

Impact of biopesticide – *Azadirachta indica* on Ovary of fresh water catfish *Heteropneustes fossilis*

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Abstract

An investigation on the effect of the *Azadirachta indica* on the ovary of fresh water catfish *Heteropneustes fossilis* was carried out in the laboratory. Fishes were exposed to sub-lethal concentrations of *Azadirachta indica* for 24, 48, 72 and 96 hrs. The LC₅₀ values of *Azadirachta indica* calculated for *Heteropneustes fossilis* for 24, 48, 72 and 96 hours are 10.5, 8.5, 7.0 and 6.5 ml/L respectively. The ovaries were removed for histological examination. The normal ovary of *Heteropneustes fossilis* showed developing follicles with large nucleus and several nucleoli, few degenerating oocytes were also seen but exposure leads to shrinkage of oocytes, vacuolated cytoplasm, degenerating vitellogenic follicles, disintegrated interstitial tissues, necrosis and damaged follicles.

Keywords: *Azadirachta indica*, *Heteropneustes fossilis*, sub-lethal concentrations, ovaries, vitellogenic follicles (DF), necrosis, oocyte

Introduction

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. *Azadirachta indica*, A Juss (Syn. *Melia. Azadirachta*) is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. *A Indica. A. Juss* and *M. azedarach* are two closely related species of Meliaceae. The former is popularly known as Indian *Neem* (Margosa tree) or Indian lilac, and the latter as the Persian lilac. The former grows as a wild tree in India, Bangladesh, Burma, Pakistan, Sri Lanka, Malaysia, Thailand and Indonesia. Presently *Neem* trees can be seen growing successfully in about 72

countries worldwide, in Asia, Africa, Australia, North, Central and South America (Isman *et al.* 2006).

Azadirachta indica, also known as *Neem* is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to India. *Neem* is hailed as a wonder tree “kalpavriksha” means miraculous tree for its versatile use.

Products made from *Neem* trees have been used in India for over two millennia for their medicinal properties. *Neem* products are trusted by Ayurvedic practitioners to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative. It is considered a major component in Ayurvedic and Unani medicine and is particularly prescribed for skin diseases.

However, insufficient research has been done to assess the purported benefits of *Neem*. In adults, short-term use of *Neem* is safe, while long-term use may harm the kidneys or liver; in small children, *Neem* oil is toxic and can lead to death. *Neem* also causes miscarriages, infertility, and low sugar (Krishnan 2009).

Materials and methods

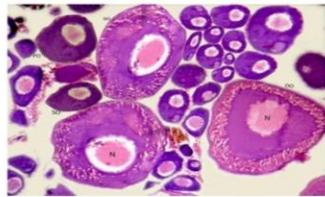
Histo-pathological Studies

Present investigation has been carried out to study the effect of sub-lethal concentration of *Azadirachta indica* on ovary of the freshwater Indian cat fish *Heteropneustes fossilis*. Healthy and sexually mature specimen of *Heteropneustes fossilis* measuring about 15-20 cm length and 50-100 gm in weight were selected for the experimental study. These collected fishes were maintained in glass aquaria containing tap water and acclimatized in laboratory conditions at room temperature for one

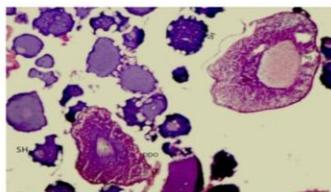
week. The water of the aquarium was changed daily and fishes are fed daily with commercial fish food. Fishes are starved for 24 hours prior to the experiment and are not fed during the period of experiment (Dalela *et al.*, 1979).

.In this experiment, the specimens were kept in two experimental groups. Control Group and Experimental Group. Each group was exposed to sublethal concentration of the *Azadirachta indica* similar set up was also maintained as control. The animals were scarified for optimal concentration of biopesticide (*Azadirachta indica*) for different exposure of 24, 48, 72 and 96 Hrs. For histological studies, fishes were scarified during the exposure period of 24, 48, 72 and 96 Hrs respectively. The toxicant was renewed after fixed period. The technique of Microtomy is being used for the histological study purpose of ovary of the fresh water catfish *Heteropneustes fossilis*.

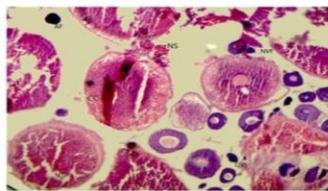
Observation and results



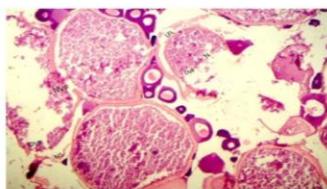
Normal Ovary



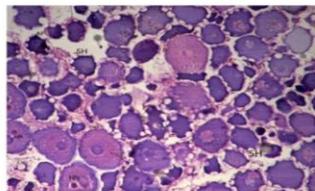
24 hours Ovary



48 hours Ovary



72 hours Ovary



96 hours Ovary

Figure: Effects of *Azadirachta indica* on Ovary of *Heteropneustes fossilis*.

Fig - Normal Ovary	DO/DF - Developing oocytes or developing follicles PO - Primary oocytes SO - Secondary oocytes N - Nucleus
Fig - 24 hours Ovary	DDO - Damage developing oocytes SH - Shrinkage of oocytes NS - Necrosis of stroma
Fig - 48 hours Ovary	NVF - Necrosis of vitellogenic follicles AF - Atretic follicles DVF - Degenerating vitellogenic follicles CC - Cytoplasmic clumping NS - Necrosis of stroma N - Necrosis
Fig - 72 hours Ovary	LFL - Loose follicular lining NVF - Necrosis of vitellogenic follicles DVF - Degenerating vitellogenic follicles N - Necrosis NS - Necrosis of stroma CC - Cytoplasmic clumping
Fig - 96 hours Ovary	SH - Shrinkage of oocyte AF - Atric oocyte R - Ruptured and slightly thin ovary wall DVF - Degenerating vitellogenic follicles CAO - Complete absorption of oocyte HN - Hypertrophied nucleoli

Labeling of ovary of *Heteropneustes fossilis* at 24,48,72 and 96 hours.

Effects of *Azadirachta indica* on Ovary of *Heteropneustes fossilis*:-

Normal ovary: The ovary of a control fish show well developed vitellogenic follicles (DF) of various sizes, developing follicles and oocytes (DO) with large nucleus (N) and several nucleoli, the primary oocytes (PO) and Secondary oocytes (SO) can also observed.

24 hours ovary: At 24 hours exposure, the developing oocytes took excessive stain, shrinkage and damaged of oocytes (SH / DO) and the cytoplasm was seen vacuolated (V). The vitellogenic follicles were seen degenerating (DF). Interstitial tissues disintegrated forming necrosis (N).

48 hours ovary: At 48 hours exposure, the vitellogenic follicles start to degenerate (DVF) and show necrosis of vitellogenic follicles (NVF). The atretic follicles (AF),

cytoplasmic clumping (CC), necrosis of stroma (NS) and interstitial tissues disintegrated forming necrosis (N) was also observed.

72 hours ovary: At 72 hours exposure show the loose follicular lining (LFL), necrosis of vitellogenic follicles (NVF), degenerating vitellogenic follicles (DVF) with cytoplasmic clumping (CC) and interstitial tissues disintegrated forming necrosis (N) with necrosis of stroma (NS).

96 hours ovary: At 96 hours exposure, histopathological changes were most pronounced, progressive and prominent. The oocytes were shrunked (SH), atric oocytes (AO), complete absorption of oocytes (CAO), ruptured wall of ovary (R), degenerating vitellogenic follicles (DVF), distinct nucleus showing hypertrophied nucleoli (HN).

Discussion

The exposure to sub lethal concentration of Biopesticide *Azadirachta indica* for 24, 48, 72 and 96 Hrs. respectively showed the histo-pathological alternation in reproductive structure of fresh water fish, *Heteropneustes fossilis*. Ovary is an egg producing reproductive organ, often found in pairs as part of the female reproductive system that employs Sexual reproduction (Santhakumar and Balaji 2000). Ovary of teleosts is also often hollow, but in this case, the eggs are shed into the cavity, which opens into the oviduct. The ovary of a control fish sowed developing follicles with large nucleus and several nucleoli, few degenerating oocytes were also seen but exposure leads to shrinkage of oocytes, vacuolated cytoplasm, degenerating vitellogenic follicles, disintegrated interstitial tissues, necrosis and damaged follicles (Jyoti and Natrayan 1999, Saxena and Gupta 2003)

Conclusion

The Neem is one of the best Biopesticides which shows very less hazards to environment. But the present study on *Heteropneustes fossilis* and previous research made on the aquatic organisms especially fresh water fishes proved the hazardous effects of *Azadirachta indica* on reproductive biology. So, in future there is need of more research to assess the purported benefits of Neem which is a *Kalpavriksha* for all the mankind. So it suggest that may *Azadirachta indica* has several medicinal values as well having utility like biopesticide but its exposure

showed adverse effect on the ovaries of fresh water catfish *Heteropneustes fossilis*.

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