International Journal of Fisheries and Aquatic Research
ISSN: 2456-7248; Impact Factor: RJIF 5.44
Received: 15-10-2020; Accepted: 30-10-2020; Published: 20-11-2020
www.fishjournals.com
Volume 5; Issue 4; 2020; Page No. 50-53

Effect of neem oil on the adenohypophysis of *Glossogobius giuris*

Narayanaswamy SY
Department of Zoology, KLEs, S. Nijalingappa College Bengaluru, Karnataka, India

Abstract
The adenohypophysis of the fish *G. giuris* histopathology was observed during post spawning phase, on treatment with sub lethal concentrations of Neem oil (0.05 ppm, 0.25 ppm and 0.5 ppm) for 24, 48, 72 and 96 hrs intervals. The results of the present study showed PRL cells were spherical or ovoid, the nuclei were found beneath the nuclear membrane. Cells were partially granular. The RPD of the pituitary PRL cells became small and their nuclei were frequently pycnotic with diffused chromatin material. GTH cells were completely vacuolated and appeared oval or spherical in shape. The fishes at higher concentration of neem oil showed signs of degranulation and vacuolization. Intercellular spaces became conspicuous. The nuclear diameter decreases, cells were small and densely granulated cytoplasm. Most of the cells in the RPD undergone degranulation. GTH cells became totally degranulated, appearance of vacuolization, large intercellular spaces were observed due to coalition of small vacuoles. The various stages of degranulation which were distributed sparsely. A reduction in the number and diameter of the gonadotrophs indicate possible reduction in release of gonadotroph hormone.

Keywords: Neem oil, *G. giuris*, prolactin (PRL) and gonadotrophs (GTH)

1. Introduction
Neem has been used worldwide in traditional medicine for various therapeutic purposes, antibacterial, antifungal and antifertility properties (Jegede and Fagbenro, 2007) [13]. Sinna and Riai (1985) reported that in Rhesus monkey and human, spermatozoa became totally immobile in 30 seconds of contact with undiluted neem oil. In vivo studies showed that intravaginal application of neem oil prior to coitus can prevent pregnancy (Sinha *et al.,* 1984) [25]. The neem extracts can affect aquatic organisms including fish and tadpoles. Patnaik *et al.,* (1987) observed that higher larval mortality of *Crocidolomia binotalis* with neem oil for laboratory condition. Azam (1991) [3] reported that neem oil causes more than 80 percent larval mortality of *Liriomyza trifolii* in cucumbers. Shanmugapriyan and Kingsly (2001) [1] reported that neem oil at 0.5, 1.0 and 2.0 % of concentration was effective on third instar larvare of *Earias vitteila* than in the fourth and fifth in stars. Antifertility effect of neem oil has also been studied and suggested to be a novel method of contraception (Upadhyay *et al.,* 1990 and 1994, Kaushic *et al.,* 1995) [15, 29]. Oral administration of aqueous extract of neem leaf also shows antifertility effect in mice (Despande *et al.,* 1980) [6]. Purified neem seed extract (praneem) has also been demonstrated to abrogate pregnancy in both baboons and bonnet monkeys, when administered orally (Mukherjee *et al.,* 1996) [16]. Neem is a membr of the Meliaceae family. Among the herbal pesticide, neem oil considerd to be an important pesticide in controlling pests and insects (Kraus *et al*., 1995, Devakumar *et al.,* 1996) [7]. The effect of neem oil and its products on fishes have been studied by Temitope *et al.,* (2008) [28]. Kumarantunga *et al*., (1989), on mosquitoes (Fredros *et a*l., 2007, Rao *et al.,* 1992, on rats Upadhyay *et al*., 1993, Omkar *et al.,* 1997, B.P. Kale 2003, Majumdar 1998, Masood *et al*., 2008) [8, 5, 15, 18, 4]. Neem oil shows toxicity of fish like tilapia and carp (Jacobson, 1995) [10], in rats and rabbits (Gandhi *et al.,* 1988), in humans (Jacobson 1995, Singh *et al*., 1985) [10, 26]. Neem leaf exhibits oral toxicity in mice (Kanungo, 1996) [14], showing signs of ill health and discomfort, gastrointestinal spasms, apathy, hypothermia and terminal convulsions, leading to death. Neem leaf extract when administered for 48 days in albinorats causes decrease in sperm count, sperm mobility, probably due to androgen deficiency (Aladakatti *et al*., 2001) [1], Stinkbug podisusnigripinus (Zanuncio, J., Mourão, S., Martínez, L. *et al*.., 2016). Hence, the present investigation, histopathological studies in the adenohypophysis (Gonadotrophs and prolactin secreting cells) *Glossogobius giuris* has been made during post spawning phase.

2. Material and Methods
The fresh water gobiid fish *Glossogobius giuris* (HAM) were randomly collected in and around Bangalore using cast and gillnets (10 mm). The fishes were brought alive to the laboratory and were kept in 50 L aquaria containing aerated tap water and acclimitated in laboratory conditions for 15 days prior to using them in experiments. They were keep under natural photoperiod and room temperature of 26 ± 4 ˚C and were fed daily with earth worms. These fishes were treated with 0.1% potassium permanganate solution for 15 min to get rid of dermal infections. The large and sexually mature female fishes were used in this study. Females were identified externally by the presence of urinogenital papillae. The body length and weight of each fishes were recorded. The female fish weighing about 20-50 g and length 110 to 220 mm were selected for the study adenohypophysis of *Glossogobius giuris*. The pesticide neemoil was dissolved in acetone and then added to test water to obtain the desired concentration. The stock solution of 1 mg/L is prepared and the desired concentration is obtained by adopting the dilution technique outlined by APHA (1995). The acclimitated fishes were divided into three experimental groups of six fishes each. The first three groups of fishes are placed in 0.05, 0.25 and 0.5 ppm of neem oil, while fourth group kept in fresh water (control). Six fishes in each concentration in 10 L

capacity glass trough. For all experiments the acclimitated Fishes were starved for 24 hr prior to their exposure to neem oil use in experiment and were not fed during the course of the experiment (Dalela *et al.*, 1971). The water was changed on alternate days and the concentration of pesticide was maintained.

The pesticide treated fishes (24, 48, 72 and 96 hr) were sacrificed by decapitation and pituitary along with brain are fixed in different fixatives. Serial sagittal sections were cut 5 to 6 µ and stained for staining techniques as for the pituitary staining.

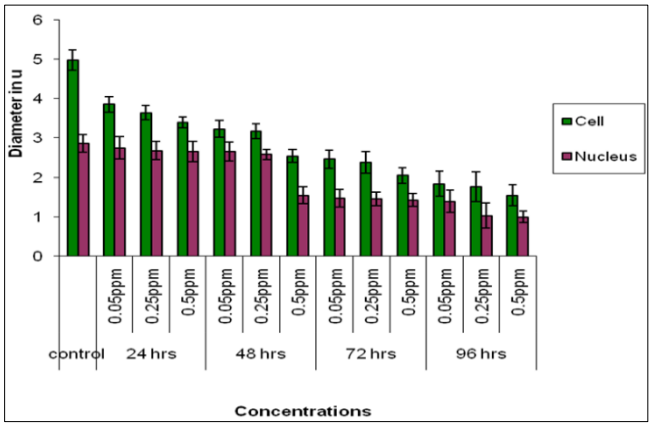
3. Result

3.1 Control

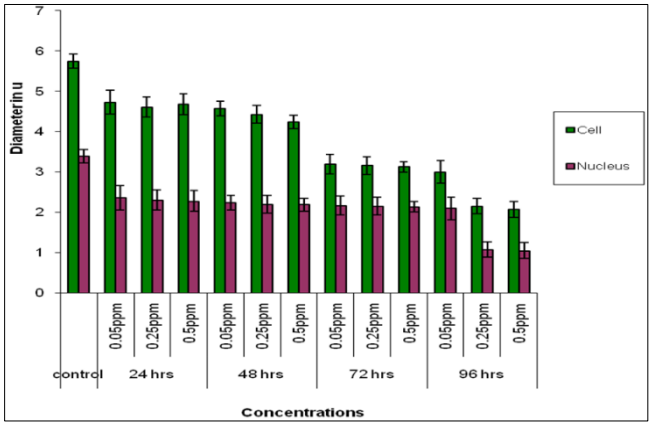
Prolactin (PRL) cells were spherical or ovoid, the nuclei were found beneath the nuclear membrane. Cells were partially granular. The cell nuclear diameter (2.86 ± 0.22µ). Gonadotrophin (GTH) cells were completely vacuolated and appeared oval or spherical in shape. The cytoplasm contain less number of granules (nuclear diameter: 3.39 ± 0.30µ)

3.2 Treated

When the fishes were treated with 0.05 ppm of neem oil for 24 hrs, PRL cells showed smaller cells and their nuclei frequently pycnotic with diffused granulated cells. The nuclear diameter decreased when compared to that of control (2.75±0.28µ). Fishes exposure to 0.05 ppm neem oil for 24 hrs, GTH cells showed, cytoplasm was less number of granular cells. The cytoplasmic region was degranulation. The nuclear diameter decreases.



Graph 1: Effect of neem oil on PRL cells and nuclear diameter of gobiid fish *G. giuris* during post spawning phase



Graph 2: Effect of neem oil on GTH cells and nuclear diameter of gobiid fish *G. giuris* during post spawning phase

The PRL cells exposed to 0.25 ppm of neem oil for 24 hrs, the cytoplasm of PRL cells in the RPD region showed degranulation with vesicular fluid. The cellular and nuclear diameter are recorded and presented in the table – 23. Exposure of fishes to 0.25 ppm of neem oil for 24 hrs, GTH cells showed slightly increased the degeneration of granulated cells in the cytoplasm of the basophils. In the fishes treated with 0.5 ppm of neem oil for 24 hrs, showed signs of degranulation and vacuolization. Intercellular spaces became conspicuous. The nuclear diameter decreases when compared to the control (3.79 ± 0.14 µ). On treatment of the fish with neem oil for 24 hrs exposure to 0.5 ppm of neemoil exhibited degranulated and a few vacuoles were found towards the central region. When the fishes were treated with 0.05 ppm of neem oil for 48 hrs, PRL cells showed various stages of degranulation consequently; most of the cells had undergone degranulation and showed vacuoles. 48 hrs treatment of 0.05 ppm of neem oil caused vasculization and degranulation posses small spherical nuclei (2.24 ± 0.21µ) and showed chromatin material in the nucleoplasm. Number of vacuoles also increased. Exposure of fishes to 0.25 ppm of neem oil for 48 hrs, PRL cells cytoplasm undergone degranulation, the cells are destructured and there was no clear boundaries. Neem oil treatment with 0.25 ppm for 48 hrs, GTH cells exhibited nuclei became smaller containing lesser chromatin material. Appearance of vacuolization. There was reduction in the nuclear diameter (2.20 ± 0.18µ). In the RPD of the pituitary at 48 hrs of treatment with 0.5 ppm of neem oil, PRL cells became smaller and their nuclei were frequently pycnotic. 48 hrs treatment with 0.5 ppm of neem oil, GTH cells exhibited more vasculization and decreases the cell and nuclear diameter (2.19 ± 0.27µ), the cells of the region arranged loosely, degranulation found towards periphery of the cell.

Fishes exposure to 0.05 ppm of neem oil for 72 hrs, PRL cells had heavy degranulations. Large intercellular spaces were observed along with a few blood vessels. The nuclear diameter decreased. The PPD of the pituitary at 72 hrs of treatment with 0.05 ppm of neem oil, the GTH cells were degranulated and showed reduction in the nuclear diameter (2.17 ± 0.35µ) when compared to control. Neem oil treatment with 0.25 ppm for 72 hrs, PRL cells showed nuclei and chromatin material were not distinct. The nuclear diameter decresed (1.45 ± 0.17µ) when compared to control. Exposure of fishes to 0.25 ppm of neem oil, GTH cells exhibited cells are clearly visible with degranulated with indistinct cell boundaries. The nuclei are small with prominent nucleoli. When fishes exposure to 0.5 ppm of neem oil for 72 hrs PRL cells were small and densely granulated cytoplasm. Most of the cells in the RPD undergone degranulation. Nuclear diameter decreases (1.43 ± 0.16µ). The PPD of the pituitary, 0.5 ppm of neem oil for 72 hrs, GTH cells appeared degranulated in higher concentration of neemoil cells were inconspicuons. On treatment of the fish with 0.05 ppm of neem oil for 96 hrs, PRL cells exhibited intercellular spaces became conspicuous and cellular disturbances were noted. There was an increase in the quantity of degranulated cells.

Treatment with 0.05 ppm of neem oil for 96 hrs, GTH cells of PPD decreased in size gradually and the cells were loosely arranged. Intercellular spaces had towards the central region of the gland. The RPD of the pituitary at 96 hrs of 0.25 ppm of neem oil, PRL cells had large number of vacuoles with degranulated cells, the nuclear diameter (1.33 ± 0.32µ). 96

hrs treatments with 0.25 ppm of neem oil, GTH cells contain large number of degranulated cells. There was a significant decrease in the size of nuclei (1.08 ± 0.27µ). The RPD of the pituitary at 0.5ppm of neem oil for 96 hrs, PRL cells became small and their nuclei were frequently pycnotic with diffused chromatin material. Treatment with 0.5 ppm of neem oil for 96 hrs, GTH cells became totally degranulated, appearance of vacuolization. Large intercellular spases were observed due to coalition of small vacuoles. The nuclear diameter decreases. (1.05 ± 0.11 µ).

4. Discussion

During the post-spawning period of *G. giuris* (January to February) the GTH cells exhibited maximum vacuolization. This coincided with increase in the number of atretic follicles and oogonial proliferations in the ovary. This phenomenon suggests that lower content of gonadotropin in the basophils, with a simultaneous increase in the circulation is responsible for the development of oocytes in *G. giuris*. Rai (1966a) [22] who worked on Tor (barbus) tor also reported that extensive depletion of cyanophilis was accompanied by an increase in the number of atretic follicles and oogonial proliferations in the ovaries. Saxena (1980) [27] suggested that the resumption of the degranualtion and degeneration process in the cytoplasm of the basophils take place during the late post spawning period in *G. giuris*. Similar degenerating changes have been described in the pituitary of Salmo gairdnerii (Robertson and Wexler, 1962a), *Onchorhynchus* (Robertson and exler 1962 b) and *Tor* (Barbus) *tor* (Rai, 1966b) [24]. The decrease in the amount of gonadotropin in the hypophysis of the spent fish resulted in the formation of corpora atretica.

Neem oil has interfered with maturation and growth of the follicles in the ovary which has also been showed by the microscopic sections of the ovary in which the number of follicles decreases (Masood Ahmed *et al*. 2009. Mukherjee *et al*. (1996 and 1999) [16]. Talwar *et.al* (1997) reported a contraceptive effect during early post implantation period as theys observed the complete resorption of embryos in Wister rats after oral administration of the NIM-76, a pure active fraction of neem seeds.

The present study on prolactin cells in *G. giuris* exposed to different concentrations of neemoil (0.05 to 0.5 ppm) for an interval of 24, 48, 72 and 96 hrs showed marked cytological changes. In 24-96 hrs treated fish prolactin cells showed degranulation and vacuolization in the cytoplasm and conspicuous intercellular spaces. These cellular disturbances in the RPD may be directly related to increase in the concentration of neemoil. This suggests an acute stress response, since prolactin is known to be released under stress condition. Similar observations have been made by Jagadeesh and Sahai, (1986 and 1988) in *M. vittatus* and *H. fossilis*.

5. References

1. Aladakatti RH, Nazeer Ahmed R, Ahmed M, Gosewami MG. Sperm parameters changes induced by *Azadirachta indica* in albino rats. J Basic clin. Physiol. Pharmacol. 2001; 12:69-76.
2. APHA. Standard methods for examination of water and waste water. American Public Health Associatated. Washington. New York, 1985.
3. Azam KM. Toxicity of neem oil against leafminer. *Liriomyza trifolii* (Burgess) on cucumber. Pl. port. 1991; 6:196-197.
4. Kale BP, Kothekar MA, Tayade HPJB, Jaju M. Mateenuddin. Effect of aqueous extract of *Azadirachta indica* leaves on hepatotoxicity induced by antitubercular drugs in rats. Indian Journal of Pharmacology. 2003; 35(3):177-180.
5. Rao DR, Reuben R, Venugopal MS, Nagasampagi BA, Schmutterer H. Evaluation of neem, *Azadirachta indica*, with and without water management, for the control of culicine mosquito larvae in rice-fields. Medical and Veterinary Entomology. 1992; 6(4):318-324.
6. Despande VY, Mendulkar KN, Sadre NL. Antifertility activity of *Azadirachta indica* in mice. J Postgrad. Med. (Bombay). 1980; 26:167-170.
7. Devakumar C, Sukhdev. In neem (eds Randhawa and Parmar, B. S.)2ND EDN, 1996, 77-110.
8. Fredros O Okumu, Bart GJ Knols, Ulrike Fillinger. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector Anopheles gambiae. Malar J, 2007, 6:63.
9. Gandhi M, Lal R, Sankaranarayana A, Banerhjee CK, Sharma PL. Acute toxicity study of the oil from *Azadirachta indica* seed (neem oil). J Ethnopharmacol. 1998; 23:39-51.
10. Jacobson M. In the Neem Tree: Source of unique natural products for integrated pest management, Medicine, industry and other purposes (ed. Schmutterer H.), 1992, 484-495.
11. Jagadeesh CG, Sahai S. Effect of malathion on the pituitary gland of Mystus vittatus (Bloch): Abstract National conference on water pollution - A potential threat to mankind, Sagar, 1986, P. 23.
12. Jagadeesh CG, Sahai S. BHC induced effects on the adenohypophyseal histocytology in Anabas testudineus (Bloch). Abstract, second National symposium on fish and their Environment, Trivandrum, 1988, 7p.
13. Jegede KC, Fagbenro OA. Tilapia husbandry vs. midwifery: use of antifertility plants. in G.E. onibi, S.O. Agele, V.A.J. Adekunle and M.O. Olufayo (eds). Proceedings of the third annual conference of school of agriculture and agricultural techonology, Federal University of Techonology Akare, Nigrdia, 2007, 118-112,
14. Kanungo D. In Neem (EDS. Randhawa and Parmar, B.S.), 2nd edn, 1996, 77-110.
15. Kaushic C, Upadhyay S. ibid, 1995; 51:203-207
16. Mukherjee S, Lohiya NK, Pal R, Shrma MG, Talwar GP. Purified neem seed extracts (Praneem) abrogate pregnancy in primates. *Contraception*, Volume 53, Issue 6, Pages 375-378contraception. 1996; 53:375-378.
17. Mujumdar AM, Upadhye AS, Pradhan AM. Effect of *Azadirachta indica* leaf extract on carbontetrachloride-induced hepatic damage in albino rats. Indian journal of Pharmaceutical Sciences. 1998; 60(6):363-367.
18. Omkar Parshad MT, Gardner TL, WILLIAMS LAD, Fletcher CK. Antifertility Effects of Aqueous and Steroidal Extracts of Neem Leaf (*Azadirachta indica*) in Male Wistar Rats. Phytotherapy Research. 1997; 11(2):168-170.
19. Patanaik NC, Panda N, Patro ER, Mishra BK. Effect of neem oil (*Azadirachta indica*, A. juss) an mustered web-warm, *Crocidolomia binotalis* zell. (Lepidoptera: Pyralidae). Neem New letter. 1987; 4(2):15-17.
20. Robertson OH, Wexler BC. Histological changes in the pituitary gland of the rainbow trout (salmogairdnerii)

accompanying sexual maturation and spawning. J. Morph, 1962, 110:157.
21. Robertson OH, Wexler BC. Histological changes in the pituitary gland of the pacific salmn (Genus onchorhynchus) accompanying sexual maturation and spawning. J Morph, 1962b, 110-171.
22. Rai BP. Histophysiology of the pituitary gland in correlation with the ovarian cycle Tor (Barbus) tor (HAM). Z. Zellforsch. 1966; 72:574-582.
23. Shanmugapriyan R, Kingsly S. Bioefficacy of neem oil on larvae of bitter gourd beetle *Epilachna vigintiocto punctata* (Coccinellidae: coleopteran), J Ecotoxicol. Environ. Monit. 2001; 11(3):215-219.
24. Sinna KC, Rai SS. Neem oil: an ideal contraceptive. Biological, 1985.
25. Sinha KC, Riar SS, Tiwary RS, Dhawan AK, Bhadhan J, Thomas P, *et al*. Neem oil as a vaginal contraceptive. Indian J Med Research. 1984; 79:131-136.
26. Singh YP, Bhaga HS, Vijan VK. Neem Newsl, 1985, 2:17.
27. Saxena DN. On the pituitary gland and reproductive cycle of Indian fresh water goby *Glossogobius giuris* (HAM) Z. mikrosk. Anat. Forsch. Leipzig. 1980; 94(4.5):705-714.
28. Temitope Jegede, Oyedapo Fagbnro. Histology of gonads in Yilapia zillii (Gervais) fed neem (*Azadirachta indica*) leaf meal diets 8 thnt symp on Tilapiain aquaculture, 2009.
29. Upadhyay SN, Kaushic C, Talwar GP. Antifertility effects of neem (*Azadirachta indica*) oil by single intrauterine administration: a novel method for contraception. Proc Biol Sci. 1990; 242(1305):175-9.
30. Upadhyay S, Dhawn S, Sharma MG, Talwar GP. Long-term contraceptive effects of intrauterine neem treatment (IUNT) in bonnet monkeys: An alternate to intrauterine contraceptive devices (IUCD). Contraception. 1994; 49:161-169.
31. Zanuncio J, Mourao S, Martinez L, *et al.* Toxic effects of the neem oil (*Azadirachta indica*) formulation on the stink bug predator, *Podisus nigrispinus* (Heteroptera: Pentatomidae). Sci Rep 2016, 6:30261. doi:10.1038/srep30261