecture (Ratnasooriya and Fernando, 2008).

So far, no studies appear to have tested the possible effect of black tea on reproductive dysfunction due to fluoride exposure. Therefore the present work was the first attempt to evaluate the potential mitigative effect of black tea against sodium fluoride-induced behavioural and reproductive toxicity represented by modulations of territorial aggressive, and sex behaviours together with fertility profile in adult male rats. Reproductive histopathological investigations were also designed to represent the possible effects on sex organs in males.

2. Materials and Methods:

2.1. Animals and housing:

Animal care as well as the experimental protocols was in compliance with guidelines of ethical standards released by Cairo University Policy on Animal Care and Use. In order to minimize animals' suffering we intended only to use the adequate minimal number of animals requested to produce reliable scientific data.

The study was performed on a total of eighty weanling 32-days-old male Wistar rats, weighing approximately 45g. Animals were procured from the Unit for Laboratory Animals at Faculty of Veterinary Medicine, Cairo University and employed in our study. They were housed in standard polypropylene cages with stainless steel wire lids, bedded with wood shavings at a temperature (20-22°C), humidity (60%) and photoperiod (12-h light/dark cycle). Standard laboratory feed and distilled water were freely available except during the time of the experiments.

2.2. Experimental design:

All males were randomly assigned into four groups of 20, divided on 2 replicates and orally administered our treatments for a 14-weeks period, 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. Behavioural testing procedures:

At 130 days of rats' age, territorial aggression was studied first, then the sexual behaviour and finally the fertility indices in all experimental groups. All behavioural measurements were monitored by a single observer unaware of the experimental treatment.

2.3.1. Resident-intruder test of territorial aggression:

A rectangular observation cage (45 x 27 x 40 cm: length x breadth x height) was used for evaluation of aggressive behaviour in rats. A stud male rat was placed in the testing arena for 10 days and served as the resident. The tested male rat (intruder) of no previous contact with the resident was then placed into the test arena, confronted with the resident male for 5-min test period. The following aggression parameters were then recorded: lateralization by stud male (LSM), boxing bouts with stud male (BBSM), fights with stud male (FSM), ventral presenting posture (supine posture) of the stud male (VP) (Hilakivi and Lister, 1989; Bataineh et al., 1997; Bataineh et al., 1998; Khouri and El-Akawi, 2005). All testing was carried out between 09:00 and 12:00 h. The four experimental groups were tested in a random array.

2.3.2. Sexual behaviour test:

Sexual behaviour of male rats was assessed using a stimulus-receptive untreated female of the same strain. Female receptivity was induced by the sequential subcutaneous administration of 5 mg

estradiol benzoate and 0.5 mg progesterone (Misr Co. for Pharm. Ind., Cairo, Egypt), dissolved in 0.2 ml of sesame oil, at 54 and 6 h before the sexual behaviour study, respectively. Single male rat was placed alone in the mating cage (45 x 27 x 40 cm: length x breadth x height), and allowed to acclimate for 5 min. Then the sexually receptive female rat was introduced into the center of the arena. Sexual behaviour of the male was monitored during a 15-min session and the following parameters were registered;

(1) mount latency (ML): time from the introduction of the female until the first mount, (2) intromission latency (IL): time from introduction of the female until the first intromission, (3) ejaculation latency (EjL): time from the first intromission until ejaculation, (4) total mount frequency (TMF): total number of mounts during test session, (5) total intromission frequency (TIF): total number of intromissions during test session, (6) ejaculation frequency (EjF) i.e. mating potential: total number of ejaculations during test session, (7) post-ejaculatory interval (PEJI): time from ejaculation until the next intromission (Ågmo, 1997; Cagiano et al., 1998; Khouri and El-Akawi, 2005; Bataineh and Nusier, 2006). Also, the following parameter was calculated on the basis of the above data: (8) intromission ratio (IR): intromission frequency/(mount frequency + intromission frequency), (9) copulatory efficacy (CE): intromission frequency/mount frequency). All measurements were conducted between 09:00 and 15:00 h in a randomized order.

2.4. Fertility assessment:

Male rats' fertility was evaluated by natural mating. Each male was individually housed with two virgin untreated females of the same strain for ten days to ensure two successive estrus cycles (Amann, 1982). One week after removal of the males, all females were killed by cervical dislocation under light ether anesthesia. Numbers of impregnated females, implantation sites, viable fetuses as well as fetal resorption sites were recorded after cesarean sections (Bataineh et al., 1998).

2.5. Relative weights of male reproductive organs:

The final body weights of five male rats per treatment were recorded. Rats were then sacrificed by cervical dislocation under light ether anesthesia. Their sex organs; testes, seminal vesicle and prostate gland were dissected out, freed from adherent tissues and blood, and weight to the nearest milligram in relation to body weight.

2.6. Histopathological examination:

After completion of all assessments, tissue specimens from testes, seminal vesicles and prostate

glands were assembled and fixed in 10% neutral buffer formalin. The tissue specimens were processed by the convention method and stain with Hematoxylin and Eosin (Bancroft and Gamble, 2008).

2.7. Statistical analysis:

Statistical tests were performed using the general linear models procedure in SPSS[®] statistical software (SPSS, 2006). Data for behaviour, fertility as well as sex organs weights were analyzed using two-way ANOVA. Post hoc comparisons between the groups after ANOVA were made using post hoc Tukey HSD test. Differences at the probability level *P*, 0.05 were considered significant. The results were expressed as mean ± SEM.

3. Results:

3.1. Territorial aggressive behaviour parameters:

Measurements of territorial aggression in adult male rats were demonstrated in Table 1. A significant profound lessening effect was noted for all parameters including; lateralization, boxing bouts, fighting and number of ventral presenting postures (*p* < 0.001) in Na-F-exposed rats when compared with controls. Simultaneous administration of black tea to Na-F-treated rats resulted in a significant recovery in all territorial aggressive parameters, to the level of control group (*p* = 0.10, 0.14, 0.79, and 0.15), respectively. As compared with respective control group, administration of black tea alone significantly advanced lateralization and boxing bouts (*p* < 0.05). Although there was a tendency to increase fighting as well as ventral presenting postures in tea-administered group, this result did not attain a recognized significant level, when compared to control.

3.2. Sexual behaviour:

Parameters related to male rats sexual behaviour were demonstrated in Table 2. Treatment with Na-F induced significant increase (*p* < 0.001) in mount, intromission and ejaculation latencies in comparison with corresponding values of control rats. In addition, there was a significant diminution (*p* < 0.001) in frequencies of all previously declared parameters. A significant prolongation (*p* < 0.001) in post-ejaculatory intervals was also detected in Na-F-exposed rats, compared with the controls. Administration of black tea along with Na-F significantly (*p* < 0.001) ameliorated all Na-F-induced changes in sex behaviour of males. The amelioration was similar to the level observed in the control group (*p* = 0.08, 0.13, 0.19, 0.15, 0.30, 0.30 and 0.11) for all recorded parameters, respectively. Supplementation with black tea solution alone revealed significant suppression (*p* < 0.001) in mount

and intromission latencies, whereas the ejaculation latencies were not significantly varied ($p = 0.13$) from those in control group. Similarly, black tea-tingested rats displayed significantly higher ($p < 0.001$) frequencies of mounts and intromissions, with no significant influence on parameters of ejaculation frequencies as well as post-ejaculatory intervals ($p = 0.60, 0.28$), respectively.

3.3. Male rats' fertility:

As seen in Table 3, oral administration of Na-F, as compared with control group, caused significant adverse effects on all fertility indices in male rats in terms of diminished numbers of impregnations ($p < 0.001$), implantation sites ($p < 0.001$) as well as viable fetuses ($p < 0.01$) along with increased numbers of resorption sites ($p < 0.01$). The prominence of black tea was only restricted to its ameliorative effect, where concurrent administration of black tea significantly alleviated Na-F-provoked negative consequences on the entire profile of fertility in males. Black tea ameliorative effect was significantly comparable to control group for all stated parameters ($p = 0.33, 0.55, 0.85$ and 0.97), respectively.

3.4. Reproductive organs weights:

Relative weights of male reproductive organs; testes, seminal vesicles and prostate gland were illustrated in Table 4. Exposure of rats to Na-F significantly reduced ($p < 0.001$) relative weights of all collected reproductive organs, when compared to their counterparts in control group. Significant effect noticed for black tea was only ameliorative when tea was simultaneously ingested with Na-F, where comparable weights were shown in both ameliorated and control groups for testes, seminal vesicles and prostate ($p = 0.89, 0.77, 0.82$), respectively.

3.5. Histopathological examination:

No pathological changes could be detected in the testes, seminal vesicle and prostate glands of rats in control group as well as their counterparts received black tea alone.

The testes of Na-F-treated rats showed severe pathological lesions represented by severe disorganization and denudation of germinal epithelial cells of most seminiferous tubules with absence of sperm in the lumina (Fig. 1). Only the basement membranes were detected with multiple numbers of spermatid giant cells. Congestion of blood vessels in tunica albuginea and edematous fluid were detected in-between the interstitial tissues (Fig. 2). Some tubules were completely destructed.

The seminal vesicle revealed hyperplasia of the epithelial lining with desquamated epithelial cells in the lumen mixed with its secretion (Fig. 3). There was edema in the lamina propria and congestion of submucosal blood vessels.

The prostate gland exhibited edema in the interstitial tissues dispersed the glands. There was severe hyperplasia of epithelium lining as folds in the lumen (Fig. 4). Few numbers of inflammatory cells were detected in the interstitial tissues.

Regarding the rats exposed to Na-F along with black tea, their testes displayed mild pathological changes represented by decrease the numbers of mature sperms in the lumen of some seminiferous tubules (Fig 5). Most of tubules were appeared with normal germinal epithelium and large numbers of matures sperms in their Lumina (Fig. 6). No pathological alterations could be detected in seminal vesicles of rats of the ameliorated group (Fig. 7), while prostate gland showed mild hyperplastic changes with edema in interstitial tissues (Fig. 8).

Table 1. Effect of Na-F and its amelioration by black tea on territorial aggression in adult male rats during a 5 min session.

	Experimental Groups			
	(C) Group	(Na-F) Group	(T) Group	(Na-F+T) Group
LSM	6.10±0.74 ^a	1.60±0.51 ^b	8.20±0.53 ^c	4.30±0.42 ^a
BBSM	4.90±0.31 ^a	1.00±0.32 ^b	6.80±0.61 ^c	3.50±0.50 ^a
FSM	2.60±0.43 ^{ac}	0.60±0.19 ^b	3.70±0.30 ^c	2.10±0.46 ^a
VP	1.50±0.22 ^{ac}	0.10±0.03 ^b	1.80±0.20 ^c	0.90±0.23 ^a

(C) Group: Animals received plain water without any treatment and served as a control.

(Na-F) Group: Animals received 100 ppm Na-F. (T) Group: Animals received 2% black tea solution alone.

(Na-F+T) Group: Animals received 100 ppm Na-F + 2% black tea solution.

^{a-c}Values within row with unlike superscripts differ significantly ($p < 0.05$), according to ANOVA. Data are expressed as mean±SEM of 10 animals per treatment.

(LSM = lateralization by stud male, BBSM = boxing bouts with stud male, FSM = fights with stud male, VP = ventral presenting posture (supine posture) of the stud male.

Table 2. Effect of Na-F and its amelioration by black tea on sexual behaviour in adult male rats during a 15 min session.

	Experimental Groups			
	(C) Group	(Na-F) Group	(T) Group	(Na-F+T) Group
ML (s)	109.2±15.64 ^a	248.3±12.07 ^b	65.00±4.21 ^c	149.2±10.18 ^a
IL (s)	118.80±12.49 ^a	263.5±10.94 ^b	69.70±3.91 ^c	146.7±4.03 ^a
EjL (s)	143.10±9.02 ^{ac}	289.00±17.40 ^b	102.50±10.32 ^c	179.9±12.67 ^a
TMF	13.00±0.30 ^a	4.1±0.81 ^b	15.90±0.38 ^c	11.20±0.36 ^a
TIF	12.30±0.26 ^a	2.80±74 ^b	15.10±0.35 ^c	10.40±0.34 ^a
EjF	4.90±0.46 ^{ac}	0.80±0.25 ^b	5.50±0.34 ^c	4.00±0.26 ^a
PEjI	73.10±2.95 ^{ac}	245.6±15.91 ^b	50.70±3.60 ^c	101.6±4.98 ^a
IR	0.49±0.01	0.34±0.06	0.49±0.01	0.48±0.01
CE	0.95±0.03	0.62±0.12	0.95±0.02	0.93±0.02

(C) Group: Animals received plain water without any treatment and served as a control.

(Na-F) Group: Animals received 100 ppm Na-F.

(T) Group: Animals received 2% black tea solution alone.

(Na-F+T) Group: Animals received 100 ppm Na-F + 2% black tea solution.

^{a-c}Values within row with unlike superscripts differ significantly ($p < 0.05$), according to ANOVA. Data are expressed as mean±SEM of 10 animals per treatment.

(ML = mount latency, IL = intromission latency, EjL = ejaculation latency, TMF = total mount frequency, TIF = total intromission frequency, EjF = ejaculation frequency (mating potential), PEjI = post-ejaculatory interval (latency period), IR (intromission ratio) = no. of intromissions/(no. of intromissions + no. of mounts), CE (copulatory efficiency) = no. of intromissions/no. of mounts).

Table 3. Effect of Na-F and its amelioration by black tea on fertility in adult male rats.

	Experimental Groups			
	(C) Group	(Na-F) Group	(T) Group	(Na-F+T) Group
No. of males	10	10	10	10
No. of females	20	20	20	20
No. of pregnant females	18/20 ^a (90%)	7/20 ^b (35%)	20/20 ^a (100%)	14/20 ^a (70%)
No. of implantation sites	7.85±0.70 ^a	3.00±1.02 ^b	8.35±0.69 ^a	6.25±0.97 ^a
No. of viable fetuses	6.10±0.57 ^a	2.45±0.85 ^b	7.45±0.48 ^a	5.30±0.82 ^a
Rats with resorptions	1/20 (5%)	7/20 (35%)	1/20 (5%)	3/20 (15%)
No. of resorption sites/total no. of implantation sites	1/157 ^a (0.64%)	15/60 ^b (25%)	1/167 ^a (0.60%)	3/125 ^a (2.4%)

(C) Group: Animals received plain water without any treatment and served as a control.

(Na-F) Group: Animals received 100 ppm Na-F.

(T) Group: Animals received 2% black tea solution alone.

(Na-F+T) Group: Animals received 100 ppm Na-F + 2% black tea solution.

^{a-c}Values within row with unlike superscripts differ significantly ($p < 0.05$), according to ANOVA. Data are expressed as mean±SEM.

Table 4. Effect of Na-F and its amelioration by black tea on reproductive organs weights (g/100g b.wt) in adult male rats.

	Experimental Groups			
	(C) Group	(Na-F) Group	(T) Group	(Na-F+T) Group
Testes	1.47±0.05 ^{ac}	0.91±0.06 ^b	1.52±0.04 ^c	1.29±0.04 ^a
Seminal vesicles	0.69±0.05 ^{ac}	0.41±0.03 ^b	0.73±0.02 ^c	0.58±0.02 ^a

Prostate gland	0.34±0.02 ^{ac}	0.20±0.01 ^b	0.37±0.01 ^c	0.28±0.02 ^a
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(C) Group: Animals received plain water without any treatment and served as a control.

(Na-F) Group: Animals received 100 ppm Na-F.

(T) Group: Animals received 2% black tea solution alone.

(Na-F+T) Group: Animals received 100 ppm Na-F + 2% black tea solution.

^{a-c}Values within row with unlike superscripts differ significantly ($p<0.05$), according to ANOVA. Data are expressed as mean±SEM of 5 animals per treatment.

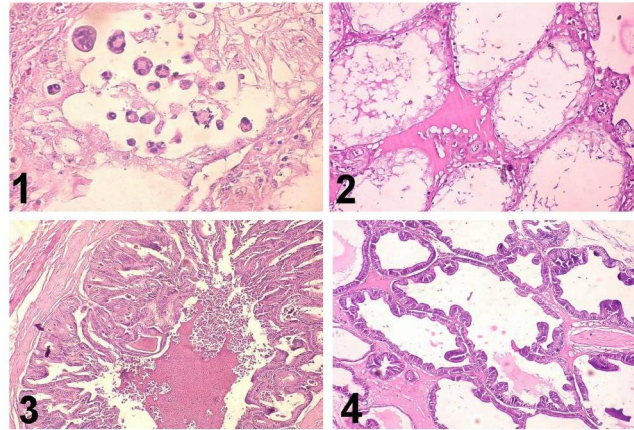


Figure 1: Testes of Na-F-treated rats, showing complete necrosis of germinal epithelium of seminiferous tubules with multiple numbers of spermatid giant cells. H&E X 400.

Figure 2: Testes of Na-F-treated rats, showing edema in-between the seminiferous tubules. Notice the complete absence of germinal epithelium and Sertoli cells of seminiferous tubules. H&E X 200.

Figure 3: Seminal vesicles of Na-F-treated rats, showing hyperplasia of the epithelial lining with desquamated epithelial cells in the lumen mixed with its secretion. H&E X 200.

Figure 4: Prostate gland of rat Na-F-treated rats, showing severe hyperplasia of epithelium lining forming finger like projection in the lumen. Notice the edema in-between the glands. H&E X 200.

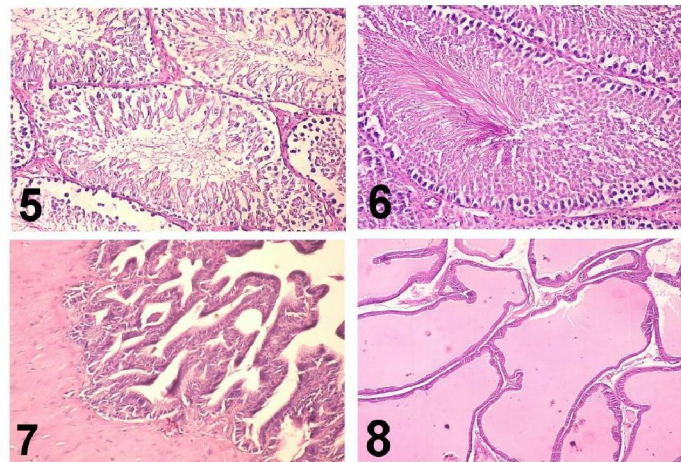


Figure 5: Testes of rats received Na-F along with black tea, showing few numbers of mature sperms in the lumen of seminiferous tubules. H&E X 200.

Figure 6: Testes of rats received Na-F along with black tea, showing normal appearance of seminiferous tubules. H&E X 400.

Figure 7: Seminal vesicle of rats received Na-F along with black tea, showing normal appearance of the glands. H&E X 200.

Figure 8: Prostate gland of rats received Na-F along with black tea, showing mild hyperplasia of the epithelial lining of glands. H&E X 100

4. Discussion:

Animals having predictive validity to human responses or physiological processes are good models (Giraldi et al., 2004). The basic neural and behavioural mechanisms controlling sexual desire or motivation are similar in rodents and humans, thus a valid reliable model of rodent would be of great utility for studying sexual behaviour (Agmo et al., 2004). In addition, rats were selected as subjects because there are several homologies between human copulatory behaviour and that of rat including the mechanism of penile erection (Pfaus, 1996).

Confirming our previous findings, an intense suppression of all territorial aggression parameters was observed in the current study following exposure to Na-F (El-Iethy et al., 2011). Bataineh and Nusier (2006) also reported lowered levels of territorial aggressive responses in Na-F-exposed rats. Fluorosis has been found to attenuate levels of serum testosterone in rats and mice; the hormone being accountable for modulating male aggression (Huang et al., 2007; Reddy et al., 2007). Strong evidence suggested that aggression increases with a corresponding elevation in testosterone levels (Simpson, 2001). Aggression has also been shown to depend upon the characteristics of the opponent "opponent effect", which include olfactory characteristics (Guillot and Chpouthier, 1996). Therefore the propensity of resident male to attack may be explained by differential olfactory recognition and discrimination of the intruder as a stranger through a differential processing cues provided by opponent. Since testosterone has been implicated to influence odour coding, this might elucidate the lowered intensity of olfactory cues with consequent less recognition of the Na-F-treated intruders, and finally decreased confrontation aggression and defensive behaviour displayed by the resident.

The alleviating effect of black tea on suppressed aggressive parameters observed with Na-F might be explained on the basis of the anti-oxidant capacity of black tea. This property has a significant contribution for limitation of body exposure to Na-F with subsequent control of its effects and manifestations to mimic the level noted with Na-F-free rats (Gardner et al., 2007).

Enhanced lateralization and boxing bouts observed in black tea-supplemented individuals in the current study indicated increased levels of testosterone hormone. Further proof derived from previous studies for Zhou et al. (2003) and Ratnasooriya and Fernando (2008), where administration of black tea was accompanied by elevated testosterone levels.

Here, a marked inhibition of all parameters of sexual behaviour were noted for Na-F-ingested rats in terms of prolongation of mounts, intromissions and ejaculations latencies and reduction in frequencies of the same parameters, along with increased post-ejaculation intervals. These findings are in agreement with earlier studies with Na-F-exposed rats (Bataineh and Nusier, 2006; Bera et al., 2007; El-Iethy et al., 2011). Again, this diminution in expression of sexual behaviour might be attributable to Na-F-inflicted reduction in androgen biosynthesis through occurrence of oxidative stress-generated testicular disorders (Ghosh et al., 2002). Furthermore, an increased oxidative stress may result in a complete derangement of the nitric oxide (NO) bioavailability with increment in oxidant generations which in turn, impairs endothelium-dependent vasorelaxation (Ferri et al., 2006; Deanfield et al., 2007). This disruption in NO mechanism needed for induction of vasodilatation and relaxation of penile corpus carvenosum might be accountable for impaired copulatory performance following Na-F exposure.

Since sexual performance was affected here by overall oxidative stress status of individuals. Administration of black tea, with the highest antioxidant potency, along with Na-F resulted in great improvement in all parameters of male sex behaviour. This outcome points out for the first time to the potential impact of black tea in alleviating Na-F-induced sexual dysfunction.

Interestingly, black tea possesses aphrodisiac tendency, where remarkable shortening of mounts and intromissions latencies along with increased frequencies were observed in our black tea-supplemented rats. These indices are indicators for sexual arousability, motivation and vigor (Ratnasooriya and Dharmasiri, 2000; Yakubu et al., 2007). Comparable results derived from former study with rats (Ratnasooriya and Fernando, 2008). These observations with black tea attest to the role that androgen plays as a key factor in sexual behaviour (Schiavi et al., 1997). Polyphenols also significantly increase endothelium-dependent vasodilatation through enhancing NO activity in vascular endothelial cells (Leikert et al., 2002; Deanfield et al., 2007). With respect to tea, both black and green teas have been reported to restore endothelial function through increasing endothelial NO activity in various animal and human studies (Duffy et al., 2001; Anter et al., 2004; Jochmann et al., 2008; Grassi et al., 2008). In particular, theaflavins from black tea have been shown in human studies to favorably affect endothelial function, thus helping to maintain healthy circulation (Stangl et al., 2007). Thus, the vasorelaxation effect favored by black tea might

directly influence penile erection and facilitate copulatory performance shown in the current study. The increase in blood flow to the testes also stimulates testosterone production and secretion, which in turn acts on the central nervous system and gonadal tissues to modulate male sexual behaviour (Wang et al., 1983). Improvement of sex performance could be also attributable, at least partly, to the anxiolytic activity of black tea providing a state of physical relaxation which makes the body more receptive to sensations (Ratnasooriya and Fernando, 2007). Further, black tea contains theanine (Modder and Amarakoon, 2002), which is known to suppress anxiety (Lu et al., 2004; Ozeki et al., 2006; Kimura et al., 2007).

Impairment of male rats' fertility after ingestion of Na-F has been proven in the present study. Fluoride-treated group exhibited the lowest numbers of impregnations, implantations and viable fetuses together with the highest numbers for resorptions. These results are consistent with former studies with rats and mice (Elbetieha et al., 2000; Bataineh and Nusier, 2006; El-Iethy et al., 2011). Further support for reduced fertility derived from the remarkable regression in weights of male sex organs evidently shown by Na-F in the presented study. Similar outcomes were reported in rats by Gupta et al. (2007) and El-Iethy et al. (2011).

Defective sperm function is the most prevalent cause of male infertility and is difficult to treat (Hull et al., 1985). Oxidative stress status-generated reactive oxygen species (ROS) has been implicated in the poor sperm function and infertility (Sikka, 1996). Oxidative stress down regulates the steroidogenic activity leading to altered testicular function (Maneesh et al., 2005a). 25-40% of infertile men had high levels of ROS in semen samples. When spermatogenesis is impaired, spermatozoa are thought to be immature and functionally defective (Thomas et al., 1997). Mammalian spermatozoa are very sensitive to free radicals-induced damage, mediated by lipid peroxidation, as they are rich in polyunsaturated fatty acids (Agarwal et al., 1994; Maneesh and Jayalekshmi, 2006). ROS attack results in a decreased sperm motility and viability along with increased mid piece morphological defects with deleterious effects on sperm capacity (Lenzi et al., 1993). Furthermore, ROS-induced DNA damage accelerates the germ cell apoptosis (Maneesh et al., 2005b). Unfortunately, limited endogenous mechanisms exist to reverse these damages induced by excessive ROS, where spermatozoa lack the cytoplasmic enzymes required to accomplish the repair (Maneesh and Jayalekshmi, 2006). This is one of the features that make spermatozoa unique in their susceptibility to oxidative insult (Krausz et al., 1994).

Hence, treatment strategies must be directed toward continuous inactivation and lowering of ROS levels to keep only a small amount necessary to maintain normal cell function. Supporting this notion, current ingestion of black tea had a high protective role against testicular oxidative stress and steroidogenic dysfunction induced by Na-F. This defensive effect was namely reflected in augmented fertility parameters and weights of male reproductive organs. Researches have reported that using antioxidants can protect sperm DNA from free radicals and increase blood testis barrier stability (Wolff et al., 1991; Palmeira et al., 2001). Flavonoids such as quercetin present in black tea could affect sperm quality (Taepongsorat et al., 2008; Duen~as et al., 2010). This trend was also encouraged by other studies, where green tea protects the testicular function, acting against sperm morphology changes in rats (Kang et al., 2000; El-Shahat et al., 2009).

Changes in testicular lipid profile were strongly correlated to testicular degeneration (Chowdhury et al. 1990). These changes were also associated with increased lipids, DNA oxidative damage and depletion of lipid-soluble antioxidants (Lucesoli and Fraga 1995). Both animal and human studies have provided evidence that tea polyphenols modulates lipid metabolism through reduction of triglycerols, inhibition of fat accumulation and enhancement of energy expenditure (Murase et al., 2002; Nagao et al., 2005; Matsuyama et al., 2008; Grove and Lambert, 2010). Moreover, theaflavins-enriched black tea has been reported to possess a unique lipid-lowering property through inhibition of a key enzyme in the pathway of cholesterol synthesis (Leung et al., 2001). Therefore, it seems reasonable that the impact of black tea on lipid profile might also make a significant contribution against Na-F-inflicted testicular disorders.

Finally, our histopathological investigations revealed prominent degenerative changes in seminiferous tubules as represented by severe disorganization and denudation of germinal epithelium along with complete absence of sperms in the lumina confirming the altered testicular function presently observed with Na-F. Moreover, severe hyperplastic changes in booth seminal vesicle and prostate gland were also shown to further verify abolished fertility detected next to Na-F. These findings are consistent with our former study with rats (El-Iethy et al., 2011). Further support derived from Tiwari and Pande (2009) where Na-F treatment has been reported to entail damaging effect on testicular histoarchitecture along with disfigured tubular structure accompanied by histological changes in other organs viz. epididymis, vas deferens, seminal vesicle and prostate gland. The structural

changes observed in the testicular tissues could be attributable to Na-F-dampened spermatogenesis and steroidogenesis in the testes as a result of oxidative stress-generated large amount of reactive free radicals oxygen species (Chinoy and Sharma, 1998; Pusphalatha et al., 2005; Ge et al., 2006). This damage might also be enlightened by Na-F-induced vascular insufficiency as discussed earlier.

Combined treatment of black tea along with Na-F significantly ameliorated the histological alterations in testicular tissues, currently provoked by fluoride alone. This was proved by normal appearance of germinal epithelium and sperms in the seminiferous tubules. The ameliorating effect of black tea currently shown might be clarified on the basis of its antioxidant properties formerly proved in animal models and human (Erba et al., 2003; Henning et al., 2004; Satoh et al., 2005; Ojo et al., 2007).

Concluding, in view of the results obtained, drinking black tea definitely has a beneficial influence in impeding Na-F-induced reproductive toxicity in rats. Further, our animal study provides some scientific support to the anecdotal belief that black tea enhances male sexual competence, acquiring marked aphrodisiac activity. However, more researches in support of this insight are still solicited. This research has important insinuations, particularly in developing countries where incidence of fluorosis and ease of use of black tea subsist.

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