Euphorbia royleana, a botanical pesticide affects ultimobranchial gland of the catfish Heteropneustes fossilis

ManiRam Prasad, Abhishek Kumar, Sunil Kumar Srivastav & Ajai K Srivastav

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273009, India

Abstract

Heteropneustes fossilis were subjected to high and low doses of the latex of Euphorbia royleana for short-term and long-term exposures, respectively. The blood was analyzed for plasma calcium levels, and the ultimobranchial glands fixed and examined. Serum calcium levels declined after 48 h following short-term exposure, and this decrease continued until the end of the experiment. The ultimobranchial cells exhibited a decrease in the cytoplasmic staining response after 96 h following treatment, and the nuclear volumes slightly decreased. Chronically exposed fish also exhibited a decline in serum calcium levels, but much later, on day 7, again progressively declining until the close of the experiment. Up to day 14 following treatment there was no change in histological structure of the ultimobranchial glands, but then there was a decrease in nuclear volume and the cytoplasm displayed a weak staining response. There was some vacuolization and degeneration.

Keywords: Botanicals, calcitonin, calcium, teleost

Introduction

Botanical pesticides pose little threat to the environment or to human health, and for this reason they have long been touted as attractive alternatives to synthetic pesticides. Euphorbia royleana (Euphorbiaceae) is a common plant having molluscidal and insecticidal properties (Singh & Agrawal 1984, 1990, Abdel-Hamed et al. 2003, Tiwari et al. 2004). It has analgesic and antipyretic properties in rat and rabbits (Bani et al. 1998). Euphorbia latex is cathartic, anthelmintic and used for a variety of conditions such as skin and eye diseases and snakebite (Chopra et al. 1958, Basak et al. 2009). The latex of Euphorbia royleana produces an intense conjunctival, corneal and iritic inflammatory reaction (Sood et al. 1971). Significant antiarthritic activity has been observed in subacute and chronic models of formaldehyde–induced arthritis, adjuvant-induced developing and established arthritis (Bani et al. 1998).


Thus the present investigation studied the effect of a botanical pesticide, the latex of Euphorbia royleana, on the serum calcium and histological changes in the ultimobranchial gland of a freshwater catfish, Heteropneustes fossilis.

Materials & Methods

Adult freshwater teleost Heteropneustes fossilis (both sexes, body weight 27–38 g) were collected locally. Healthy fish showing no external signs of injury and disease were selected for experiments and were acclimatized to laboratory conditions (under natural photoperiod;
In the present study, the latex of *Euphorbia royleana* was used, which has a 96-h LC$_{50}$ value of 3.090 mg/L for *H. fossilis* (Prasad et al. 2010c). For short-term exposure, the fish were subjected to 2.47 mg/L of latex (80% of the 96-h LC$_{50}$ value), while for the long-term exposure experiment the fish were subjected to 0.618 mg/L (20% of the 96-h LC$_{50}$ value). Simultaneously, a control group was also run for comparison, using tap water containing ethanol. Fish were kept in groups of 10 in 40 L media. Latex of *Euphorbia royleana* was weighed and a stock solution (4 mg/ml) prepared in 100% ethanol. Six fish were sacrificed at each time interval from control and experimental groups after 24, 48, 72 and 96 h in the short-term experiment, and after 7, 14, 21 and 28 days in the long-term experiment.

Blood samples were collected by sectioning of the caudal peduncle of fish. The sera were separated by centrifugation at 3500 rpm and analyzed for calcium levels (calcium kit, RFCL Limited India). After the collection of blood samples, the area adjoining the heart along with the oesophagus were removed and fixed in aqueous Bouin’s fluid. Fixed tissues were routinely processed in a graded series of alcohols, cleared in xylene, and then embedded in paraffin wax. Serial sections were cut at 6 µm and stained with hemotoxylin-eosin (HE).

Nuclear indices (maximal length and maximal width) of ultimobranchial cells (50 nuclei per specimen; thus 300 nuclei were measured from six specimens) were taken with the aid of an ocular micrometer, and then the nuclear volume calculated as volume = $4/3 \pi ab^2$, where ‘a’ is the major semiaxis and ‘b’ is the minor semiaxis.

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

---

**Fig. 1:** Serum calcium levels in the fish *Heteropneustes fossilis* exposed over the short term to high concentrations (80% of 96-h LC$_{50}$) of the latex of *Euphorbia royleana*. Values are mean ± SE of six specimens. Asterisks indicate significant differences (p < 0.05) from the control group.
Results

In the short-term experiment, no alteration was noticed in the serum calcium levels of *H. fossilis* at 24 h following exposure to *Euphorbia royleana* latex. The levels then progressively decline from 48 h to 96 h (Fig. 1). Analysis of variance indicated that the levels of serum calcium were significantly different between groups (between intervals $F_{3,43} = 20.8, p<0.0001$; between treatments $F_{1,43} = 169.5, p<0.0001$).

![Graph showing serum calcium levels](image)

**Fig. 2:** Serum calcium levels in the fish *Heteropneustes fossilis* exposed over the long term to low concentrations (20% of 96-h LC$_{50}$) of the latex of *Euphorbia royleana*. Values are mean ± SE of six specimens. Asterisks indicate significant differences ($p < 0.05$) from the control group.

In the long-term experiment, 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 $F_{3,43} = 14.4, p<0.0001$ between treatments $F_{1,43} = 152.2, p<0.0001$).

![Images of ultimobranchial gland](image)

**Fig. 3.** Ultimobranchial gland of control *H. fossilis* exhibiting follicles (arrow) and cell cords (broken arrow). HE × 200.

**Fig. 4.** Ultimobranchial gland of treated *H. fossilis* (72 h) exhibiting follicles (arrow) and cell cords (broken arrow). HE × 500.
The histological structure of the ultimobranchial glands of control fish usually consisted of solid parenchyma composed of cell cords and small follicles (Fig. 3). All the cells are alike and their cell boundaries are indistinct. When stained with HE, the cytoplasm of these cells are noticed slightly eosinophilic.

In the short-term experiment, no histological change was seen in the ultimobranchial glands up to 72 h following treatment with latex. After 96 h following treatment, a decrease in the staining response of the cytoplasm of ultimobranchial cells was noticed (Fig. 4). The nuclear volume of these cells decreased slightly (Fig. 5) with time ($F_{3,43} = 3.67$, $p<0.02$), but did not differ between treatments ($F_{1,43} = 2.81$, $p<0.10$).

In the long-term experiment, the ultimobranchial gland exhibited no change up to 14 days following latex exposure. A slight decrease in the staining response of the cell cytoplasm was seen at day 21 (Fig. 6), and the nuclear volume decreased (Fig. 7). After 28 days the nuclear volume decreased further (Fig. 7), and degeneration and vacuolization appeared (Fig. 8).

![Graph showing nuclear volume of ultimobranchial cells of H. fossilis treated with high-dose short-term amounts of latex of Euphorbia royleana. Mean ± SE (n=6). Asterisks indicate differences (p < 0.05) from control group.](image)

**Fig. 5:** Nuclear volume of ultimobranchial cells of *H. fossilis* treated with high-dose short-term amounts of latex of *Euphorbia royleana*. Mean ± SE (n=6). Asterisks indicate differences (p < 0.05) from control group.

![Image of ultimobranchial gland 21 days after treatment with latex of Euphorbia royleana, showing decreased staining response of the cytoplasm HE × 500.](image)

**Fig. 6:** Ultimobranchial gland 21 days after treatment with latex of *Euphorbia royleana*, showing decreased staining response of the cytoplasm HE × 500.

![Bar graph showing nuclear volume of ultimobranchial cells of H. fossilis treated with low-dose long-term amounts of latex of Euphorbia royleana. Mean ± SE (n=6). Asterisks indicate differences (p < 0.05) from control group.](image)

**Fig. 7:** Nuclear volume of ultimobranchial cells of *H. fossilis* treated with low-dose long-term amounts of latex of *Euphorbia royleana*. Mean ± SE (n=6). Asterisks indicate differences (p < 0.05) from control group.

![Image of ultimobranchial gland of 28 days after treatment with latex of Euphorbia royleana exhibiting degeneration (arrow) and vacuolization (broken arrow). HE × 500.](image)

**Fig. 8:** Ultimobranchial gland of 28 days after treatment with latex of *Euphorbia royleana* exhibiting degeneration (arrow) and vacuolization (broken arrow). HE × 500.
The ultimobranchial glands exhibited inactivity in latex-treated fish, expressed by the diminished staining response and decreased nuclear volume of their cells. The glands of treated fish also exhibited vacuolization and degeneration. Inactivity of the ultimobranchial glands could be attributed to the prolonged hypocalcemia caused by exposure to the latex of *Euphorbia royleana*. No comparable report is available regarding the effects of the latex of *Euphorbia royleana* on the activity of fish ultimobranchial glands. Inactivity of these glands in treated fish is similar to the observations of other investigators who exposed the fish to different toxicants - deltamethrin (Srivastav et al. 2002), metacid (Mishra et al. 2004), cypermethrin (Mishra et al. 2005), and cadmium (Rai et al. 2009).

Hypoactivity/inactivity of the ultimobranchial glands has also been observed in response to experimentally induced hypocalcemia after calcitonin administration to fish - *Anguilla anguilla* (Peignoux-Deville et al. 1975), *Gasterosteus aculeatus* (Wendelaar Bonga 1980), *Clarias batrachus* (Srivastav et al. 1989), *Amphipnous cuchia* (Tiwari 1993) and *Heteropneustes fossilis* (Srivastav et al. 2009). The observed degeneration and vacuolization in the gland may be due to the continuous disuse of the ultimobranchial gland caused by prolonged hypocalcemia in treated fish.

It can be concluded from the present study that *Euphorbia royleana* is effective in inducing histological changes in the ultimobranchial gland of fish and causing physiological disturbances.

**Acknowledgements**

**References**


Prasad et al.: *Euphorbia royleana* affects the ultimobranchial gland of catfish


الملخص العربي

(ioforbia royleana) (Heteropneustes fossilis)

مانيرام براساد، أبهيشك كومار، سونيكل كومار سريفاستاف & أجاي سريفاستاف
قسم علم الحيوان، جامعة دنالي ابابادهارا، الهند

المعضلة

تعرض سمكة القط (Heteropneustes fossilis) لجرعات عالية ومنخفضة من عصارة نبات (Euphorbia royleana) لفترات قصيرة و طويلة على التوالي. تم تحليل مستويات الكالسيوم في بلازما الدم، كما تم فحص الغدة الخيضوية. ومن خلال الفحص والتحليل وجد أن مستويات الكالسيوم قد انخفضت في بلازما الدم بعد 48 ساعة من التعرض لفترات قصيرة، وظلت منخفضة حتى نهاية التجربة. أظهرت خلايا الغدة الخيضوية انخفاضاً في الاستجابة لعملية صبغ السينوبلازم بعد 96 ساعة من المعالجة، كما أن الأندية قد تقلصت في الحجم قليلاً. أما بالنسبة للأسماك التي تعرضت بشكل مزمن لعصارة النبات فقد أظهرت كلاً من الكالسيوم في الدم، و قبل ذلك وبداية من اليوم السابع على وجه التحديد بدأت مستويات الكالسيوم في التراجع مرة أخرى بشكل طبيعي حتى نهاية التجربة. وصولاً إلى اليوم الرابع عشر بعد المعالجة لم يكن هناك أي تغير في التركيب النسيجي للغدة الخيضوية، ولكن بعد ذلك ظهر انخفاض في حجم الأندية، كما أصبح السينوبلازم ضعيف الاستجابة للفحص. كان هناك بعض الفجوات الموجودة في النسيج وبعض التلف.