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Fenitrothion Impaired Sexual Behaviour and Reproductive Performance in Male Sprague-Dawley Rats

(Fenitrotion Menjejaskan Tingkah Laku Seksual dan Prestasi Reproduktif Tikus Jantan Sprague-Dawley)

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ABSTRACT

Fenitrothion (FNT) induces oxidative damage in various organs including male reproductive organ such as testis and sperm; however, its effects on the male sexual behaviours and reproductive performance remain unclear. Therefore, this study aimed to evaluate the effects of FNT administration on male sexual behaviour and reproductive performance. Fertile male Sprague-Dawley rats were randomly divided into three groups (n=8/group): Control - receiving corn oil (1 mL kg^{-1}); FNT-10 and FNT-20 receiving 10 and 20 mg kg^{-1} of FNT, respectively. FNT was administered via *oral force feeding for 28 consecutive days prior to mating with untreated female rats. After mating, the rats were sacrificed to obtain plasma and sperm for further evaluation. Results showed that FNT-20 alone decreased AChE activity (P<0.05) compared to the control and FNT-10. Both doses of FNT also decreased sperm quality compared to control (P<0.05). A significant difference was found (P<0.05) for the presence of intromission and ejaculation among all groups. The mount and intromission latencies as well as post-ejaculation interval were increased (P<0.05) in FNT groups compared to the control group. The total mount and mount frequency were decreased (P<0.05) in FNT-10 and FNT-20 compared to the control group. For reproductive performance, there was a significant difference (P<0.05) for the mating and pregnancy indexes among all groups. Furthermore, both doses of FNT reduced the number of dams, delivered pups and male pups compared to the control (P<0.05). In conclusion, FNT impaired sexual behaviour and reproductive performance of male rats.*

Keywords: Infertility; organophosphate; parturition; sexual behaviour; sperm damage

ABSTRAK

Fenitrotion (FNT) mengaruh kerosakan oksidatif dalam pelbagai organ termasuklah organ reproduktif lelaki seperti testis dan sperma; walau bagaimanapun, kesannya terhadap tingkah laku seksual dan prestasi reproduktif masih belum diketahui. Oleh itu, kajian ini bertujuan untuk menilai kesan pemberian FNT terhadap tingkah laku seksual dan prestasi reproduktif. Tikus jantan Sprague-Dawley subur dibahagikan secara rawak kepada tiga kumpulan (n=8/kumpulan): Kawalan - menerima minyak jagung (1 mL kg^{-1}); FNT-10 dan FNT-20 menerima 10 dan 20 mg kg^{-1} FNT, setiap satunya. FNT diberikan secara oral paksa berturutan selama 28 hari sebelum pengawanan dilakukan dengan tikus betina tidak dirawat. Selepas pengawanan, tikus dikorbankan untuk mendapatkan plasma dan sperma bagi penilaian selanjutnya. Keputusan menunjukkan FNT-20 sahaja menurunkan aktiviti AChE (P<0.05) berbanding kumpulan kawalan dan FNT-10. Kedua-dua dos FNT juga menurunkan kualiti sperma berbanding kumpulan kawalan (P<0.05). Terdapat perbezaan signifikan (P<0.05) bagi kehadiran intromisi dan ejakulasi antara semua kumpulan. Latensi pemanjatan dan intromisi serta selang selepas ejakulasi turut meningkat (P<0.05) pada kumpulan FNT berbanding kumpulan kawalan. Sebaliknya, hanya FNT-20 yang menyebabkan pengurangan frekuensi intromisi dan peningkatan latensi ejakulasi berbanding kumpulan kawalan (P<0.05). Jumlah dan frekuensi pemanjatan adalah menurun (P<0.05) pada kumpulan FNT-10 dan FNT-20 berbanding kumpulan kawalan. Bagi prestasi reproduktif, terdapat perbezaan signifikan (P<0.05) pada indeks pengawanan dan kehamilan antara semua kumpulan. Tambahan lagi, kedua-dua dos FNT telah mengurangkan bilangan betina hamil, progeni yang dilahirkan dan progeni jantan berbanding kumpulan kawalan (P<0.05). Kesimpulannya, FNT menjejaskan tingkah laku seksual dan prestasi reproduktif tikus jantan.

Kata kunci: Kelahiran; ketaksuburan; kerosakan sperma; organofosfat; tingkah laku seksual

INTRODUCTION

Infertility remains a critical public health problem globally. According to United Nation Department of Economic and Social Affairs (2017), the global fertility rate (GFR) has declined from 3 babies to 2.5 babies born from a healthy reproductive woman in 2017. It is unfortunately predicted to further decrease to 2.2 by the year 2045. Most infertility cases that occur are reported due to infertile

men as the contributor (WHO 2014). In accordance with Agarwal et al. (2015), at least 30 million men are infertile around the world. Some causes of male infertility are anatomical abnormalities, hormonal imbalance, genetic defects, improper diet, unbalanced lifestyle, and oxidative stress (D'Souza 2017; Singh et al. 2014). Furthermore, the exposure to noxious agents such as drugs, radiation and pesticides play a significant role in infertility (Al-Gubory et al. 2014).

Fenitrothion (FNT) *[O,O-dimethyl–O–(3–methyl–4–nitrophenyl) phosphotioate]* is one of the most common organophosphate (OP) pesticides that act by inhibiting the acetylcholinesterase enzyme (AChE) activity (Pope 2006). AChE acts by hydrolysing acetylcholine into choline and acetate after activation of acetylcholine receptors at the postsynaptic membrane. It serves to terminate synaptic transmission, preventing continuous nerve firings at nerve endings (Lionetto et al. 2013). It is widely used in public health sector as a vector control agent for malaria and filariasis as well as in agricultural sector to control insects, pests, and mites on rice, fruits and vegetables to increase the quality of food production (Malhat 2012; Uygun et al. 2005; WHO 2008). Due to extended used and persistence in the environment, exposure to FNT and its metabolites is unavoidable (Damalas & Koutroubas 2016). Humans are potentially exposed to FNT either directly through occupational exposure or indirectly via food consumption (Elhalwagy et al. 2008). FNT caused undesirable effects on human health including to the liver (Jayusman et al. 2014), lungs (Budin et al. 2012) and kidney (Budin et al. 2013), as well as testis and sperm (Taib et al. 2013) of rats.

Several studies reported that FNT is not only inhibiting AChE activity; but FNT and its metabolite also caused defects in the male reproductive system via antiandrogenic effects (Li et al. 2006; Tamura et al. 2003) and epididymal phospholipidosis (Miyake et al. 2018). The AChE is conventionally known for terminating cholinergic neurotransmission and on top of that has been shown to be a potential marker and regulator of apoptosis in some cells (Zhang & Greenberg 2012). Studies have also revealed the functions of AChE during spermatogenesis, including interactions with the receptor of activated protein kinase C (RACK1) to promote apoptosis and with the glycolytic enzyme enolase–α, increasing enolase activity which lead to reduction in sperm differentiation and sperm counts (Mor et al. 2008). The level of AChE in seminal plasma have an inverse relationship with sperm motility, indicating that acetylcholine has a role in sperm motility (Mor et al. 2001). A defect in testosterone production will impair spermatogenesis particularly in terms of sperm maturation and sexual behaviour such as mating process. In males, healthy sexual function is characterised mainly by the erection of the penis and ejaculation. The inability of men to perform reproductive function effectively is known as male sexual dysfunction (MSD) (Rastrelli & Maggi 2017). The prevalence of MSD is about 10-52% among men worldwide including in Malaysia, affecting about 27% of population (Ho et al. 2011). Sexual dysfunction in males could be manifested from unregulated sexual behaviour, decrease libido, arrested ejaculation and inability to sustain an erection and respond to erectile stimulation as well as failure to accomplish regular sexual intercourse (Lotti & Maggi 2018).

Besides involved in the termination of impulse transmission, AChE also plays an important role in spermatogenesis and sperm motility (Mor et al. 2008, 2001). Therefore, AChE inhibition influences the sperm quality. Furthermore, FNT at the dose that did not inhibit AChE activity has been reported to cause spermatotoxicity (Ito et al. 2014); however, its effects towards male reproductive performance and sexual behaviour is scarcely reported. Hence, this study aimed to assess the effects of different doses of FNT on male reproductive performance and sexual behaviour of rats. This study might stretch a new input on the male reproductive damage focusing on male reproductive performance and sexual behaviour when exposed to the non-injurious dose of FNT.

Materials and Methods

Chemicals

FNT (98.66% purity) was purchased from LGC Labor GmbH (Augsburg, Germany; Lot No. G144531) and was diluted in corn oil prior to administration at 10 and 20 mg kg^{-1}, respectively. Other chemicals and reagents used in this study were of high purity grade purchased from Sigma Aldrich (United States of America), unless otherwise stated.

Experimental animals

Male Sprague-Dawley rats (n=24, 9 weeks old) weighing 240-270 g were obtained from the Laboratory Animal Research Unit of Health Campus in Universiti Sains Malaysia (USM), Malaysia. All rats were housed in polycarbonate cages (2 animals per cage) and maintained at 20-24 °C with reversed 12 h light/dark cycle and relative humidity of 50 ± 5%. They were provided rodent chow pellets (Gold Coin Sdn. Bhd. Kuala Lumpur, Malaysia) and water *ad libitum.* The rats were acclimatised for one week prior to experimentation. All the animal handling procedures strictly adhered to ethical guidelines approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) with reference no. FSK/2016/IZATUS/23-NOV./807-NOV.-2016-FEB.-2019 and Animal Ethics Committee of USM (USM/IACUC/2018/(112)(921)).

Experimental design

All male rats were randomly divided into three groups (n = 8/group): Control (C), FNT-10 and FNT-20. Control group received corn oil (1 mL kg^{-1}) alone; whilst both FNT-10 and FNT-20 received 10 mg $kg^{-1}day^{-1}$ FNT (1/60

LD_{50}) (Ito et al. 2014) and 20 mg $kg^{-1}day^{-1}$ FNT (1/30 LD_{50}) (Taib et al. 2013), respectively. Taib et al. (2013) found that 20 mg kg^{-1} of FNT cause inhibition of AChE parallel with the oxidative damages on sperm and testis while Ito et al. (2014) found that 10 mg kg^{-1} of FNT cause spermatotoxic with less inhibition effects on AChE activity. All the regimens were administered via oral forced feeding between 09:00 and 10:00 am for 28 consecutive days prior to mating with untreated female rats. After the assessment of reproductive performance and sexual behaviour, all rats were fasted overnight, anaesthetised with a single intraperitoneal injection of ketamine and xylazine cocktail (KTX) and sacrificed. Blood samples were collected via cardiac puncture into heparinised tubes and were centrifuged at 3000 rpm for 10 min at 4 °C. Plasma samples were collected and stored at -40 °C for AChE analysis. Sperm samples were collected from cauda epididymis for evaluation of sperm characteristics.

PLASMA AChE ENZYME ACTIVITY ANALYSIS

Plasma AChE was measured using Ellman assay (Ellman et al. 1961). Briefly, AChE found in the samples hydrolyses the propionythiocholine to form thiocholine, that reacts with 5,5,9-dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow solution containing 5-thio-2-nitrobenzoate. The formation of the yellow colour was monitored at 405 nm wavelength, and the AChE activity was expressed in U/L.

SPERM CHARACTERISTICS ANALYSIS

All the methods used in assessing the sperm characteristics were based on Seed et al. (1996). Sperm suspension was obtained from each cauda by centrifuging it at 1000 rpm for 3 min at 4 °C. Ten microliters of sperm suspension was then used for calculation of motility and concentration using the Makler counting chamber under 10 × magnification using a light microscope. Sperm motility and concentration were expressed in percentage and 10^6 cells per mL, respectively. Sperm viability was evaluated by assessing the penetration ability of the eosin on the non-viable cells. Sperm morphology was also evaluated using a thin smear of sperm suspension. The dried smear was stained with 2% of eosin, and the sperm morphology was evaluated in 200 sperms per slide. Sperm cells presenting with normal morphology were reported in percentage.

SEXUAL BEHAVIOUR ASSESSMENT

Sexual behaviour of each male rat was assessed after 3 days of rest. Each rat was paired with one untreated female rat in oestrous phase to assess the male sexual behaviour for 20 min (between 09:00 and 12:00 h) in the following week as reported earlier (Mohamed et al. 2013). Each male rat was placed in a transparent cage, in a silent room and was recorded under dim white light illumination using a video camcorder (HDD Handycam DCR–SR42, Sony Corporation, Japan). After 10 min of adaptation, a receptive female in oestrous phase was introduced gently into the cage (Mohamed et al. 2013). The following parameters were recorded and calculated: Presence of pre-coital exploratory activities such as sniffing, nose-to-nose contact and genital exploring; presence of mount; presence of intromission; presence of ejaculation; presence of two or more ejaculations; mount and intromission latencies (the time interval between the introduction of female and the first mount or intromission by the male); ejaculatory latency (the time interval between the first intromission and the first ejaculation); and mount and intromission frequencies (the number of mounts or intromissions until the first ejaculation); post-ejaculatory interval (time from first ejaculation and next intromission).

REPRODUCTIVE PERFORMANCE ASSESSMENT

After 28 days of treatment, each male rat was separated into a single cage and cohabited for 3 h daily (from 0900 to 1200) for up to 2 weeks with three 8-10 weeks old, untreated female rats (3:1) weighing 180-220 g. Vaginal smear was performed daily at the end of cohabitation period and examined under a light microscope (Olympus BX41, Olympus Corporation, Tokyo, Japan) for the presence of spermatozoa. Female rats with sperm-positive vaginal smear were considered successfully mated and were separated from male rats with denotion as Day 0 of gestation (Mohamed et al. 2013). A male rat was considered unable to mate if no mating was recorded after three attempts. The following parameters were evaluated for male reproductive performance: mating index: (number of males that make any of his female partner sperm-positive within 14 days/total number of males involved in mating) × 100; fertility index: number of days elapsed until the male rats had first fertilised its female partner; libido index: (number of sperm-positive females/total number of females involved in mating) × 100; pregnancy index: (number of pregnant females/number of sperm-positive females) × 100.

PARTURITION AND PREGNANCY OUTCOMES EVALUATION

Female rats with sperm-positive vaginal smears were treated as pregnant rats. Weekly body weight was recorded, and the signs of pregnancy such as enlargements of nipples and abdomen were observed. All pregnant female rats (dams) were allowed for normal delivery starting on Day 21. The following parameters were recorded and calculated for the parturition and pregnancy outcomes: number of the dams, gestational index, gestation length, number of delivered pups per litter, number of live pups per litter based on sex, number of dead pups per litter, and sex ratio.

STATISTICAL ANALYSIS

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 23 and GraphPad PRISM version 7.0 for Windows (GraphPad Software Inc., La Jolla, CA, USA). One-way analysis of variance (ANOVA) was used with Tukey post hoc test to analyse data with normal distribution and homogenous variance and the results were expressed as mean ± standard error of the mean (SEM). Kruskal-Wallis test was otherwise used for data with non-normal distribution and non-homogenous variance. Dunn's multiple comparisons test was then used and the results were expressed as median (interquartile range). Categorical data were analysed using Pearson Chi-square or Fisher's exact test and presented in percentage. Differences were statistically significant at $P<0.05$.

RESULTS

BODY WEIGHT GAIN

All the groups showed increased body weight throughout the experimental period (Figure 1). However, the rats treated with FNT at both doses showed a significantly lower weight gain compared with the control ($P<0.05$). Furthermore, FNT-20 group also showed a significantly lower weight gain compared to the FNT-10 group ($P<0.05$).

SIGNS OF TOXICITY

Table 1 shows the signs of toxicity in experimental rats. No deaths were recorded in all experimental groups. However, cholinergic signs were only observed in 6/8 rats from FNT-20 group. No toxicity signs and symptoms were observed in control and FNT-10 groups.

PLASMA AChE ACTIVITY

Figure 2 shows the AChE activity in plasma of experimental rats. A marked reduction in AChE activity was found in the FNT-20 group compared to the control and FNT-10 groups ($P<0.05$). However, no significant difference was observed in AChE activity between the FNT-10 and control groups.

SPERM CHARACTERISTICS

Sperm characteristics of experimental rats are shown in Figure 3. The sperm count, viability, motility, and normal morphology were significantly decreased in both FNT groups compared to the control group ($P<0.05$). Furthermore, when compared against FNT-10 group, all the sperm characteristics (sperm count, viability, motility, and normal morphology) were significantly lowered in FNT-20 group ($P<0.05$).

SEXUAL BEHAVIOUR

The findings on the sexual behaviour of male rats are presented in Table 2. Rats in all the experimental groups exhibited pre-coital exploratory activities and mounting. However, only 50% of the rats from FNT-20 group and 87.5% of the rats from FNT-10 group intromitted and ejaculated ($P<0.05$) compared to the control group (100%). All rats in the control group were present with more than one ejaculation, while only 37.5 and 12.5% of rats from FNT-10 and FNT-20, respectively, were present with more than one ejaculation ($P<0.05$). Both doses of FNT significantly increased ($P<0.05$) mount latency (ML), intromission latency (IL) and post-ejaculatory interval (PEI) compared to the control group. However, the mounting frequency (MF) was significantly reduced ($P<0.05$) in FNT-treated groups compared to the control group. Only FNT-20 group showed significant increase ($P<0.05$) in ejaculatory latency (EL) compared to the control group. A marked reduction ($P<0.05$) in both total intromission (TI) and intromission frequency (IF) was seen in FNT-20 group as compared to the control group. Furthermore, FNT-20 group showed significantly increased ($P<0.05$) in ML, IL and PEI and significantly decreased ($P<0.05$) in MF, TM and TI when compared to the FNT-10 group. Further results showed no significant differences ($P>0.05$) for mount and intromission ratios across all groups.

REPRODUCTIVE PERFORMANCE PARAMETERS

Reproductive performance parameters of male rats are shown in Table 3. The rats from FNT-treated groups had significantly ($P<0.05$) lower mating and pregnancy indexes compared with those from control groups. Even though no statistically significant difference was seen ($P>0.05$), the ratios of sperm–positive females (libido index) and the fertility index appeared to decline in FNT-treated groups in a dose-dependent manner.

PARTURITION AND PREGNANCY OUTCOMES

Table 4 shows the parturition and pregnancy outcomes in all experimental groups. Both doses of FNT significantly decreased number of dams, number of delivered pups per litter and number of live male pups per litter at $P<0.05$ when compared with control group. Meanwhile, a significant reduction ($P<0.05$) in the number of dams and the number of delivered pups per litter was seen in FNT-20 group compared to the FNT-10 group.

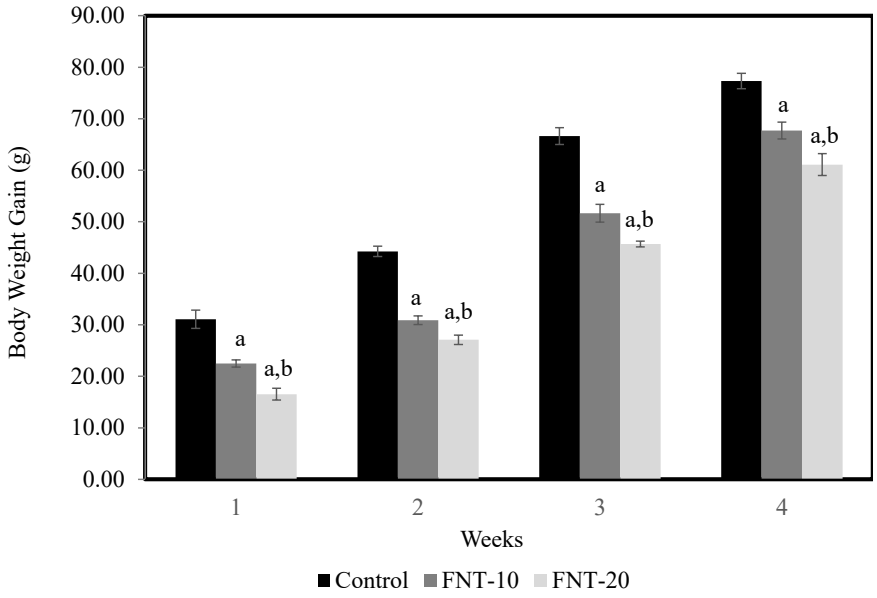

FIGURE 1. Body weight gain in all experimental groups. Data are represented as the mean ± SEM. [a]p<0.05 compared to control group, [b]p<0.05 compared to FNT–10, *n* = 8 for each group

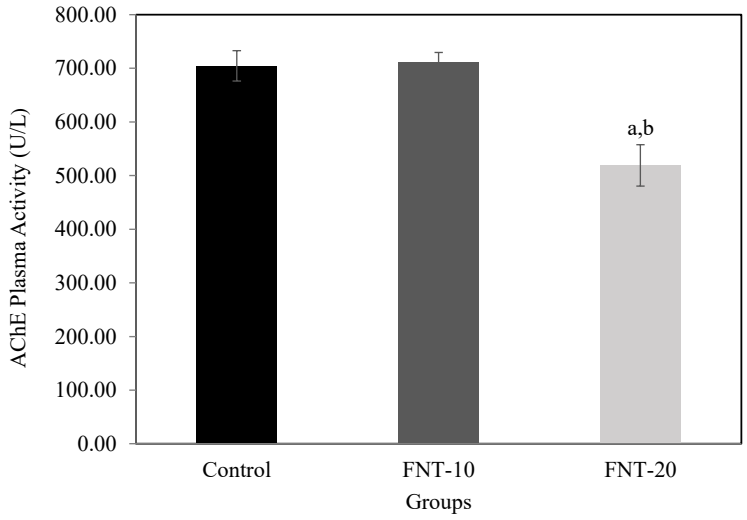

FIGURE 2. Plasma AChE enzyme activity in all experimental groups after 28 days of study. Data are represented as the mean ± SEM. [a]p<0.05 compared to control group, [b]p<0.05 compared to FNT–10, *n* = 8 for each group

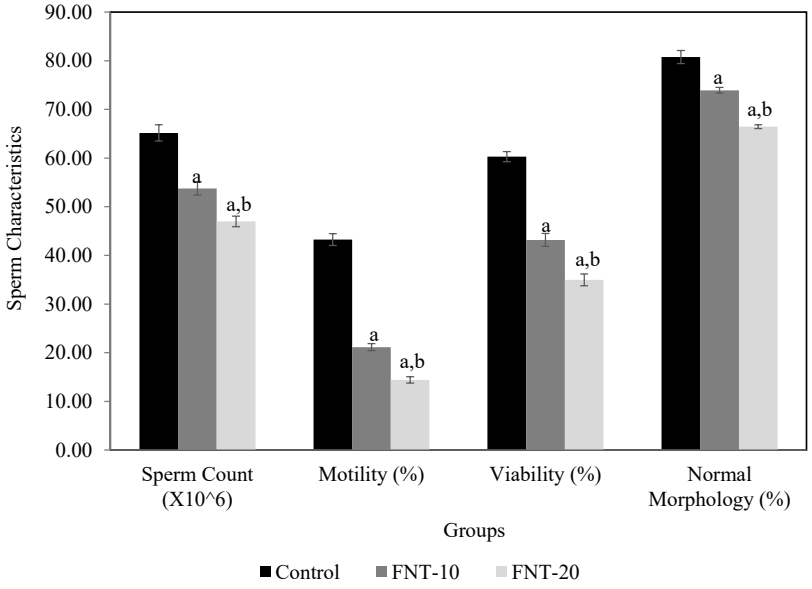

FIGURE 3. Sperm characteristics in all experimental groups after 28 days of study. Data are represented as the mean ± SEM. [a]$p<0.05$ compared to control group, [b]$p<0.05$ compared to FNT–10, $n = 8$ for each group

TABLE 1. Toxicity sign of male rats in all experimental groups

Groups	Mortality rate (n/T)	Toxicity signs (n/T)	Toxicity signs	Observation time (h)
Control	0/8	0/8	Nil	Nil
FNT–10	0/8	0/8	Nil	Nil
FNT–20	0/8	6/8	Tremor	0.25 – 3
			Lacrimation	0.75 – 1
			Piloerection	0.30 – 3
			Hypoactivity	1 – 2

TABLE 2. Sexual behaviour of male rats in all experimental groups

Parameter	Control	FNT–10	FNT–20
Presence of pre-coital exploratory activities[$]	100.00 [8]	100.00 [8]	100.00 [8]
Presence of mount[$]	100.00 [8]	100.00 [8]	100.00 [8]

Presence of intromission[$]	100.00 [8]	87.50* [7]	50.00* [4]
Presence of ejaculation[$]	100.00 [8]	87.50* [7]	50.00* [4]
Presence of >1 ejaculation[$]	75.00 [6]	37.50* [3]	12.50* [1]
Mount latency (min)[#]	0.22 ± 0.02 [8]	0.40 ± 0.01[a] [8]	0.79 ± 0.04[a,b] [8]
Intromission latency (min)[@]	0.58 (0.26) [8]	1.25 (0.17)[a] [7]	3.68 (0.75)[a,b] [4]
Ejaculatory latency (min)[@]	12.18 (2.27) [8]	14.28 (3.78) [7]	16.77 (2.67)[a] [4]
Mount frequency until first ejaculation (per 30 min)[#]	26.13 ± 1.06 [8]	19.63 ± 0.91[a] [8]	15.88 ± 0.81[a,b] [8]
Intromission frequency until first ejaculation (per 30 min)[#]	17.75 ± 0.84 [8]	16.29 ± 0.87 [7]	12.75 ± 0.85[a] [4]
Post ejaculatory interval (min)[#]	8.15 ± 0.17 [8]	11.99 ± 0.36[a] [7]	13.97 ± 0.54[a,b] [4]
Total mounts (per 30 min)[#]	52.75 ± 2.14 [8]	40.38 ± 1.07[a] [8]	33.88 ± 1.58[a,b] [8]
Total intromission (per 30 min) [#]	31.00 ± 2.33 [8]	27.86 ± 1.24 [7]	19.50 ± 0.65[a,b] [4]
Mount/Intromission ratio[#]	1.763 ± 0.13	1.470 ± 0.07	1.688 ± 0.12

Values are in percentages. Number in [ ] represents the number of rats per group which recorded positive results in each parameter. [$]Pearson's Chi–square test. [&]Fisher's exact test. [@]Data are presented as median (interquartile range), Kruskal-Wallis test, followed by Dunn's multiple comparison test. [#]Data are presented as mean ± SEM (One–way ANOVA, followed by Tukey post–host). *significant different among group, [a]$p<0.05$ vs control, [b]$p<0.05$ vs FNT–10

TABLE 3. Reproductive performance of male rats in all experimental groups

Parameter	Control	FNT–10	FNT–20	p–value
Fertility Index (day)[@]	2.00 (1.75)	3.00 (6.00)	4.00 (1.00)	>0.05
Mating Index (%)[&]	100.00 [8]	91.67 [7]	66.67 [4]	<0.05
Libido Index (%)[&]	91.67 [22]	79.17 [19]	62.50 [15]	>0.05
Pregnancy Index (%)[&]	100.00 [22]	94.73 [18]	73.33 [10]	<0.05

[@]Data are presented as median (interquartile range), Kruskal-Wallis test, followed by Dunn's multiple comparison test. Values are in percentages. Number in [ ] represents the number of rats per group which recorded positive results in each parameter. [&]Fisher's exact test. *significant different among group

TABLE 4. Parturition/Pregnancy outcomes of rats in all experimental groups

Parameter	Control	FNT–10	FNT–20
Number of Dam[#]	22.00 ± 0.53	18.00 ± 0.69[a]	10.00 ± 0.91[a,b]
Gestational Index (%)[&]	100.00 [22]	100.00 [18]	90.00 [9]
Gestation Length (day)[@]	22.00 (0.00)	22.00 (2.00)	22.50 (1.00)
Number of delivered pups per litter[#]	9.13 ± 0.40	7.00 ± 0.31[a]	5.00 ± 0.41[a,b]
Number of live pups per litter[#]			
Male	5.13 ± 0.64	3.00 ± 0.43[a]	1.75 ± 0.29[a]
Female	4.00 ± 0.57	4.00 ± 0.62	3.00 ± 0.41
Number of dead pups per litter[#]	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.25
Sex Ratio (%)[#]	55.91 ± 6.05	43.96 ± 7.21	34.58 ± 3.56

[@]Data are presented as median (interquartile range), Kruskal-Wallis test, followed by Dunn's multiple comparison test. [#]Data are presented as mean ± SEM (One-way ANOVA, followed by Tukey post–hoc test). [&]Fisher's exact test. Number in [ ] represents the number of rats per group which recorded positive results in each parameter

DISCUSSION

Indiscriminate, extensive use of OP insecticides can be detrimental to the mammals. Several studies have reported toxic effects of FNT on different facets of the male reproductive system. Indeed, oxidative stress, antiandrogenic effects and phospholipidosis, were all identified as mechanisms involved in FNT-induced male reproductive system damage (Ito et al. 2014; Taib et al. 2013; Tamura et al. 2003). This present study showed that repeated exposure to FNT only inhibits AChE activity at the dose of 20 mg kg^{-1}; but failed to inhibit AChE activity at 10 mg kg^{-1}. Thus, it might explain although the signs of toxicity that were observed in both of FNT groups were correlated with dosage.

Inhibition of AChE enzymes prevents break down of acetylcholine, increasing both the level and duration of the neurotransmitter action in cholinergic synapses (Čolović et al. 2013). Therefore, cholinergic signs including the hypoactivity, lacrimation, piloerection, and tremor were observed. Lacrimation and piloerection were the most prominent signs indicating a disturbance in autonomic nervous systems. Tremor, on the other hand, is a common sign indicating neurotoxicity (Mueller 2001). A significant decrease in body weight gain was observed in the FNT-treated groups which might be associated with the toxic symptoms, such as cholinergic signs. High energy is required in the biotransformation of FNT into its metabolites such as fenitrooxon and 3 methyl-4-nitrophenol (3MNP). This energy is obtained through breakdown of carbohydrates from food, thus resulting in absence of fat storage (Franklin & Yost 2000). Furthermore, the reduction in body weight gain may be due to the overall increase in degradation of lipids and proteins (Mansour & Mossa 2011). All these reasons might explain the significant reduction of body weight gain in FNT-intoxicated rats.

FNT at both doses reduced the sperm quality by reducing the sperm count, motility, viability, and normal morphology. These findings were in line with previous studies which found that OP pesticides reduce sperm quality through oxidative stress, disturbance in phospholipids and hormonal imbalance (Ito et

al. 2014; Mehrpour et al. 2014; Taib et al. 2013). In normal conditions, the plasma testosterone level in male rat remains elevated throughout breeding session to relatively increase the sexual interest in receptive females (Numan 2015). Therefore, any disturbance in the testosterone level might influence the sexual behaviour and reproductive performance of male rats.

Sexual behaviour in a male is a complicated phenomenon controlled by endocrine, central and peripheral nervous systems. Sexual behaviour in animal models is considered useful in predicting the similar potential effects of chemicals in human (Hull & Dominguez 2007). The mating behaviour of a male rat when cohabited with oestrous (receptive) female consists of a repeated series of mounts and intromissions, ultimately leading to ejaculation. Male rats from all groups showed pre-coital exploratory and mounting activities in agreement with the previous study (Mohamed et al. 2013). Following the sexual motivation and libido, penile erection and the sustainability of this action can be assessed through the percentage of intromission and ejaculation. The persistent incapability to accomplish or to sustain the penile erection for satisfactory sexual performance is defined as erectile dysfunction (ED) (NIH 1993). In this study, both doses of FNT reduced the percentage of rats achieving intromission and sustained the ejaculation; suggesting that the FNT could cause ED in rats. These findings are consistent with previous findings among farmers who were exposed to pesticides (Kaur et al. 2015).

Sexual arousal is usually inversely proportional to sexual motivation or desire (Besong et al. 2018). Both doses of FNT impaired sexual arousal of the rats by increasing the mount and intromission latency. However, only FNT at the highest dose increased ejaculatory latency. Sexual arousal is a stage influenced by dopamine and testosterone. Testosterone increases the release of dopamine in the medial preoptic area of the hypothalamus; and enhances the sexual arousal (Wan Yaacob et al. 2013). The rate of recovery from exhaustion after the first series of mating can be evaluated by the post-ejaculatory interval (Cao et al. 2012); which both doses of FNT significantly increased. The post-ejaculatory interval, as well as mount and intromissions, are considered as a reflection of libido, strength, potency, sexual performance, and vigour in male rats (Kpomah et al. 2012). Following sexual behaviours, the reproductive performance assessment is usually done to evaluate the success of mating that produces feasible offsprings (Swaney et al. 2012). The present findings indicated that male rats exposed to both doses of FNT significantly reduced mating and pregnancy indexes. According to the previous study, a high concentration of chlorpyrifos-ethyl reduced the reproductive performance in male rats by reducing the quality of sperm (Kenfack et al. 2015). Reduction in reproductive performance suggests penile tumescence, rigidity, and accessory muscle that help in sustaining an erection of the male sexual organs were not fully functioning (Yakubu & Afolayan 2009). It was suggested that the sexual capability of FNT-treated rats was decreased given that day elapsed for male rats to make their females sperm-positive were longer compared to control rats. Libido index remained statistically unchanged although there was a trend for percentage reduction in FNT-treated groups as shown in the previous study (Dasuki et al. 2012). The interference during the process of male brain sexual differentiation after perinatal exposure to picrotoxin influenced deleterious effects of this toxicant on the fertility and sexual behaviour of male rats (Yasuhara et al. 2005). In contrast, no adverse effects were seen for amitraz, a formamidine insecticide given in the drinking water on the fertility, mating, libido and pregnancy indexes of male rats (Lim et al. 2010). The discrepancy in this finding may be explained by the differences of the animal sex, dose and the method of exposure.

In this study, both doses of FNT reduced number of dams, delivered pups and the number of male pups; thus, impairing the parturition and pregnancy outcomes. The impairment of parturition and pregnancy outcomes was also found in the rats exposed to the mixtures of endocrine disruptors (Jacobsen et al. 2012). According to Jacobsen et al. (2012), this impairment is due to the anti-androgenic effects which might also impair parturition and pregnancy outcomes in FNT-intoxicated rats. From the findings, FNT defected the sexual behaviours and reproductive performance in dose-dependent manner. Even at a dose with no inhibition of AChE activity reported, FNT reduced mounting and intromission of male rats; while these defects were extended to the disability of ejaculation at the higher dose of FNT. In contrast, no dose–response relationship was found in the sexual behaviour and reproductive performance defects among rats induced by mixtures of pesticides previously (Jacobsen et al. 2012). According to Jacobsen et al. (2012), sexual behavioural defects are due to the anti-androgenic effects of pesticides. In conclusion, FNT particularly at the dose that did not inhibit AChE activity impaired sperm quality, reproductive performance, sexual behaviour as well as parturition and pregnancy outcomes. These defects are more visible at the higher dose of FNT. Therefore, the use of FNT needs more precaution and observations taken. A future study might be done to evaluate the genetic disorders and chromosomal aberrations in pups exposed to FNT.

Conclusion

In conclusion, even at the harmless dose, FNT results in significant reproductive impairment and decreased

fertility potential, which may be attributable to poor sperm quality. Moreover, it also affecting pregnancy outcomes in healthy female rats. The findings might influence the progeny health status as FNT might cause genetic disorders which leads to malignancies. Hence, the effects of FNT in male rats could be further investigated perhaps in term of genetic profile to know the exact mechanism that involve in the impairment.

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