



## IS NEEM OIL HARMFUL TO FISH G GIURIS

## Zoology

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## ABSTRACT

Histopathological effects of neem oil were studied in fish. The fish were treated with neem oil; control fish received the emulsifying agent with or without peanut oil. Peritoneal lavage, collected on subsequent days, showed a results of this study neem oil acts as a non-specific immunostimulant and that it selectively activates the skin Integument (is a active secretory organ forms the primary barrier external covering of the body) with Neem oil treatment for short duration. Alteration of normal organization in mucous glands and its secretion, club cells initiates reaction. Where as in melanocytes of fish, *G giuris* exhibit further aggressive colouration. Further physiological functions including respiration, excretion and osmoregulation are also disturbed. Skin supposed to keeps the body surface moist and acts as antibacterial and antifungal agent, it fails this action due to neem toxicity even at lower dosages. The aim of this work is to focus on histopathology of skin of fish, (*Glossogobius giuris*,) being a bottom dwelling habitation and exposed to sediment toxicity. cell-mediated immune (CMI) mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge.

## KEYWORDS

Skin, *G.giuris* and Azadirachtin.

## Introduction

The aquatic risk assessment process showed that the risk values of tested pesticides did not exceed the criteria, and therefore, no ecological hazard is likely to result from their use. Extensive work has been carried out on skins normal physiological functions like mechanical injury, respiratory, excretory and osmoregulatory. It is a self active secretory organ which provides number of useful products. Mucous glands whose secretion plays a major role in protection against stress factors, imposed by changes in the nature and chemical composition of surrounding water (Zuchelkowski, E. M. & et al., 1985.). It keeps the body surface moist, acts as antibacterial and antifungal agent (Gona, o., 1979.). Also, the skin houses the alerted content of club cells which initiates alarm reaction (Pfeiffer, W., 1971). The skin contains melanocytes which provide fish with its specific colouration (Lagler, K.F., 1977). *Glossogobius giuris* fish differs from that of higher vertebrates in that the epidermis is composed of non-keratinized living cells. Epidermal integrity is vital to fish in maintaining osmotic balance and excluding micro-organisms. The epidermal healing response in this fish is extraordinarily low, even at low temperatures (Mohan, 1991 and Bullock et al., 1978). Where as work on neem toxicity on Bottom dwelling fish, *G giuris* is lacking, Therefore in this contest the need for such a work in indeed need of an hour.

The importance of studying this fish is to evaluate its very existence and survival in Bangalore region (Mohan 2000) and to discuss the implications for its exit in this particular area.

Azadirachtin, is an herbal/plant pesticide derived from the neem tree, *Azadirachta indica* is one of the most promising natural compounds (Winkaler et al., 2007), where it is less harmful to the environment than the synthetic pesticides (sundaram, 1996). The neem tree has been described as *A. indica* as early as 1830 by De jussieu. However, neem has been found to be toxic to non-target organisms where it induces marked alterations in experimental animals (Mahboob et al., 1998; Panda and kar, 2000; Rahman et al., 2002 and Hassanein et al., 2007). These Azadirachtin Pesticides are used to prevent the pest and there by increase the agricultural production. They are carried away by rains and floods to water bodies and alter the physicochemical properties of water (Ansari and Ahmad, 2010). These Pesticides are potentially more toxic to fish and other aquatic organism owing to the excessive use of synthetic pesticides, the environment and water resources are being polluted, thus endangering aquatic life directly and human life indirectly. Although, safe dosage to higher animals, (both synthetic and natural pesticides) are toxic to fish.

To overcome the hazardous effects of organic chemicals natural pesticides of plant origin are used. Plants are virtually inexhaustible sources of structurally diverse and biologically active substances (18). Neem (*Azadirachta indica* *Azadirachta juss*) is also a traditional and

highly esteemed medicinal tree for the people of Indian Subcontinent ( ). Azadirachtin (a tetraterpenoid ) is one of the major components of neem, which have pesticidal properties. Deshmukh and Pariyal. The neem is used in organic manure as with insecticide properties. It is a potential source of organic manure and is rich in many plant nutrients namely nitrogen (2-3%), phosphorus (1%) and potassium (1,4%). Recently neem based pesticides are popularized due to their effectiveness, cheaper price and comparatively safe for users, which is used widely in several states of India (Anjaneyulu and Mishra, 1999) Neem toxicity is more evident even at lower dosages in the skin of Fish.

## Materials and Methods

The fishes, *Glossogobius giuris* (10-12 $\pm$ 2 gms wt and 10-12  $\pm$ 3 cms in length) were collected from the Kaveri delta region of Karnataka, India. Then fish were acclimatized prior to subject them to experiments for 15 days in the laboratory conditions in glass aquaria. During the experiment fish were fed with tube-fix. Acclimatized fish were divided into 4 groups of 10 each as first group and served as the control, while remaining three groups were exposed for 3 sub lethal concentration of 0.25, 0.5 and 1ppm of neem oil for 24, 48, 72 and 96hrs. Fish were sacrificed periodically and their skin was excised, fixed in Bouin's fluid and were processed following the standard histological techniques. Tissues were embedded in paraffin wax of 56 -58 c and serial section of 4-6 micrometer thickness (22) were cut, stained in Haematoxylin and counter stained with Eosin. The sections were examined under light microscopy and taken Microphotographs by using a minisec camera.

## Results

Anatomy of The *Glossogobius giuris* skin comprising the cuticle, epidermis, basement membrane, dermis (comprising *stratum laxum*(spongiosum) *stratum compactum*), and hypodermis (sub-cutis).

Cuticle, the external layer, the cuticle is a mucopolysaccharide layer approximately 1micrometer thick. It is secreted by epithelial surface cells and is a complex of cell protoplasm, sloughed cells and any goblet cell mucus that has been secreted onto the surface. The cuticular layer contains specific immunoglobulins, lysozyme and free fatty acids, all of which have anti- pathogen activity. Epidermis, The fundamental unit of the epidermis of the *Glossogobius giuris* is the fibrous malpighian cell. They are rounded cells at all levels except the outermost, where they are flattened horizontally, with a cytoplasm composed of an accumulation of elongated vesicles, degenerating mitochondria and dense bundles of fibres. The epidermis, respond to non-specific irritation by a thickening of the cuticle or a hyperplasia of the malpighian cells, minimizes the chances of epidermal disruption. The epidermis is composed of a non-keratinized stratified squamous epithelium of variable thickness (4to20 cells thick). The outermost epidermal cells (Malpighian cell layer) retain the capacity to divide.

Other cells present in the epidermis are goblet cells, large eosinophilic club cells or alarm cells (present in most species of fish), eosinophilic granular cells (unknown function), leukocytes and macrophages. Pigment cells and sensory structures are the characteristic features of Teleost skin. Epithelial cells The basal layer of epithelial cells, arranged in a single layer on a thin non-cellular basement membrane, are usually low columnar in shape with centrally placed rounded nuclei, sometimes appearing flattened with flat nuclei. The middle layer epithelial cells, which are arranged less compactly in two to four layers, are, in general, polygonal with centrally placed, rounded nuclei. Epithelial cells, which are below mucous or eosinophilic granular cells, appear flattened with flat nuclei. In the superficial layer, epithelial cells present a flattened aspect with flat nuclei. Occasionally, exfoliated epithelial cells isolated or in groups, are also seen on the surface. The outermost epidermal fusiform cells remain viable and retain the capacity to divide: this has obvious implications for healing processes. The epidermis is formed of thickened stratified squamous epithelium having a well definite basal layer of columnar cells, stratum germinativum, which is situated on a thin basement membrane. On the top of stratum germinativum lies several layers of fusiform cells covered by a superficial squamous cells. Mucous cells, The mucous cells are distributed in the middle layers of the epidermis and have its base on the basement membrane. These cells directly open onto the surface. Cells found within the epidermis include: the filament containing or malpighian cells that represent the major component: mucous cells responsible for secreting glycoprotein (mucus) forming a slimy protective coat. The functions include predator evasion and isolation of superficial epithelial cells from bacteria. Immunoglobulin, also present in mucus, provide additional protection against infection. In addition to this the skin contains wandering leucocytes and macrophages. Club cells, Club cells are large, usually round cells, found in the lower and middle layers of the epidermis. The club cells are the Shreckstoffzellen found in the epidermis, secrete a potent alarm substance. The Sense organs were found to be numerous detected as superficial organized neuromasts, fusiform pale staining cells, rested on fibrous dermal folds. Lymphocytes, Rounded or irregular lymphocytes, usually distributed in the intercellular spaces of the deeper epidermal layers of other fishes were observed sparsely in this fish. Pigment cells (melanocytes), showed well developed, highly branched, and are distributed randomly throughout the deeper layers of the epidermis and in the dermis. These cells are most evident as an almost continuous layer just beneath the basement membrane. The pigment cells are filled with dark brown or black coarse granules. Eosinophilic granular cells, large number of very well developed eosinophilic granular cells (EGC), spherical or ovoid, are present in the upper, and occasionally in the lower layers of the epidermis. However, these cells could not be located in the deeper layers of the dermis. Taste buds, Very few taste buds could be located on the skin surface. These are pear-shaped containing tall columnar sustentacular cells with ovoid darkly stained nuclei, alternating with fusiform sensory cells having lightly stained nuclei. They are associated with small pyramidal basal cells. Structures consisting of spindle shaped neuro-epithelial cells and supporting cells each positioned above a well-developed dermal papilla. Long taste hairs arise on the free surface from these neuro-epithelial cells. Dermis, is about 15-20 times as thick as the epidermis and hence is the largest component of the dorsal skin. It consists of two distinct layers – the outer stratum laxum (spongiosum) and the inner stratum compactum. The dermal layer is formed of dense compact parallel bundles of collagenic fibers. Stratum laxum, The stratum spogiosum is a loose network of collagen and reticular fibres continuous with the epidermal basement membrane. It contains the pigment cells (Chromatophores), mast cells and also the scales. *Stratum compactum*, Comparatively thin, this tissue consists of layers of coarse collagen fibre bundles arranged parallel to the skin surface with a few fine elastic fiber bundles. Many fibrocytes are scattered among the collagen fibers. Branches from the main blood vessels, pigment cells and nerves in the subcutis run through this layer and supply the capillaries in the *stratum laxum*. In the inner surface of the *stratum compactum*, a layer of small-branched pigment cells is discernible. Beneath the dermal layer is a loose, adipose tissue, which is generally more vascular than the overlying dermis. This is the hypodermis. It is a frequent site of development of inflammatory processes. However, the hypodermis is not distinguishable in many regions and some author say that this layer does not exist in fish and is part of the deep dermis. Subcutis is the innermost region of the skin and lies in between the *stratum compactum* and the muscles. It is composed of loose connective tissues and is richly infiltrated with fat cells that in routine HE preparations appear as empty spaces. Many blood vessels

and nerves may be found in this region. Scales are calcified plates originating in the dermis and covered by the epidermis. Cycloid scales of teleost fish have a smooth outer surface and are laid down in concentric a ring that makes them useful in determining the age of some fish. Scales also represent a source of calcium for fish; some fish will utilize the calcium in the scales in preference to the calcium in their skeleton during times of starvation or pre-spawning activity.

## Discussion

Skin is continuously exposed to internal and external influences that may alter its condition and functioning. As a consequence, the skin may undergo alterations leading to photo aging, inflammation, immune dysfunction, imbalanced epidermal homeostasis, or other skin disorders. The objective of these studies was to evaluate the present knowledge about the interrelation of neem oil and skin, Skin being the functional organ to protect the mechanical injury, and to perform respiratory and excretory and osmoregulatory functions. It is a self active secretory organ which provides number of useful products. Mucous glands whose secretion plays a major role in protection against stress factors, imposed by changes in the nature and chemical composition of surrounding water (Zuchelkowski, E. M. & et al., 1985.) It keeps the body surface moist, acts as antibacterial and antifungal agent (Gona, o., 1979.). Also, the skin houses the alerted content of club cells which initiates alarm reaction (Pfeiffer, W., 1971). The skin contains melanocytes which provide fish with its specific colouration (Lagler, K.F., 1977). It has sense organs like neuromasts, taste buds and lateral line canal (Jakubowski, M., 1974. Hong, C., 2000. & El-Morsy, S., 2001.). *Glossogobius giuris* fish differs from that of higher vertebrates in that the epidermis is composed of non-keratinized living cells. Epidermal integrity is vital to fish in maintaining osmotic balance and excluding micro-organisms. The epidermal healing response in this fish is extraordinarily low, even at low temperatures (Mohan, 1991 and Bullock et al., 1978).

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