Haryana Vet. (Dec., 2019) **58(2),** 273-277

Research Article

TOXIC EFFECTS OF INDOXACARB ON RELATIVE BODY WEIGHT GAIN, REPRODUCTIVE ORGAN WEIGHT AND SPERMS OF MALE WISTAR RATS AFTER ORAL EXPOSURE FOR 28 DAYS AND UP TO DAY OF MATING WITH FEMALE RATS

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Received: 15.07.2017; Accepted: 03.07.2018

ABSTRACT

Indoxacarb is a non-systemic, synthetic novel oxadiazine pesticide used against a wide range of pests. In the present study indoxacarb was evaluated for its effects on relative body weight gain, reproductive organ weight and sperms parameters (sperm motility, sperm vitality and morphology) in male Wistar albino rats at two dose levels (6 and 12 mg/kg b.wt.) administered orally by gavage for 28 days and up to day of mating. The result of present study indicated that indoxacarb feeding in male rats significantly decreased relative body weight gain and reproductive organ weight, sperm motility, viability and increased sperm abnormality.

Keywords: Body weight gain, Indoxacarb, Reproductive organ weight, Sperms, Wistar rats

Indoxacarb (Indo) is first broad spectrum insecticide of oxadiazine group synthesized from pyrazoline class of compound (Wing *et al.*, 1998). It is used on vegetables to control lepidopterous insects, like moths, in their larval stages (USEPA, 2000). Indoxacarb's selective toxicity towards pests, and insects is due to its rapid bio-activation to active metabolites DCJW, while higher animals primarily degrade indoxacarb to inactive metabolites. It kills insects by inhibition of mammalian sodium channels which is voltage dependent, causing a significant hyperpolarizing shift in the voltage dependence of inactivation (Tsurubuchi and Kono, 2003). The injudicious use of this pesticide is likely to contaminate feed and fodder and to gain access to animals. Till now very scanty reports are available on indoxacarb governed reproductive toxicity in laboratory animals. The present study is aimed to evaluate effects of indoxacarb in male Wistar rats on relative body weight gain and reproductive organ weight, sperm motility, viability and sperm abnormality.

MATERIALS AND METHODS

In total, 36 adult male Wistar rats weighing between 100-174 g were used in this study. One male was used for mating with two females. Prior approval of Institutional Animal Ethics Committee of LUVAS Hisar was obtained for use of animals before the experiment was conducted.

Indoxacarb was administered in male rats daily by oral gavage at the dose rate of 6 mg/kg (1% of MTD = 600 mg/kg b.wt., Shit (2008)) and 12 mg/kg (2% of MTD) for four weeks prior to mating and up to the day of mating with indoxacarb treated females. The time interval of dosing beyond 28th day of dosing upto the day of mating was variable for each male rat. Control groups were administered distilled water (dw) orally daily by gavage. Each group of male rats comprised of twelve animals. Male rats were killed under ether anaesthesia after mating with females. Following parameters were studied.

Relative Body Weight gain: The body weight was recorded on day 0 and at the weekly interval during treatment of male rats for 28 days and up to the day of mating with female rats. Relative body weight gain of each male rat was determined on 7, 14, 21 and 28 days of treatment period and was expressed in g/100 g b.wt.

Relative weight of testes and epididymis: After sacrifice testis and epididymis of both sides were removed and weighed using digital weighing balance and expressed as g/100 g body weight of male rats.

Sperm Evaluation: Epididymal sperm suspension/ sperm aliquot were prepared by mincing cauda portion of epididymis in 3 ml phosphate buffered saline (PBS, pH = 7.4). The suspension was then filtered through muslin cloth. Following sperm parameters were studied.

(a) **Sperm motility (% Motility):** Manual motility estimation was done by Wet Mount technique which involves placing a drop of diluted semen from cauda epididymis in PBS on a glass slide and covered with cover slip. At least 200 spermatozoa were examined under light microscope at magnification of 40x. The results are expressed as % motility.

(b) **Sperm vitality (% of live sperm):** Eosin-Nigrosin stain was used to count dead and live spermatozoa. It is based on the principle that eosin stains the dead spermatozoa as pink because of damage of plasma membrane in case of dead sperms and nigrosin provide background for the live spermatozoa which remain unstained. The staining solution for the one

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step technique contained mixed solution of 5% aqueous Eosin-Y and 10% aqueous Nigrosin (Dougherty *et al.*, 1975). The result was expressed as percent of live and dead spermatozoa in a semen sample.

Procedure for Staining: Approximately equal volumes of caudal epididymal aliquot and stain were mixed. The suspension was incubated for 30 second at room temperature. After incubation, smear was prepared from this mixture on a clean and dry glass slide. Two smears were made from each sample. The smears were air dried and examined directly. At least 200 sperm were assessed at magnification of 100x under oil immersion. Sperms that were white (unstained) were classified as live and those that showed any pink or red colouration were classified as dead.

(c) Sperm morphology: Sperm head and tail are clearly visible under oil emersion lens when stained with Eosin-Nigrosin stain. Abnormal sperms were counted and percent abnormality was determined.

RESULTS AND DISCUSSION

The results were expressed as mean ± standard error followed by One way ANOVA along with Bonferroni multiple comparison tests using Graph Pad Prism Version - 5.03 software and Microsoft Excel ($p \leq 0.05$) was the critical criterion for the statistically significant differences between the data (Mead and Curnow, 1982).

1. Effect on relative body weight gain: The effects of indoxacarb on body weight and relative body weight gain of male Wistar albino rats at two dose levels are presented in Table 1.

The relative body weight gain was significantly ($p<0.05$) lower in male rats treated with Indo at 6 mg/kg b.wt. at 7, 14 & 21 days of treatment and in male rats treated with Indo at 12 mg/kg b.wt. at 7, 14, 21 & 28 days of the treatment as compared to control group. The decrease in relative body weight gain was dose dependent. This reduction might be due to decreased feed intake due to indoxacarb, as this drug might adversely affect the feeding centre in the brain which reduced the appetite of the animals and on motor co-ordination due to which access of animals to the feed get decreased (USEPA, 2006).

Reduction in body weight and body weight gain is used as an indicator for the deterioration of general health status of experimental rats and it serves as an index of growth rate in male rats (Palani *et al.*, 1999). It is evident that monitoring of body weight provides information on general health level of animals which can also be an important interpretation of reproductive effects (Aly *et al.*, 2009). The studies conducted by Malek (1997) in mice fed with diets containing 0, 35, 75, 150 or 300 ppm indoxacarb for 90 days showed reduced body weight gain, food consumption and food efficiency in males and females fed at 300 ppm. Similar findings were reported by Reynolds (1993) in mice fed with diets containing indoxacarb. In present study, indoxacarb showed negative effect on relative body weight gain in dose dependent manner.

2. Relative weight of testes and epididymis: The effects of indoxacarb on relative weight of testes and

Table 1

Toxic effects following oral exposure of male rats to indoxacarb in terms of body weight (g) and relative body weight gain (g/100 g b.wt.) (Mean±S.E., n = 12 in each group)

Days of treatment	Control (DW)	Indoxacarb (6 mg/kg b.wt)	Indoxacarb (12 mg/kg b.wt.)
Body Weight			
Starting day of dosing (day 0)	149.70±5.32	146.50±4.77	141.80±4.23
7th day	164.00±5.57	163.00[a]±5.76	162.00[a]±4.52
14th day	188.00±5.90	175.00[a]±4.70	174.00[a]±4.20
21th day	194.30±5.71	182.30[a]±4.37	176.20[a]±4.05
28th day	207.33±5.72	189.50[a]±4.01	184.58[a]±3.99
On the day of sacrifice	233.00±9.37	243.75±9.16	255.17±9.13
Relative body weight gain			
7 day	10.33±0.82	7.06[a]±1.10	6.18[a]±0.68
14 day	17.55±1.62	12.36[a]±1.25	11.37[a]±1.26
21 day	24.90±2.15	17.91[a]±1.44	15.75[a]±1.63
28 day	33.11±2.55	24.08±1.52	19.68[a]±2.19

a, b vs Control, Indo (6 mg/kg b.wt.), respectively ($p<0.05$)

epididymis are presented in Table 2. Relative weights of both right and left testis in different treatment groups were observed to be significantly ($p<0.05$) decreased at both doses of Indoxacarb as compared to control group in dose dependent manner. There was also significant ($p<0.05$) dose dependent decrease in relative weight of right testis within treatment groups.

Relative weights of both right and left epididymis decreased significantly ($p<0.05$) in male rats treated with Indoxacarb at 6 mg/kg b.wt. as compared to control group animals. There was significant ($p<0.05$) decrease in relative weight of both right and left epididymis in animals treated with Indoxacarb at 12 mg/kg b.wt. as compared to animals treated at 6 mg/kg b.wt. and animals of control group.

Relative weights of testis and epididymis may be utilized in evaluation of risks from toxic effects on male reproductive system (Clegg *et al.*, 2001). The reduced weight of testis in Indo-treated rats of present study might be due to decreased number of germinal cells and elongated spermatids in the testis, which caused reduction in the weight of testis (Chapin *et al.*, 1997). The decline in testicular weight in pesticides treated rats indicates impairment at testicular level (Chitra *et al.*, 1999). In addition, studies on insecticides also reported that insecticides reduce tubule size, arrest spermatogenesis and inhibit steroid biosynthesis of Leydig cells (Sujatha *et al.*, 2001). The epididymis and accessory sex organs are androgen-dependant organs and require a continuous androgenic stimulation for their normal growth and functions (Klinefelter and Hess, 1998). Moreover, decrease in weight of epididymis may be due to decrease in number of spermatozoa.

3. Sperm parameters: Sperm motility, vitality and abnormalities (Table 2) decreased significantly ($p<0.05$) in indoxacarb-treated male rats at both doses as compared to control. Significant ($p<0.05$) decrease in sperm parameters was observed in animals of treatment groups in a dose-dependent manner. This might be due to the oxidative stress in the testis (site of sperm production) and epididymis (storage site of sperm), spermatozoa and Leydig cells in mammals are rich in PUFAs, so Reactive oxygen species (ROS) easily attack the unsaturated bonds of the lipids of sperm membrane, and destroys the structure of lipid matrix in the membranes of spermatozoa, which causes rapid loss of intracellular ATP leading to damaged flagellum which is an important machinery for the sperm motility and it also directly impairing spermatogenic cell development, also impair maturation or spermiation, decreased sperm viability and increased sperm abnormalities. Various types of morphological alterations of sperms were observed absent, asbent head, apical head, coiled tail, bifided head, coiled over middle piece, pin head, bent neck, spoon head, prism head, double head, bifurcated tail, flat head with protoplasmic droplet, bent tail, hook shaped head and amorphous head as depicted in Fig. 1.

Some studies reported a significant reduction in the number of motile sperms with a considerable increase in the percentage of dead sperm in the cases with chronic insecticide exposure (Bustos and Gonzalez, 2003 and Saiyed *et al.*, 2003). A decrease in sperm motility may seriously reduce fertilizing ability (Bedford, 1983), which is related to low level of ATP content (Bai and Shi, 2002). Decreased sperm motility, vitality and increased sperm abnormalities in dose dependent manner in response to oral exposure of Indoxacarb in the present study are in accordance with the findings of some other researchers investigating effects of pesticides on reproductive system (Zhang *et al.*, 2011 and Nigam, 2015).

Table 2

Toxic effects following oral exposure of male rats to indoxacarb reproductive parameters (Mean ± S.E., n= 12 in each group)

Parameter	Control (DW)	Indoxacarb (6 mg/kg b.wt.)	Indoxacarb (12 mg/kg b.wt.)
Relative organ weight (g/100 g b.wt.)			
Right testis	0.75±0.059	0.53^{a}±0.066	0.43^{ab}±0.021
Left testis	0.71±0.035	0.46^{a}±0.036	0.45^{a}±0.027
Right epididymis	0.22±0.010	0.18^{a}±0.010	0.14^{ab}±0.005
Left epididymis	0.21±0.011	0.18^{a}±0.009	0.14^{ab}±0.004
Sperm Parameters (Per cent)			
Sperm Motility (motile sperm)	76.91±0.67	65.37^{a}±0.88	55.14^{ab}±1.45
Sperm vitality (live sperm)	84.59±0.44	70.08^{a}±0.82	61.15^{ab}±1.28
Sperm morphology (abnormal sperm)	7.50±0.33	11.08^{a}±0.35	13.08^{ab}±0.28

a, b vs Control, Indoxacarb (6 mg/kg b.wt.), respectively ($p < 0.05$)

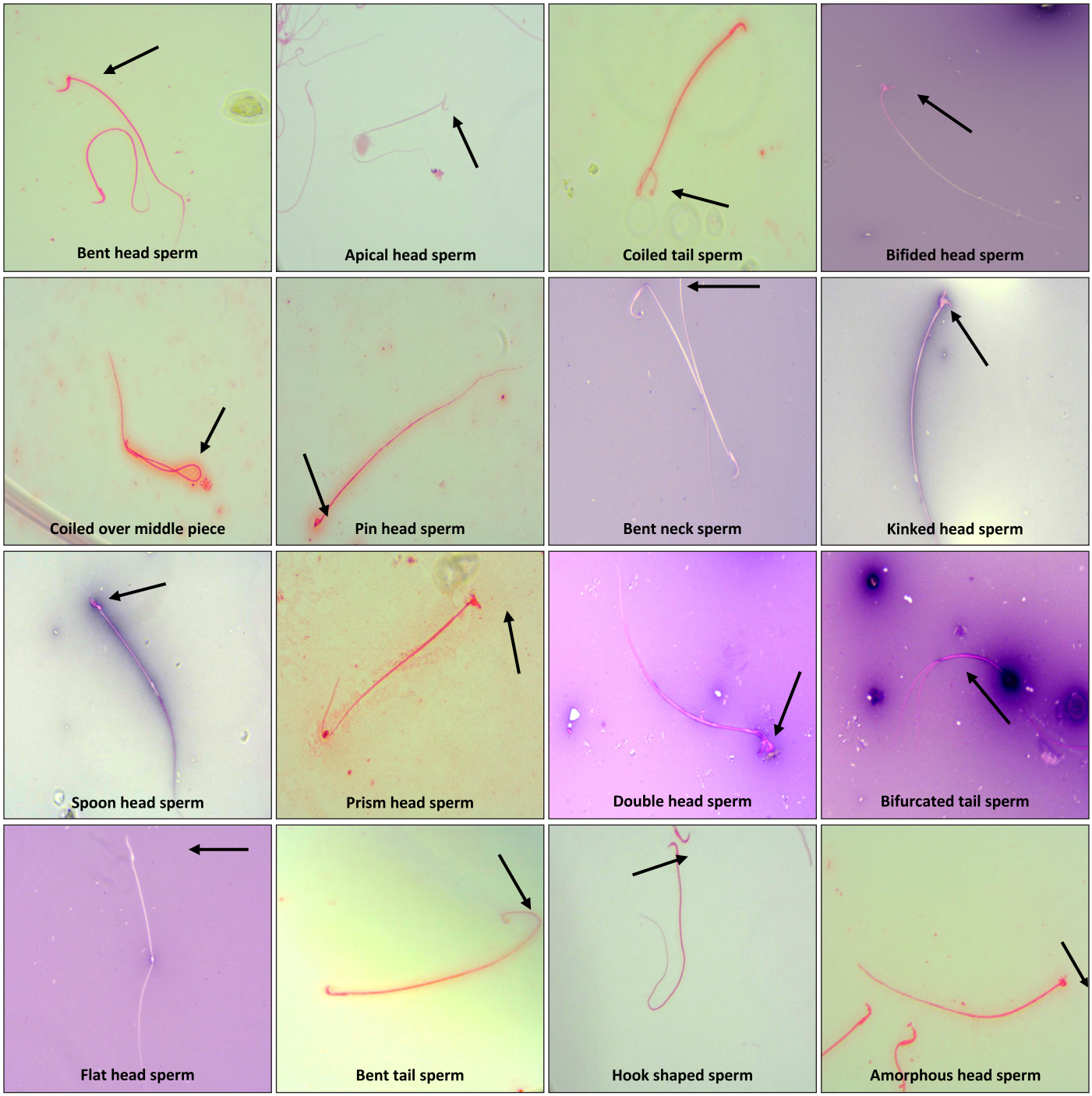

Fig. 1. Representative pictures showing some common abnormalities in the morphology of sperm of indoxacarb treated animals.

Therefore, it can be concluded that indoxacarb feeding at the dose rate of 6 mg/kg and 12 mg/kg b. wt. for 28 days showed negative effects on relative body weight gain, relative reproductive organ weight and on sperms parameters of male Wistar albino rats.

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