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Research Article

DETERMINATION OF MAXIMUM TOLERATED DOSE AND SUBACUTE TOXIC EFFECT OF INDOXACARB ON BODY WEIGHT AND ORGAN WEIGHT OF FEMALE WISTAR RATS AND ITS AMELIORATION WITH QUERCETIN

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ABSTRACT

In this study, the subacute toxic effect of indoxacarb on body weight and organ weight was determined at its two doses i.e., 2.5% of maximum tolerated dose (MTD; 3.1 mg/kg b.wt.) and 5% of MTD (6.2 mg/ kg b.wt.) in female Wistar rats and ameliorative effect of quercetin was studied at 100 mg/ kg body weight. Control group was administered distilled water at the dose of 1ml/200g body weight. Another group received quercetin at dose of 100 mg/ kg body weight. Female rats were treated daily over a period of 14 days and 28 days. Female Wistar rats (n=144) were divided in 6 groups, each group comprising of 24 rats. Twelve rats from each group were killed on 15th and 29th days of experiment. A significant decreasing trend in body weight gain was observed in indoxacarb treated rats at both (3.1 mg/ kg b. wt. and 6.2 mg /kg b.wt.) dose levels after 14 day and 28 day treatment. A significant increase in relative liver and spleen weight in female rats treated with indoxacarb at 6.2 mg/ kg b. wt for 14 days and 28 days was observed as compared to control and quercetin treated rats. The relative wt. of various other organs viz. uterus, ovary, adrenal gland, heart, and kidney did not show any change in relative organ weight in both treatment schedules. Quercetin showed significant ameliorative effect on decrease in body weight gain after 28 days treatment at both doses of indoxacarb. While no significant ameliorative effect was observed on relative weight of liver and spleen.

Key words: Indoxacarb, MTD, body weight, organ weight

Indoxacarb (INDO) is the first commercialized insecticide of the oxadiazine group. It acts by blocking the sodium channel in insect neuron and was designated as a reduced risk product by the Environment Protection Agency (Anon, 1998; McCann *et al*., 2001). It has been used for controlling insect pests of cotton, fruits and vegetables in many countries. It is also effective as insecticidal baits against cockroaches and ants.

Quercetin (3, 32 ,42 , 5-7-pentahydroxyflavone; Qu), is a flavonol and is widely distributed in plants, and is probably the most abundant of the flavonoid molecules in the plant kingdom. It possesses a catalogue of pharmacological actions, including cardio-protection, cataract prevention, anti-cancer activity, anti-ulcer effects, anti-inflammatory, anti-allergic, antiviral and antibacterial activities, and so forth (Bronner and Landry, 1985; Stavric, 1994). Therefore, quercetin may have ameliorative effect on subacute toxicity due to indoxacarb in rats.

In an effort to get more agriculture produce the injudicious use of this pesticide is likely to contaminate feed and fodder as residue and to gain excess to animals and therefore, needs to be evaluated for its toxicological potentials. Perusal of literature shows very few toxicity studies on indoxacarb and no study on the ameliorative effect of quercetin in indoxacarb induced toxicity. Therefore, it is imperative to study the subacute toxic effect of indoxacarb alone and in presence of quercetin.

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MATERIALS AND METHODS

Female Wistar rat weighing 120-140 g were procured from Disease Free Small Animal House, LUVAS, Hisar. Prior approval of Institutional Animal Ethical Committee was obtained for the use of animals in this study. These were housed in the Departmental Animal House in polyacrylic cages. The animals were provided with feed and water *ad libitum* and were maintained at 22 to 27°C with a natural light dark cycle. The animals were acclimatized to laboratory conditions for 7 days before the experiment.

Indoxacarb (Kingdoxa®), purchased from local market, was used in the present study. Its aqueous suspension was prepared in distilled water and was administered orally at 3.1 mg/ kg b.wt. (2.5% of maximum tolerated dose; MTD) and 6.2 mg/ kg b.wt. (5.0% of MTD). Quercetin at a dose rate of 100 mg/kg b. wt. was given orally as a co-treatment with both doses of indoxacarb. The aqueous suspension of quercetin was prepared with vigorous trituration and stirred homogenously before administration.

Determination of MTD: MTD was determined in female Wistar rats through oral route following the method of Moser and Padilla (1997). For this, the pilot dose range finding study was conducted in rats (n=3) using several doses including lethal dose. Each group of rats was administered single oral dose. Out of these doses, a maximum dose was selected which could produce clear sign of toxicity but not result in lethality i.e. maximum tolerated dose.

Subacute Toxicity of Indoxacarb and Amelioration with Quercetin: Subacute toxicity of indoxacarb and ameliorative effect of quercetin was studied in 144 adult female rats weighing between 120-140 g. Rats were divided in 6 groups, each comprising of 24 rats. A gap of 12 h was maintained between quercetin and indoxacarb administration. Twelve rats from each group were killed on 15th and 29th days of treatment to collect the blood and different vital organs for further toxicological studies. Following parameters were studied.

Relative Body Weight Gain: Body weight of female rats were recorded on day 0 and at an interval of two days till the completion of experiment and relative weight gain was expressed as gram/100g b. wt. at the end of the study. Relative weight gain (g/100g) = [Final body weight (g) - Initial body weight (g)]/ Initial body weight (g) × 100

Determination of Relative Organ Weight: After killing of animals under ether anaesthesia, vital organs viz. liver, heart, kidney, spleen, ovary, uterus, and adrenal gland were excised free from surrounding tissues and were weighed individually. Relative organ weights were calculated as: Relative organ weight (g/100g body weight)= Organ weight (g)/Body weight (g) × 100

Statistical Analysis: The results were expressed as mean ± standard error (S.E.) followed by one way ANOVA along with Bonferroni multiple comparison tests using Graph Pad PrismVersion-4.0 software and Microsoft Excel. P≤0.05 was the critical criterion for the statistically significant differences between the data.

RESULTS AND DISCUSSION

The dose range values and corresponding mortality data in determining the MTD of indoxacarb in female rats by conducting pilot dose range finding study are as presented in Table 1. Indoxacarb treated rats showed a dose dependent onset and severity of toxic symptoms. Grossly observable symptoms after indoxacarb administration started with staggering gait, motor incoordination and prostration. Vigorous rolling, salivation, restlessness, head tilt, intermittent clonic convulsions, depression, open mouth breathing and death were also seen. Animals were observed daily up to 14 days and they showed decrease in body weight gain, normal activity and feed consumption. Indoxacarb showed delayed toxicity, mortality was observed on day second to seventh post single dose administration. On the basis of mortality data (Table 1), the MTD was determined as 125 mg/ kg b. wt. in adult female rats. Sarver (1996) reported oral median lethal dose (LD_{50}) of indoxacarb technical grade as 1730 and 267 mg/kg b.wt.in male and female rats, respectively. However, initial reports of acute toxicity of pyrazoline-type insecticides suggested that they were very neurotoxic, with acute oral LD_{50} values in excess of 3000 mg/kg b. wt. in rats (Jacobson, 1990). Shit (2008) found 24 hours oral MTD of indoxacarb to be 500 mg/ kg b. wt. in male mice and 600 mg/kg b.wt. in male rats.

Effect of sub acute oral exposure of female rats to indoxacarb, quercetin and their combination on relative body weight gain (g/100g b.wt.) of female rats in different treatment groups of both treatment schedules are presented in Table 2. The body weight gain serves as an index of growth rate (Palani *et al.,* 1999). From many decades several studies have revealed that pesticides adversely affect the body weight gain (Makita *et al*., 2003; Sharma *et al*., 2005; Brkic *et al*., 2008; Bhardwaj *et al*., 2010; Bal *et al.,* 2012). Significant decreasing trend in body weight gain was observed at both dose levels of indoxacarb i.e. 3.1 mg/ kg b.wt. and 6.2 mg/ kg b.wt. administered daily for 14 days and 28 days. This may be due to decreased feed intake as a result of toxic effect of indoxacarb on motor co-ordination due to which access of animals to the feed must have abandoned or feeding centre in brain which probably reduced appetite of the animal. However, these findings are in accordance with earlier reports in mice by Reynolds (1993a) and

Table 1
Maximum tolerated dose (MTD) of indoxacarb administered orally in adult female wistar rats

Dose of indoxacarb (mg/ kgbody weight)	Number of rats died/ Number of rats administered	Percent mortality (%)
1000	3/3	100
600	3/3	100
500	3/3	100
400	3/3	100
300	2/3	66.6
200	2/3	66.6
150	1/3	33.3
125	0/3	0
125	0/3	0
100	0/3	0

Table 2
Effect of sub acute oral exposure of indoxacarb, quercetin and their combination on relative weight gain (g/100 g b.wt.) in adult female rats

Parameters	No. of days	Control (D.W.)	2.5% MTD of INDO (3.1 mg/kg b.wt.)	5% MTD of INDO (6.2mg/ kg b.wt.)	Quercetin (100 mg/kg b.wt.)	INDO (3.1 mg/ kg b.wt.)+Qu (100 mg/kg b.wt.)	INDO (6.2 mg/kg b.wt.) +Qu (100 mg/ kg b.wt.)
Relative weight	14	19.43±1.66	13.85^{a}±1.30	8.99^{ab}±1.23	23.45^{bc}±2.14	17.01^{cd}±3.43	14.53^{cd}±0.98
gain (g/100 g b. wt.)	28	32.37±1.28	25.33^{a}±2.80	20.64^{ab}±0.77	49.92^{abc}±2.69	30.85^{bcd}±2.49	27.04^{acd}±3.92

Values are expressed as mean ± SE of 12 animals in each group. INDO=Indoxacarb; QU=Quercetin
a, b, c, d, e ($p \leq 0.05$) vs Control

Malek (1997a), in rats by Shit (2008), Reynolds (1993b) and Malek (1997b) and in dogs by Mertens (1997). Quercetin co-treatment resulted in significant ameliorative effect on body weight gain at both doses as compared to respective indoxacarb treated groups in 28 days treatment schedule. In 14 days treatment schedule significant ameliorative effect was observed at higher dose treated with quercetin in comparison to rats treated with indoxacarb at higher dose.

Effect of sub acute oral exposure of indoxacarb, quercetin and their combination on relative organ weight of animals of different treatment groups are expressed in organ weight in g/100g b. wt., as presented in Table 3. Significant increase in relative liver weight in animals treated with INDO at 6.2 mg/kg b.wt. for 14 days and 28 days was observed as compared to control and quercetin treated rats. The increased weight of liver may be due to induction of metabolizing enzyme system. Our findings are in agreement with the study of Shit (2008) in which hepatocellular hypertrophy was found due to exposure to indoxacarb.

There was a significant increase in relative spleen weight in animals treated with indoxacarb at 6.2 mg/ kg b.wt. for 14 days and 28 days as compared with control and quercetin treated animals as evidenced by gross pathological observations showing increased spleen size in indoxacarb treated female rats. A similar effect of increase in spleen weight was reported in male rats in which sub-acute toxicity at 12 mg/kg and 24 mg/kg was studied for a period of 28 days in male rats by Shit (2008),

Table 3
Effect of sub acute oral exposure of indoxacarb, quercetin and their combination on relative organ weight (g/100g b. wt.) of adult female rats

Parameters (g/100 g b. wt.)	No. of days	Control (D.W.)	2.5% MTD of INDO (3.1 mg/kg b.wt.)	5% MTD of INDO (6.2mg/ kg b.wt.)	Quercetin (100 mg/kg b.wt.)	INDO (3.1 mg/ kg b.wt.)+Qu (100 mg/kg b.wt.)	INDO (6.2 mg/kg b.wt.) +Qu (100 mg/ kg b.wt.)
Liver	14	3.54±0.11	3.86±0.06	3.99a±0.08	3.49bc±0.11	3.61c±0.10	3.78±0.06
	28	3.34±0.08	3.75±0.13	4.20ab±0.04	3.34c±0.07	3.49c±0.09	3.84a±0.21
Heart	14	0.37±0.008	0.36±0.006	0.36±0.020	0.37±0.007	0.36±0.008	0.36±0.005
	28	0.35±0.007	0.35±0.011	0.34±0.009	0.36±0.009	0.37±0.015	0.36±0.005
Spleen	14	0.25±0.016	0.27±0.012	0.29a±0.017	0.25c±0.005	0.26±0.004	0.27±0.011
	28	0.24±0.013	0.27±0.009	0.31ab±0.011	0.25c±0.006	0.26c±0.006	0.28a±0.008
Uterus	14	0.19±0.013	0.19±0.005	0.20±0.005	0.19±0.005	0.19±0.019	0.20±0.009
	28	0.20±0.009	0.21±0.006	0.23±0.009	0.20±0.004	0.21±0.006	0.21±0.005
Right kidney	14	0.30±0.0037	0.31±0.0067	0.32±0.0145	0.30±0.0062	0.30±0.0033	0.31±0.0092
	28	0.27±0.0059	0.29±0.0079	0.30±0.0066	0.28±0.0030	0.29±0.0055	0.29±0.0059
Left kidney	14	0.29±0.0131	0.30±0.0054	0.30±0.0155	0.29±0.0099	0.29±0.0201	0.30±0.0034
	28	0.27±0.0074	0.29±0.0065	0.30±0.0066	0.30±0.0095	0.29±0.0087	0.30±0.0054
Left ovary	14	0.036±0.003	0.035±0.003	0.034±0.002	0.036±0.002	0.035±0.001	0.035±0.001
	28	0.037±0.008	0.036±0.004	0.035±0.002	0.038±0.003	0.037±0.003	0.036±0.002
Right ovary	14	0.035±0.004	0.035±0.002	0.034±0.001	0.036±0.002	0.035±0.001	0.035±0.001
	28	0.037±0.005	0.037±0.003	0.035±0.002	0.038±0.003	0.037±0.004	0.036±0.001
Left adrenal gland	14	0.017±0.0018	0.017±0.0006	0.016 ±0.0011	0.016±0.0009	0.016±0.0007	0.016±0.0009
	28	0.018±0.0009	0.017±0.0009	0.017±0.0006	0.018±0.0021	0.018±0.0005	0.017±0.0017
Right adrenal gland	14	0.016±0.0032	0.016±0.0007	0.016±0.0025	0.017±0.0005	0.016±0.0006	0.016±0.0002
	28	0.017±0.0010	0.016±0.0011	0.015±0.0005	0.017±0.0005	0.016±0.0016	0.016±0.0009

Values are expressed as mean ± SE of 12 animals in each group
a, b, c, d, e ($p<0.05$) vs Control, INDO (3.1 mg/kg b.wt.), INDO (6.2 mg/kg b.wt.), Qu, INDO (3.1 mg/kg b.wt.)+Qu, respectively

and in long term toxicity study of indoxacarb in rats by Frame (1997) which suggested that this may be due to secondary physiological responses to the increased RBC turn over. The relative weight of other organs viz. uterus, ovary, adrenal gland, heart, and kidney was not changed among all treatment groups of both the treatment schedules.

Indoxacarb produced significant toxic effects in liver and spleen, and quercetin co-treatment could not decrease relative organ weight of liver and spleen. Therefore, it is concluded that the maximum tolerated dose of indoxacarb determined in adult female Wistar rats was 125 mg/kg body weight. Quercetin showed significant ameliorative effect on decrease in body weight gain after 28 days treatment at both lower dose and higher dose of indoxacarb. While no significant ameliorative effect was observed on relative weight of liver and spleen.

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