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Response of prolactin cells of stinging catfish, *Heteropneustes fossilis* to *Euphorbia royleana* treatment

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Abstract
Effect of *Euphorbia royleana* on prolactin cells were investigated in *Heteropneustes fossilis.* Fish were subjected to 2.47mg/L and 0.618 mg/L of *Euphorbia royleana* for short- and long-term, respectively. Blood was collected on 24, 48, 72 and 96 h in short-term and after 7, 14, 21, and 28 days in long-term experiment and was analyzed for serum calcium levels. Pituitary glands were fixed on 24, 48, 72 and 96 h in short-term and after 7, 14, 21, and 28 days in long-term experiment. Serum calcium levels of treated *H. fossilis* declined progressively from 48 h till 96 h. Latex of *Euphorbia royleana* provoked a decrease in serum calcium level on day 7 which progresses till 28 days. No histological alteration was observed in prolactin cells throughout the short- term exposure of *Euphorbia royleana*. Nuclear volume of prolactin cells exhibited an increase after 14 day. Also these cells exhibited degranulation. These changes increased progressively 21 day onwards. Degenerating cells were discerned after 28 day following the treatment.

Keywords: Botanical pesticide, Calcium, Teleost, Pituitary gland.

1. Introduction
Due to the prohibitive cost of synthetic pesticides and hazards caused by their persistence in the environment, there is a growing interest in the use of botanical pesticides for crop protection. Botanical pesticides are phytochemicals that have evolved in plants for defence against phytophagous insects [1, 2]. Some plants contain components that are toxic to animals. Nicotine, rotenone and pyrethrins have been widely used in both small-scale subsistence farming as well as in commercial agriculture. Therefore, they are not very selective but target a broad range of insects. This means that even beneficial and non target organisms can be affected. Furthermore, botanical pesticides are generally highly bio-degradable, so that they become inactive within hours or a few days. This reduces again the negative impact on beneficial organisms [3, 4]. However, despite being natural and widely used in agricultural systems, some botanicals may be dangerous for humans and they can be very toxic to natural enemies. For example nicotine, derived from tobacco plant, is one of the most toxic organic poisons for humans and other warm-blooded animals [5, 6]. Before a new botanical pesticide applied in large scale, its effect on ecosystem should be tested in a small field experiment.
The chemical constituents of plants of the Euphorbiaceae family include phenolic compounds (flavonoids, coumarins, lignans, tannins, quinones, phenanthrenes, phenolic acids, etc.), triterpenoids and related compounds (alcohols, sterols and hydrocarbons), alkaloids, cyanogenic glucosides and glucosinolates [7]. Various parts of *Euphorbia royleana* have insecticidal and molluscicidal properties [8, 9]. The latex of *Euphorbia royleana* has been reported as an irritant to the eye and skin [10].
Removal of pituitary gland caused a decline in the serum/plasma calcium level of freshwater fish [11, 12]. Hypophysectomized killifish kept in calcium-deficient seawater exhibited hypocalcemia [13] suggesting that killifish pituitary possess a hypercalcemic factor. Recently, a role of prolactin during organogenesis in Zebra fish [14] and in nuptial colouration in female fish has been reported Skold *et al.* [15].
Several studies were carried on the impact of environmental toxicants on fish, e.g., behavioural responses [16, 17], histopathology of vital organs [18-21], hematological anomalies [22 -27], but there exists no information regarding the impact of botanical pesticides (excluding synthetic pyrethroids) on the prolactin cells of the fish. Keeping this in view an attempt has been made to study the effects of latex of *Euphorbia royleana* on the histological alterations in the prolactin cells of a teleost, *H. fossilis.*

2. Materials and Methods

Adult freshwater teleost *Heteropneustes fossilis* (both sexes body weight 27 – 38 g) were collected from Ramgarh Lake Gorakhpur, India, Latitude- 26.765844 and Longitude- 83.364944. Healthy fish showing no external signs of injury and disease were selected for experiments and were acclimatized to laboratory conditions (under natural photoperiod 11.46 – 12.18 and temperature 26.74 ± 2.11 ^{0}C; pH 7.26 ± 0.09; hardness 167.97 ± 5.69 mg/L as $CaCO_3$; dissolved oxygen 7.85 ± 0.36 mg/L) for 15 days in dechlorinated tap water. The white milky latex of *Euphorbia royleana* was drained into glass tubes by cutting the stems and bark. The latex was lyophilized at -40 °C and the lyophilized powder was stored at -20 °C under dark until further use.

In the present study, latex of *Euphorbia royleana* was used. The 96 h LC_{50} value of latex of *Euphorbia royleana* (3.090 mg/L for the fish *H. fossilis*) have been reported by Prasad *et al.* [28]. Latex of *Euphorbia royleana* was weight and stock solution (4 mg/ml) was prepared in 100% ethanol. In short term exposure the fish were subjected to sub-lethal dose 2.47mg/L of latex of *Euphorbia royleana* (80% of 96 h LC_{50} value). In long term exposure the fish were subjected to 0.618 mg/L (20% of 96 h LC_{50} value) of latex of *Euphorbia royleana*. Simultaneously, a control group was also run for comparison by using the tap water containing ethanol (ethanol was used as the latex of Euphorbia was dissolved in ethanol so in control also ethanol was added). Fish were kept in groups of 10 in 40 L media. Six fish were sacrificed on each time intervals from control and experimental (*Euphorbia royleana*) groups after 24, 48, 72 and 96 h in short-term exposure and after 7, 14, 21 and 28 days in long term experiment.

Blood samples were collected by sectioning of the caudal peduncle of fish. The sera were separated by centrifugation at 3500 r. p. m. and analyzed for calcium levels (calcium kit, RFCL Limited India). After collection of blood samples, the pituitary glands along with the brain were fixed in aqueous Bouin's fluid and Bouin's-Hollande fixatives for histological studies. Tissues, thus fixed were routinely processed in graded series of alcohols, cleared in xylene, and then embedded in paraffin wax and serial sections were cut at 6 µm. The pituitaries were stained with Herlant tetrachrome and Heidenhain's azan techniques.

Nuclear indices (maximal length and maximal width) of prolactin cells were determined (50 nuclei were measured per specimen; thus 300 nuclei were measured from six specimens) were taken with the aid of ocular micrometer and then the nuclear volume was calculated as volume = 4/3 π ab², where 'a' is the major semiaxis and 'b' is the minor semiaxis.

All samples were estimated in duplicate. All data were presented as the mean ± S.E. of six specimens and student's 't' test was used for the determination of statistical significance. In all studies, the experimental group was compared to its specific time control group. Two-way Analysis of Variance (ANOVA) was used for multiple group comparisons.

3. Result

No alteration has been noticed in the serum calcium levels of *H. fossilis* at 24 h following the *Euphorbia royleana* exposure. The levels decline progressively from 48 h till 96h. (Fig. 1). Analysis of variance indicated that the level of serum calcium were significantly different between groups (between intervals F = 20.80, P<0.0001 between treatment F = 169.51, P<0.0001).

Latex of *Euphorbia royleana* provoked a decrease in the serum calcium level on day 7. This decrease continued progressively till the close of the experiment (28 days) (Fig. 2). Analysis of variance indicated that the level of serum calcium were significantly different between groups (between intervals F = 14.41, P<0.0001 between treatment F = 152.17, P<0.0001).

The structural details of prolactin cells of control fish (Fig. 3) are almost similar to that of normal fish.

No histological alteration has been observed throughout the short- term exposure with latex of *Euphorbia royleana* in the prolactin cells of fish *H. fossilis* (Fig. 4). Analysis of variance indicated that in short-term experiment the nuclear volume of prolactin cells were not significant (between intervals F=0.22, P<0.881, ns; between treatments F= 0.16, P<0.696, ns)

The prolactin cells remain unaltered till day 7 following exposure to latex of *Euphorbia royleana*. The nuclear volume of these cells exhibits an increase after 14 day (Fig. 5). Also these cells exhibit degranulation (Fig. 6). These changes increase progressively 21 day onwards (Fig. 6). Moreover, few degenerating cells are discerned after 28 day following the treatment (Fig. 7). Analysis of variance indicated that in long-term experiment the nuclear volume of prolactin cells were significantly different between groups (between intervals F = 21.18, P<0.0001; between treatment F = 85.78, P< 0.0001).

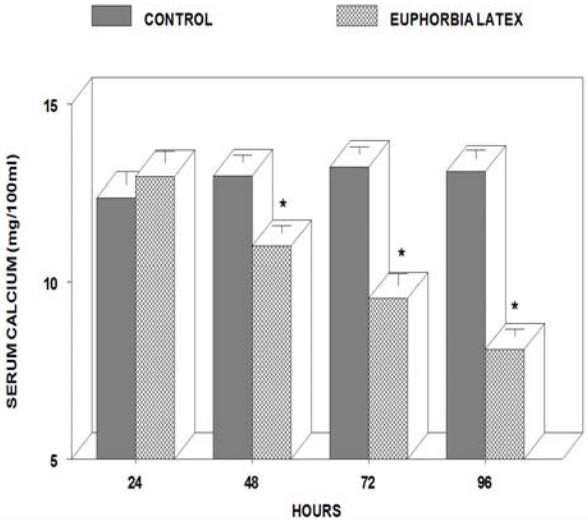

Fig 1: Serum calcium levels of short-term latex of *Euphorbia royleana* treated *H. fossilis.* Values are mean ± SE of six specimens. Asterisk indicates significant differences (P< 0.05) from control group.

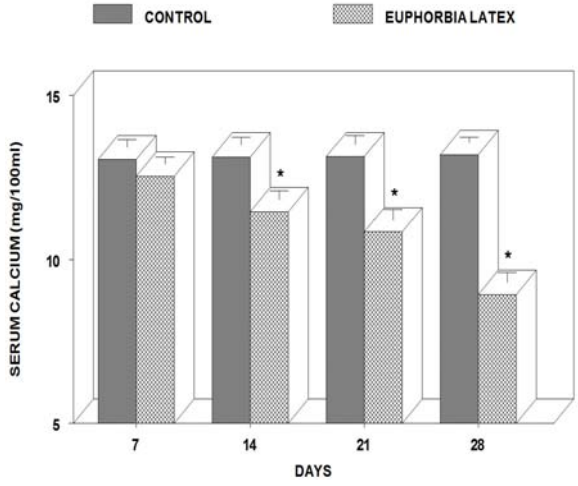

Fig 2: Serum calcium levels of long-term latex of *Euphorbia royleana* treated *H. fossilis*. Values are mean ± SE of six specimens. Asterisk indicates significant differences (P< 0.05) from control group.

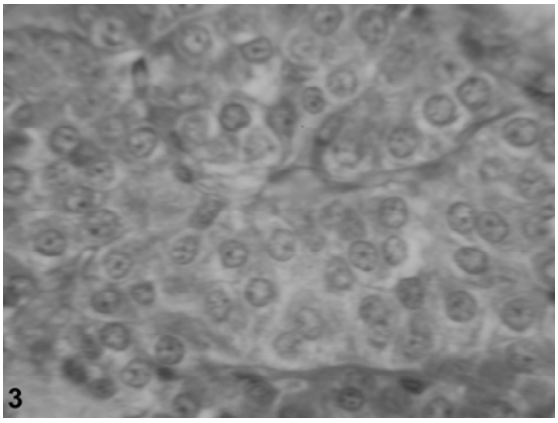

Fig 3: Prolactin cells of control *Heteropneustes fossilis*. Herlant tetrachrome X 800.

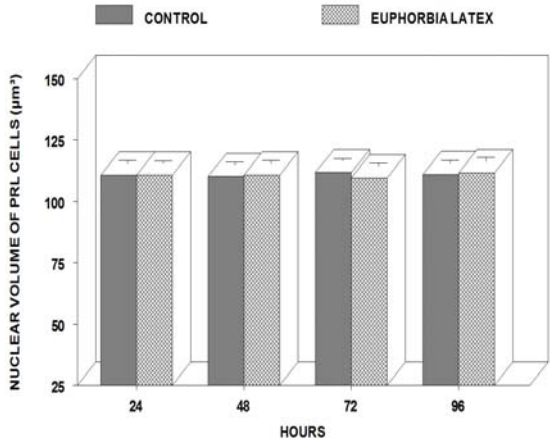

Fig 4: Nuclear volume prolactin cells of short- term latex of *Euphorbia royleana* treated *Heteropneustes fossilis.* Each value represents mean ± S.E. of six specimens.

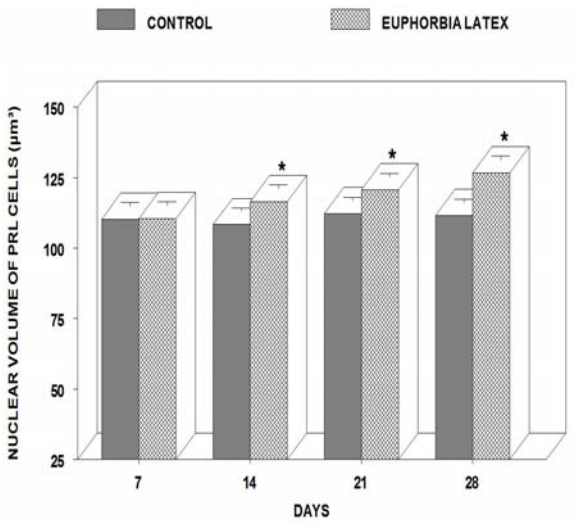

Fig 5: Nuclear volume of prolactin cells of long-term latex of *Euphorbia royleana* treated *Heteropneustes fossilis.* Each value represents mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) from control.

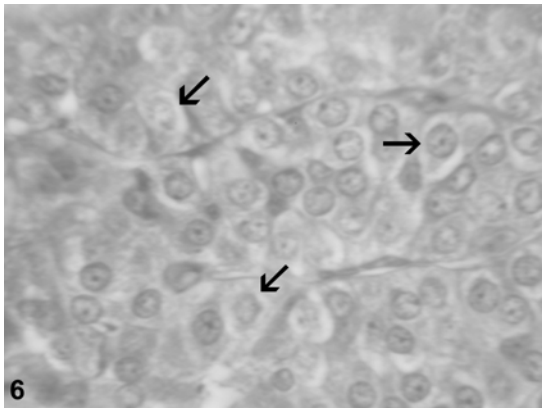

Fig 6: Prolactin cells of 14 days latex of *Euphorbia royleana* treated *Heteropneustes fossilis* showing degranulation (arrows). Herlant tetrachrome X 800.

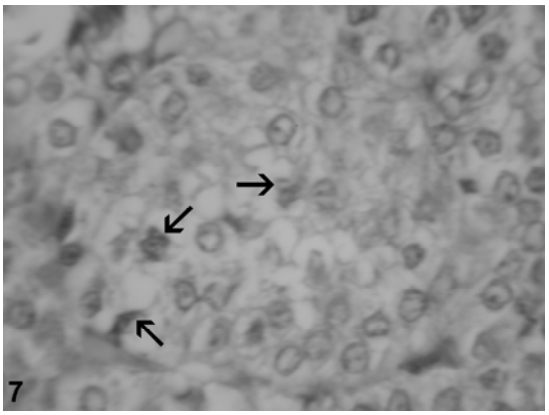

Fig 7: Prolactin cells of 28 days latex of *Euphorbia royleana* treated *Heteropneustes fossilis* showing degeneration (arrows). Herlant tetrachrome X 800.

4. Discussion

Latex of *Euphorbia royleana* exposure resulted into hyperactivated prolactin cells which is expressed by degranulation and increased nuclear volume. Response of prolactin cell activity after exposure to pollutants has been studied by James and Wigham [29]; Fu [30]; Mishra *et al.* [31, 32]; Srivastav *et al.* [33]. The observation regarding hyperactivated prolactin cells in treated fish is in agreement with the reports of earlier investigators who have also noticed increased activity of prolactin cells after exposure of fish to toxicants— cadmium (tilapia- Fu) [30], metacid (catfish- Mishra *et al.*) [31], cypermethrin (catfish- Mishra *et al.*) [32], deltamethrin (catfish- Srivastav *et al.*) [33] and *Nerium indicum* (catfish- Prasad *et al.*) [35]. James and Wigham [29] failed to observe any effect on prolactin cell activity following cadmium injection to rainbow trout. The present study derives support from the studies of Meredith *et al.* [35], Thangavel *et al.* [36, 37] Ramesh *et al.* [38] who have reported increased levels of prolactin in fish exposed to.

In fish hypercalcemia has been reported by prolactin treatment [39-45]. This response of prolactin in fish has been caused be controlling the gill epithelium permeability [46-48]. Hyperactivity of prolactin cells in latex of *Euphorbia royleana* treated fish can be attributed to the action of prolactin to maintain the ionic balance in blood of latex of *Euphorbia royleana* exposed *H. fossilis* through regulation of efflux/influx at gill, kidney and bone.

5. Conclusion

The present study reflects that *Euphorbia royleana* affect the blood calcium and activity of prolactin cells of *H. fossilis*. Calcium has been correlated with the functioning of several vital functions. Similarly prolactin controls the ionic permeability at gills. Hence any change in calcium and prolactin cells would severely affect the growth, vital functions, survival of the species in the nature and also reproductive activity of the organism. Therefore, uses of botanical pesticides near fish inhabiting reservoirs must be taken with great care and precaution

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