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Effects of pyrethroid insecticide ICON (lambda cyhalothrin) on reproductive competence of male rats

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Abstract   Aim: To assess the effect of ICON (trade name of lambda-cyhalothrin) on sexual competence and fertility of male rats. **Methods:** Male rats were gavaged daily for 7 consecutive days with different doses of ICON (63 mg/kg and 100 mg/kg) or vehicle (distilled water). Their sexual behaviour and fertility were evaluated at different time points during treatment and post-treatment using receptive females. **Results:** Treatment had no effect on fertility, but sexual competence was seriously impaired: libido (assessed in terms of pre-coital sexual behaviour, and numbers of mounting, intromission and ejaculation), sexual arousability/motivation (in terms of latencies for mounting, intromission and ejaculation), sexual vigour (judged by frequencies of mounting and intromission or copulatory efficiency). In addition, ICON suppressed intromission ratio, indicating erectile dysfunction. These effects on sexual function had a rapid onset and was reversible. ICON-induced sexual dysfunction was mediated by multiple mechanisms, mainly toxicity, stress, sedation and possibly via GABA and dopaminergic systems. **Conclusion:** Exposure to ICON may cause sexual dysfunction in male rats. *(Asian J Androl 2002 Mar; 4: 35-41)*

1   Introduction

Several currently used pesticides, especially those having endocrine disruptive properties, are known to adversely impair reproductive competence of males under laboratory, field, clinical or occupational settings [1-5]. Some of these agents are among the most commonly used pesticides/insecticides in developing countries including Sri Lanka. Lambda-cyhalothrin (trade name: ICON) is a potent, synthetic, type II pyrethroid [6,7]

recently introduced to Sri Lanka as an indoor spray against malaria vector mosquitoes (Manuweera G, Registrar of pesticides, Sri Lanka, personal communication). It is also used as an agropesticide in onion cultivation. It is a stomach, contact and a residual insecticide, which acts as a neuropoison interfering in the ionic conductance of nerve membranes by prolonging the sodium current [6, 7]. In addition, pyrethroids increase the spontaneous release of neurotransmitters such as GABA, dopamine or noradrenaline [7], and may also acts as hormone disruptor [8]. Collectively, the facts suggest that ICON may disrupt male reproductive function, but this has not been experimentally documented.

The aim of this study was to assess the potential impacts of ICON on sexual competence and fertility of rats with a view of possible extrapolation of the findings to man, as the processes and regulation of male reproduction are highly conserved in mammals [9].

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2   Materials and methods

2.1 Animals

Sexually experienced healthy adult crossbred albino rats (males weighing 225-250 g and females 200-225 g) were used. They were kept singly in plastic cages under standardized animal house conditions (28-31 ¡æ 12 h light/ 12 h darkness, relative humidity 50-55%) with free access to pelleted food (Master Feeds Lanka Ltd., Colombo, Sri Lanka) and tap water.

2.2 ICON Preparation

Two desired doses (63 mg/kg and 100 mg/kg) of ICON was prepared by mixing the commercially available ICON powder in distilled water (DW). The lower dose selected is close to the reported oral no-observed-effect level of ICON, which was 50 mg/kg [6].

2.3 ICON administration

ICON or vehicle was orally administered by gastric intubation (09:00 - 10:00 h) for specified time periods as indicated under relevant sub-sections. The first day of administration was considered day 1 of treatment.

2.4 Adverse effects

After every dosing, cage side observations were made on each rat continuously for 3 -5 h for mortality, overt signs of toxicity (salivation, wilting, tremors, convulsions, ataxia, yellowing of fur, diarrohea), stress (fur erection and exophthalmia) and changes in non-sexual behaviours (such as cleaning of face, self grooming, climbing in cages, rearings).

2.5 Fertility and general toxicity evaluation

2.5.1 Animals and grouping

Male rats were assigned to three groups: (1) ICON 63 mg/kg/day (*n* = 12), (2) ICON 100 mg/kg/day (*n* = 17) and (3) 1 mL DW/day (*n* = 12) for 7 consecutive days.

2.5.2 Effect on fertility

Rats were assessed for their libido, ejaculatory ability and fertility on days 1, 3 and 7 of treatment and then at weekly intervals for 90 days during the post-treatment period. Each male was paired overnight with a pro-oestrous female (at 16.00-16.30 h). The pre-coital sexual behaviour (chasing, nosing, anogenital sniffing, genital grooming, attempted clasping and mounting) of the paired rats was observed for 2 h right after pairing. Vaginal smears of the females were taken on the following morning (at 08:00 - 08:30 h). The presence of spermatozoa was considered day 1 of pregnancy. If spermatozoa were present, their numbers were determined in duplicate using an improved Neubauer haemocytometer (Fision, UK) and gross morphology was observed microscopically ($\times 100$ and $\times 400$). If spermatozoa were absent, then daily vaginal smearing was done for at least 10-12 days to determine the appearance of pregnancy or pseudopregnancy. At 14 days following pairing, the females were subjected to laparotomy under ether (Fluka, Switzerland) anesthesia using aseptic precautions. The number of conceptus (both viable and dead) was then determined. The following reproductive indices were computed: index of libido= (number mated/number paired)$\times 100$; quantal pregnancy= (number pregnant/number mated)$\times 100$; implantation index= (total number of implantation/number mated)$\times 100$; pre-implantation loss = [(number of corpora lutea-number of implants)/number of corpora lutea]$\times 100$; post-implantation loss = [(number of implants - number of viable implants)/number of implants]$\times 100$.

2.5.3 Effect on rectal temperature

The rectal temperature was determined on days 1 and 7 of treatment (5 h after dosing) using a clinical thermometer (Oson Duopris, Germany)

2.5.4 Effect on food and water intake

Food and water intake of the rats were determined daily on day 2-5 of treatment using standard laboratory techniques [10].

2.5.5 Effect on body weight

Body weights were determined 5 h after dosing on days 1 and 7 of treatment using an electronic balance (M.P. 6000, Chyo, Japan).

2.5.6 Effects on haematology

On day 1 post-treatment, blood was collected from tails under aseptic conditions and red blood cell (RBC) counts, white blood cell (WBC) counts, packed cell volume (PCV), and haemoglobin content were estimated as described by Cheesbrough et al [11]. Mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) was then computed.

2.6   Evaluation of sedative potential

The sedative potential of ICON was evaluated using rat hole-board technique [12]. Forty-one male rats were assigned into 3 groups and dosed similarly as in 2.5.1. Five hours after the last dosing rats were individually placed on the center of rat hole-board with a 7.5 min trial period. The number of headings, rears and locomotory activity were recorded.

2.7 Evaluation of muscle strength and co-ordination

Male rats were either orally treated with 100 mg/kg/day of ICON (*n* = 6) or 1 mL DW/day (*n* = 6) for 7 consecutive days. Five-seven hours after the last dosing, each of these rats were subjected to Bar Holding test to evaluate muscle strength [13], and the time taken for the rat to fall from the bar was determined. Immediately following this test, these rats were subjected to Bridge test to evaluate the muscle co-ordination [13] and the latency to slide off was recorded.

2.8 Effect on sexual behaviour

Forty one male rats were divided and dosed similarly as in 2.5.1. On days 1, 3 and 7 of treatment (5-7 h following oral dosing) and day 14 post-treatment rats were individually paired with a female rat brought into oestrous by subcutaneous injection of 12 μg estradiol benzoate (Sigma, USA) and 48 h later by 0.5 mg of progesterone (Sigma, USA). The pre-coital and coital behaviour were observed for 15 min or until ejaculation. During this period, the mount latency, the intromission latency, the ejaculation latency, the number of mounts and the number of intromissions were recorded. Using these parameters the percentage of rats mounted, intromitted and ejaculated, and the intercopulatory interval, the copulatory efficiency and the intromission ratio were calculated [14].

2.9 Statistical analysis

The data are expressed as mean±SEM. Statistical analyses were made using *G*- test (for quantal data), and Mann-Whitney *U*-test and Student's *t*-test wherever appropriate. Significance was set at *P* < 0.05.

3 Results

3.1 Adverse effects

Marked salivation [low dose: 6 out of 12 (50%) and high dose: 7 out of 17 (41%)], ataxia [low dose: 3 out of 12 (25%) and high dose: 5 out of 17 (29%)], mild diarrhoea [low dose: 4 out of 12 (33%) and high dose: 5 out of 17 (29%)], mild fur erection (in all treated rats), and exopthalmia [low dose: 1 out of 12 (8%) and high dose: 4 out of 17 (24%)] was evident. These effects appeared 2-3 h following each dose and persisted for 6-10 h. Nearly full recovery was evident for all the manifestations. Furthermore, all non-sexual behaviours observed were markedly impaired temporally. Treatment-related deaths: 2 with low dose and 5 with high dose.

3.2 Effects on fertility

Both low and high doses markedly suppressed all pre-coital sexual behaviours monitored on days 1, 3 and 7 of treatment. Furthermore, as shown in Figure 1, ICON caused a profound and significant (*P*< 0.05) suppression in the index of libido on days 1 (both doses) and 7 (high dose) of treatment. On the other hand, ICON did not significantly impair (*P*>0.05) other parameters of fertility during or after treatment.
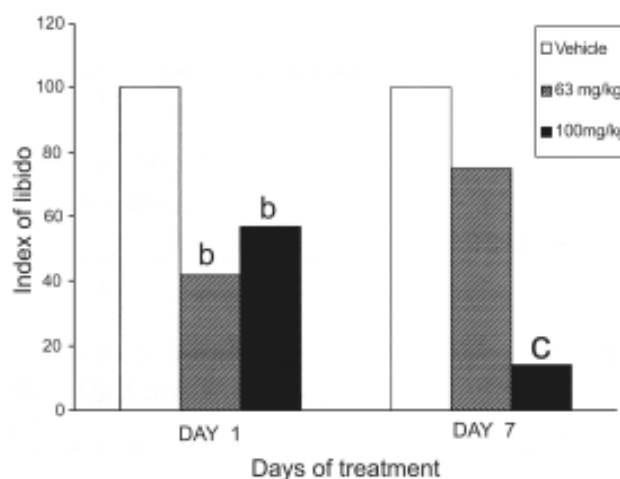

Figure 1.   Effect of 7 day oral administration of ICON on index of libido in male rats. [b]*P* < 0.05, [c]*P* <0.01, *G*-test.

3.3 Rectal temperature

ICON treatment did not significantly (*P*> 0.05) alter rectal temperature (data not shown).

3.4 Food and water intake

ICON treatment caused a significant (*P*<0.05) reduction in food intake (26-72%) on day 2 [daily food intake in the control, low dose and high dose groups being 22.2±1.0, 13.5±0.7 (by 39%), and 8.8±1.1 g (by 60%), respectively], and day 5 [23.2±0.8, 17.2±1.3 (by 26%), and 6.5±1.2 g (by 72%), respectively]. The effect appears to be dose-dependent. ICON had no significant (*P*>0.05) effect on water intake (data not shown).

3.5 Body weight

A significant (*P*<0.05) suppression in body weight gain was evident in rats treated with ICON (weight gain of the control, low dose and high dose groups being +4.1±2.9, -6.6±4.2, and -34.1±6.3 g, respectively).

3.6 Haematology

Both doses had no significant effect on WBC and differential counts (data not shown). However, high dose of ICON significantly lowered (*P*<0.05) the RBC count [the control and high dose groups being 10.7±0.4 and 9.2±0.3×10^6 cells/mm^3 (by 14%), respectively], the PCV [45.0±1.0 and 37.6±1.4 % (by 16%), respectively], the

MCHC [28.6±1.1 and 39.4±2.9 pg (by 38%), respectively] and the MCV [43.3±1.6 and 37.9±1.6 mm^3 (by 12%), respectively].

3.7 Sedative effect

As shown in Table 1, low dose of ICON significantly ($P<0.05$) impaired the locomotor activity (by 40%). On the other hand, the high dose significantly ($P< 0.05$) inhibited all the 3 parameters monitored: number of rears (by 63%), locomotor activity (by 45%), and number of head dips (by 43%).

Table 1. Effect of ICON on sedative parameters with rat hole-board technique. Range in parenthesis.

	Locomotor activity	Number of rears	Number of head dips
Vehicle (Distilled water)	19.9 ± 2.7 (9 - 40)	17.8 ± 4.0 (6 - 46)	8.3 ± 1.1 (4 - 14)
ICON 62.5 mg/kg	11.9 ± 2.4[b] (5 - 28)	14.8 ± 2.4 (4 - 22)	5.9 ± 0.8 (3 - 11)
100mg/kg	10.9 ± 1.1[b] (5 -16)	6.6 ± 1.2[b] (2-10)	4.7 ± 0.7[b] (2- 8)

As compared with control: [b] $P < 0.05$, [c] $P < 0.01$ (Mann-Whitney *U*-test, Student *t*-test or *G*-test).

3.8 Muscle strength and co-ordination

ICON did not significantly ($P> 0.05$) influenced the reaction time both in the Bar Holding and the Bridge tests (data not shown).

3.9 Sexual behaviour

Table 2 summarizes the results obtained with the sexual behaviour study. Most of the effects on the sexual behaviour were evident on days 1 and 3 of treatment and some on day 7. The number of rats attempting mounting (on day 1: low dose by 33%; high dose by 47% and on day 3: low dose by 30%; high dose by 54%), intromission (on day 1: low dose by 33%; high dose by 47% and on day3: low dose by 30%; high dose by 54%) and ejaculation (on day 1: low dose by 58%; high dose by 59% and on day 3: low dose by 30%; high dose by 54%) were significantly ($P<0.05$) impaired. The time lags required for the initiation of each of these events were also significantly ($P<0.05$) prolonged (on day 1, mounting: low dose by 249% and high dose by 294 %, intromission: low dose by 231% and high dose by 266%, and ejaculation: low dose by 43% and high dose by 48%; on day 3, mounting: low dose by 540% and high dose by 905 %, intromission: low dose by 540 % and high dose by 905%,

and ejaculation: low dose by 63% and high dose by 75%; and on day 7, mounting: high dose by 238% and ejaculation: low dose by 78%; high dose by 69%). However, the frequency of mounting (by 57%) and intromission (by 56%) were inhibited significantly ($P<0.05$) only with the high dose and only on day 1 of treatment. Further, both the low and high doses of ICON significantly ($P<0.05$) reduced the copulatory efficiency and intromission ratio. In contrast, intercopulatory interval was not significantly ($P>0.05$) changed by ICON treatments.

4 Discussion

In this study we used the trade product of lambda-cyhalothrin, ICON, which contains 10% of the active ingredient in an inert wettable powder. It is the form of pesticide that is currently used as an indoor spray in Sri Lanka providing potential for human exposure. Several other investigations also used trade products of pesticides in their studies [5,15].

The results showed that at the experimental conditions and doses used, the acute oral administration of ICON had no effect on ejaculatory competence (in terms of vaginal sperm counts), sperm quality (in terms of teratozoospermia), fertility (in terms of number of uterine implants, quantal pregnancy or implantation index), and pre- and post-implantation losses. Furthermore, unimpaired ejaculated sperm density in treated rats throughout the spermatogenic cycle [16] indicates that ICON is unlikely to interrupt testicular sperm production. This is an interesting and an important finding because exposures to many pesticides are known to cause problems related to male fertility [1-5,9]. Whilst with ICON fertility remained unaffected even when transient but distinct signs of toxicity (reduction in food intake, diarrohea, suppression in body weight gain, ataxia, lethargy, sedation, haemotoxicity) were evident.

In contrast, ICON caused a marked, although a transient inhibition of sexual competence *par se*. This is a novel finding, which indicates a possible risk of sexual dysfunction in ICON-exposed men. Irrespective of the dose, the antimasculine effect had a very rapid onset (within 5 h) and their severity decreased with repeated administrations. This was an unexpected finding. Such an action may be the result of rapid clearance through the induction of liver enzymes [6,17] as liver is the main site of ICON catabolism [6]. ICON is known to increase the liver weight [18] and the activity of the xenobiotic-metabolizing enzyme, amino pyrione-*N*-demethylase [6]. Receptor desensitization [19] is another possibility but seems unlikely to be operative here, in view of the reported modes of action of ICON and due to the

Table 2. Effect of ICON on sexual behaviour of male rats. Mean±SEM. [b]$P < 0.05$, [c] $P < 0.01$, compared with control. Range in parenthesis.

			Treatment		Post-treatment
		Day 1	Day 3	Day 7	Day 7
% mounted	Vehicle	100	100	100	100
	62.5 mg/kg	67[b]	70[b]	100	100
	100 mg/kg	53[b]	46[c]	81	100
% intromitted	Vehicle	100	100	100	100
	62.5 mg/kg	67[b]	70[b]	80	100
	100 mg/kg	53[b]	46[c]	81	100
% ejaculated	Vehicle	100	100	100	100
	62.5 mg/kg	42[c]	70[b]	80	100
	100 mg/kg	41[c]	46[c]	72[b]	100
Number of mounts	Vehicle	13.3 ± 2.0 (5 - 24)	11.8 ± 2.2 (8 - 21)	13.3 ± 2.5 (5 - 23)	13.8 ± 1.5 (9 - 19)
	62.5mg/kg	9.2 ± 2.3 (0 - 23)	11.4 ± 3.3 (0 - 32)	13.2 ± 1.0 (10 - 15)	11.7 ± 3.6 (5 - 21)
	100 mg/kg	5.7 ± 1.6[c] (0 - 18)	6.1 ± 2.8 (0 - 26)	7.6 ± 1.9 (0 - 18)	13.0 ± 1.4 (8 - 18)
Number of intromissions	Vehicle	13.1±1.9 (5 - 24)	11.7 ± 2.2 (8 - 21)	13.1 ± 2.6 (5 - 23)	13.7 ± 1.6 (8 - 19)
	62.5 mg/kg	9.0 ± 2.2 (0 - 22)	11.4 ± 3.3 (0 - 32)	11.2 ± 2.4 (0 - 15)	11.5 ± 3.6 (5 - 21)
	100 mg/kg	5.7 ± 1.6[c] (0 - 18)	6.1 ± 2.8 (0 - 26)	7.2 ± 1.8 (0 - 17)	12.9 ± 1.5 (8 - 18)
Mount latency	Vehicle	131.0 ± 32 (60 - 300)	60.0 ± 5.0 (60 - 90)	102.9 ± 29.2 (60 - 270)	95.0 ± 19.6 (60 - 180)
	62.5 mg/kg	457.5 ± 99.7[c] (90 - 900)	384.0 ± 114.0[b] (60 - 900)	288.0 ± 131.0 (60 - 900)	67.5 ± 7.5 (60 - 90)
	100 mg/kg	517.1± 91.0[c] (90 - 900)	603.0 ± 111.0[c] (60 - 570)	348.0 ± 125[b] (60 - 900)	77.1 ± 8.9 (60 - 120)
Intromission Latency	Vehicle	141.1 ± 30.6 (60 - 300)	60.0 ± 5.0 (60 - 90)	107.1 ± 29.9 (60 - 270)	100.0 ± 18.4 (60 - 180)
	62.5 mg/kg	467.5 ± 97.7[c] (90 - 900)	384.0 ± 114.0[b] (60 - 900)	310.0 ± 135.0 (60 - 900)	75.0 ± 8.7 (60 - 180)
	100 mg/kg	517.1[c] ± 91.0 (90 - 900)	603.0 ± 111.0[c] (60 - 570)	85.7 ± 102 (60 - 900)	366.1 ± 119[b] (60 - 120)
Ejaculation latency	Vehicle	516.7 ± 74.5 (150 - 810)	455.0 ± 78 (150 - 660)	390.0 ± 59.6 (150 - 660)	560.0 ± 97.6 (240 - 840)
	62.5 mg/kg	737.5 ± 66.5[b] (390 - 900)	741.0 ± 55.3[c] (510 - 900)	695.0 ± 110[b] (210 - 900)	465.0 ± 154 (150 - 870)
	100 mg/kg	764.1 ± 53.9[b] (390 - 900)	796.4 ± 56.5[c] (390 - 900)	660.0 ± 76.9[b] (240 - 900)	565.7 ± 68.8 (300 - 840)
Copulatory efficency	Vehicle	98.5 ± 1.0 (91 - 100)	98.9 ± 1.0 (93.8 - 100)	98.7 ± 1.3 (91 - 100)	98.9 ± 1.5 (91 - 100)
	62.5 mg/kg	65.1 ± 13.9[b] (0 - 100)	61.0 ± 15.9[b] (0 - 100)	81.1 ± 16.4 (0 - 100)	96.8 ± 2.7 (81 -98)
	100mg/kg	52.5 ± 12.4[c] (0 - 100)	45.5 ± 15.7[c] (0 -100)	66.7 ± 14.6 (0 - 100)	89.3 ± 1.8 \(83 - 100)
Intromission ratio (%)	Vehicle	49.7 ± 0.2 (48 -50)	49.7 ± 0.3 (48.4 50)	49.6 ± 0.3 (47.6 -50)	48.9 ± 0.3 (43.5 - 50)
	62.5mg/kg	32.9 ± 7.03[b] (0 - 50)	35.0 ± 7.6 (0 - 50)	41.0 ± 8.2 (0 - 50)	42.5 ± 3.2 (0 - 50)
	100 mg/kg	26.3 ± 6.2[c] (0 - 50)	22.7 ± 7.8[c] (0 - 50)	34.1 ± 7.5 (0 - 50)	39.9 ± 3.8 (17.3 - 83)
Inter copulatory interval	Vehicle	43.4 ± 7.3 (51 - 78)	44.2 ± 9.5 (14.3 - 71.3)	36.4 ± 8.6 (15.8 78)	42.6 ± 1.7 (38.4 - 80)
	62.5 mg/kg	32.8 ± 7.5 (0 - 75)	34.7 ± 10.7 (0 - 12.5)	43.4 ± 13.6 (0 - 90)	45.1 ± 3.4 (0 - 88.3)
	100 mg/kg	43.1± 14.0 (0 - 220)	48.0 ± 27.9 (0 - 300)	29.8 ± 10.4 (0 - 82.5)	38.2 ± 6.7 (12.5 - 90)

lack of a dose-dependent relationship.

Rats treated with ICON had markedly impaired libido as measured by suppression of pre-coital sexual behaviour (qualitatively), index of libido, and numbers of rat mounting, intromitting or ejaculating. The rapid onset and equally rapid reversibility of these effects suggest that the anti-libido action of ICON was not owing to changes in the blood testosterone or prolactin levels. Both testosterone deficiency [20,21] and hyperprolactaemia [21,22] inhibit libido. On the other hand, ICON, a pyrethroid II [6,7], can release GABA and dopamine [7]. GABA [23], GABA agonists [23,24], dopamine [25] or dopamine agonists [25] inhibits sexual behaviour in rats more or less in a similar fashion. Thus ICON may induce anti-libido effects probably via brain GABAergic and/or dopaminergic systems [24,25]. In addition, ICON through its neurotoxicity [6,7] could directly inhibit the sexual center in the hypothalamus [26] and thereby suppress libido. Thus, it is possible that ICON induced antilibido action also through sedation, since sedatives inhibit libido [27]. ICON treatment was associated with overt signs of stress. Further, ICON treatment in female rats increases adrenal weight and induces enlargement of adrenal cortex [18], possibly indicating glucocorticoid release as immediate increase in glucocorticoid levels occurs in stress [28] and glucocorticoides impair sexual behaviour [28,29]. Thus the antilibido effects in this study could also results from stress. Alternatively, ICON may induce antilibido effects through cholinergic mechanisms, as pronounced cholinergic side effects were evident in the study and cholinergic agonist suppress libido[30]. ICON treatment induced detrimental changes on health and behaviour, which are usually considered as manifestation of general toxicity. Thus, a strong possibility exists that ICON induced reduction of libido is secondary to its general toxicity.

ICON induced a marked prolongation in the latencies of mounting, intromission or ejaculation. This is indicative of inhibition of sexual arousability/motivation [31]. An inverse relationship exists between the latencies of these parameters and sexual arousability/motivation [22]. Two arousal mechanisms are believed to be involved in male copulatory behaviour of rats, a specific sexual arousability mechanism and a nonspecific arousal mechanism [33]. Reduction in both pre-coital and non-sexual behaviours (such as autogrooming, rearing) and induction of sedation in ICON treated rats suggest that both these arousal mechanisms may have been inhibited.

ICON also triggered a substantial impairment in mounting and intromission frequency and copulatory efficiency, which is suggestive of impaired sexual vigour [31,34]. In addition, ICON suppressed intromission ratio,

which reflects disruption in penile erections [24,31,34]. This may inhibit sexual performance as proper erections are essential for proper vaginal penetration [35]. However ICON had no effect on inter copulatory interval, implying that the sexual performance remains virtually unaltered [31,34].

In conclusion, this study shows, for the first time, that exposure to ICON pose a potential threat to sexual competence but not to fertility in male rats.

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