



## Effect of *Ipomea Carnea* (JACQ) Leaf Extract on the Brain Tissue of *Heteropneustes Fossilis*

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### Abstract

*Ipomea carnea* was found abundantly in lower Assam, was observed to be neurotoxic effect to animal specially central nervous system. In the present investigation ethanolic extract of *ipomoea carnea* exposed Indian cat –fish *H. fossilis*. Fishes were divided in to four groups-one control group and three experimental groups.(Af1,AF2,AF3,). Fish were exposed to 5mg/ml concentration for 96 hours. Neurotoxic behavior was observed in the experimental groups such as behavioral response of movement, unconsciousness, loss of weight, reduced intake of food was observed as compared to control. Histopathological study showed damage of histological and cytological picture of brain. In AF2 group neurons cell exhibited marked histopathological alteration as normal cell converted to round, pear shaped and elongated structure. Occurrence of vacuolation around the nucleus and the nucleus shifted to the side of vacuoles were significant feature. In AF3 and AF4 group most of the nucleus to round large and vacuolar the number of nucleus were appear to be more than control. Which indicate meiosis of cell. Finally they demyelization, necrosis in inner modullarly region, blood capillary dilation and swelling as well as hyalinization of the cell body, along with astrocytoma like alteration of histological structure of the brain. All these finding indicated that *Ipomea carnea* is a potent neurotoxic agent; It can be used as biopesticide.

Further investigation is suggested.

**Keyword:** *Ipomea carnea*, neurotoxic effect, *h.fossilis*, brain tissue, vacuolation, acentri nucleus, demyalinatation.

### Introduction

The locoweed *Ipomea carnea* (Jacq) subspecies *fistulosa* coisy is abundantly growing in several tropical countries of the world and grow abundantly in India, particularly in the Northeastern region of the country<sup>1</sup>. The plant is slender growing upto 8 to 9 feet in height. It grows in the adverse conditions<sup>2</sup>. On the other hand, it is found to be toxic to animals such as cattle, sheep and goats<sup>3</sup>.

Alkaloids are the main toxic principle such as poly hydroxylated alkaloids Swainsonine, 2-epilentiginosine, N-methyl trans-4 hydroxyl-L-prolin and calystegines A1, B2, C1 and 2a and 2a dihydroxy nortropane<sup>4</sup>.

The leaf intake induces neuro behavioural effects in goat, cattle and sheep, as alkaloid in leaf has sedative, hypnotic central depressant activity and muscular relaxant property<sup>5</sup>. The concentration of alkaloids swansomine and calystegines in the nervous system may be lower, because these two alkaloids are weakly basic compounds<sup>6</sup> and the barriers of the brain is relatively rich in lipids and swansomine is less lipid soluble<sup>7</sup>. But they somehow migrate to cross the brain barrier.

The present study was undertaken to investigate the detailed histopathological changes induced in brain tissue when *Heteropneustes fossilis* is exposed to sublethal concentration of *Ipomea carnea* in short term study of 72 hours.

### Material and Methods

*Carnea* leaves were collected in October, 2006 from Barpeta district of Assam, India where it grows abundantly in low lying and marshy areas. The authenticity of the plant species was confirmed by the Department of Botany, M.C. College, Barpeta, Assam.

The sun dried fresh leaves were powdered. An amount of 600g powdered leaves were triturated with absolute ethyl alcohol and then macerated for 72 hours in ethyl alcohol. The solution thus obtained was filtered in a glass container and the filtrate was evaporated under reduced pressure at a temperature below 50°C. The product thus obtained was stored. This process was repeated thrice and the final dark green extract was suspended in distilled water (90 g ± 8% W/W) to the remove waxy residues. It was then treated with n-butanol saturated with water. This fraction of leaf extract contains active toxic principle.

**Animals:** Air breathing cat fish *Heteropneustes fossilis* of body weight 20-25g and length 8-10cm. were collected from local ponds of Barpeta district in Assam and acclimatized to laboratory conditions in glass aquaria for 15 days<sup>8</sup>. Fishes were fed on a laboratory standard diet during the period of acclimatization and toxicity test.

Fishes were divided into four groups of which one is maintained as control. The residues of *I. carnia* were rediluted<sup>6</sup> for

experimental purpose. In our Investigation AF1 (5ppm), AF2 (10ppm), AF3( 30ppm) doses of rediluted residues of *I. carnia* were exposed to the fish for a time period of 3 days.

The doses were used for oral administration. Control and Test groups were kept in 60L glass aquaria. Swimming and other abnormal behavior were observed for the time period of 72 hours. After 72 hours brain of the control and experimental groups were removed quickly and fixed in Carnoy's fixative followed by standard histological techniques. Sections were made and stained in eosine and haemotoxyline for the histopathological examination of brain tissues.

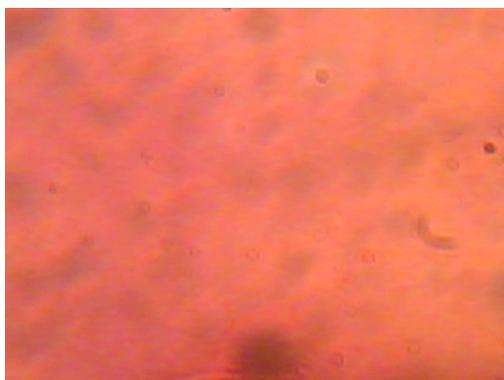
## Results and Discussion

The fishes established marked abnormal behavioural responses due to *Ipomea* exposure. Visible de-pigmentation along with profound mucous secretion occurred over the entire body of the exposed fish.

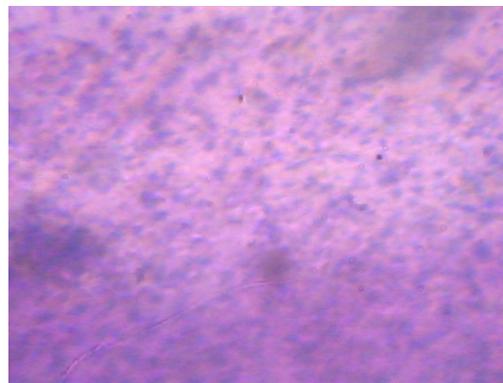
In AF1 test group the toxic symptoms were indicated by visible changes in various physiological activities that occurred early in exposure period and reduced during later exposure period as revealed by changes in opercular movements. This indicated that *I. carnea* had an immediate effect on the gills, leading to impaired oxygen uptake.

At higher concentration of AF1 and AF2 the fishes swam erratically with rapid jerky movements including spiral convulsion and hyper excitability. Later on, the fish struggled hard for aerial breathing. They also showed restricted swimming movements and indicated poor response to external stimuli. These symptoms were followed by a loss of equilibrium and the fish slowly moved forward in vertical directions and became progressively lethargic. Ultimately they laid down at the bottom of the container with their belly upward.

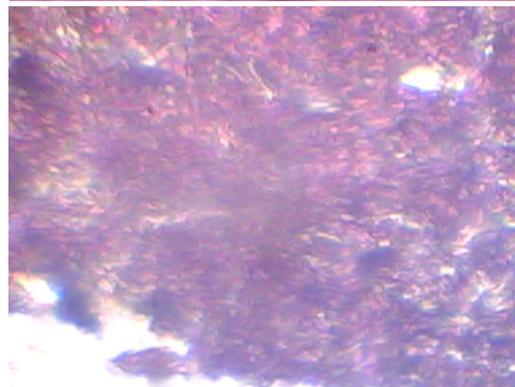
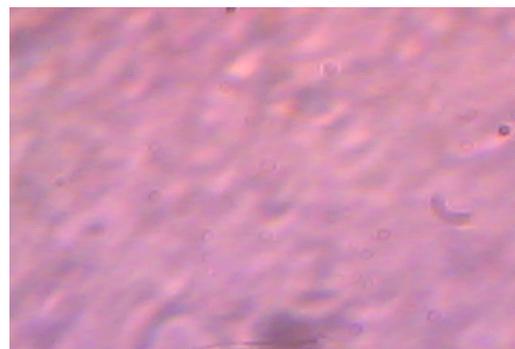
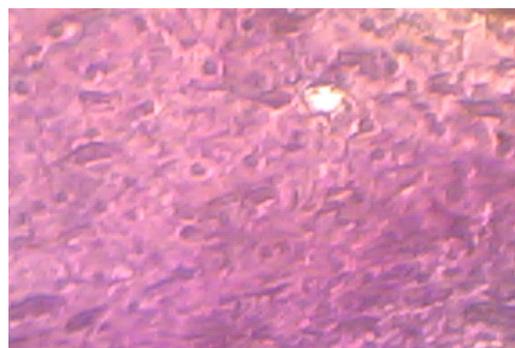
Their feeding habits were also reduced in all of the test groups which suggested that there were adverse effect on the normal physiological activity including digestive process.



**Figure-1**  
Showing histology of Brain in Control fish (×400)



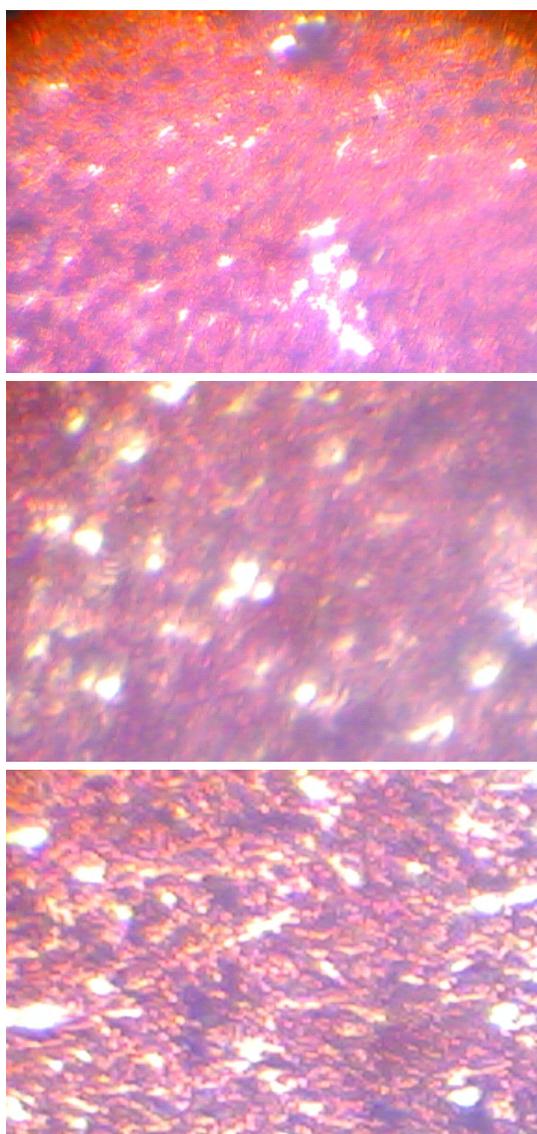
**Figure-2**  
Showing histology of Brain in Control fish (×400)



**Figure-3, 4 and 5**  
Showing histology of Brain in fish exposed to AF1 of I.C. marked with vacuolation, nuclear fragmentation and acentric nuclei (×400)

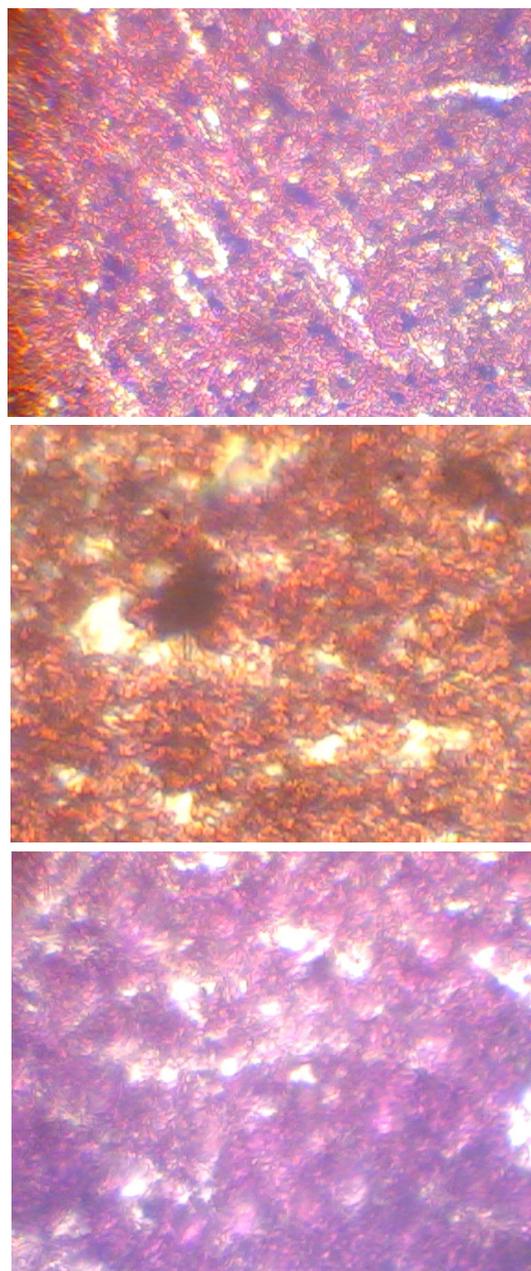
**Histopathological examination of Brain tissue:** A histopathological picture of control (normal) tissue is shown in figure-1 and 2. The neurotoxic effect of AF1 concentration of *I. carnea* extract was observed in the histopathological picture of the brain tissue of *H. fossilis*. Degeneration of brain cell was observed in the test group along with abnormal cell size and shape. Cytomorphological changes in brain tissue were also observed. Neuron cells were enlarged and oval with marked vacuolation around the nucleus. The nucleus shifted to the side of the vacuole and showed granular structures inside it. An indication of karyolysis was evident by nuclear fragmentation.

At AF2 test group, progressive chromatolysis with profuse vacuolation and lipidosis was observed. Infiltration of macrophage polymorpholeucocytes indicated a serious infiltration in brain tissue.



**Figure-6, 7 and 8**  
Showing fish exposed to AF2 of I.C. marked with acentric nuclei, vacuolation

The histopathological picture of the brain tissue of the AF3 test group indicated total loss of cellularity with profuse vacuolar degeneration. The loss of cellularity with profuse vacuolation and necrosis was also marked in intermedullary regions. This indicated extreme neurotoxicity with demyelination. Due to degeneration of nerve cells, swelling and hyalinization of cell bodies were observed. The cells were separated by numerous glial fibrils. These changes revealed astrocytoma of the brain cells.



**Figure-9, 10 and 11**  
Showing fish exposed to AF3 of I.C. Showing total loss of cellularity, fibrosis and lipidosis (×400)

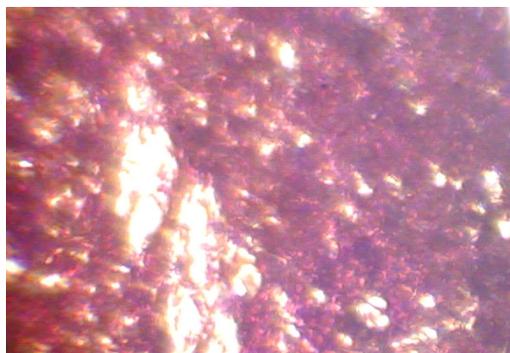


Figure- 12

Showing fish exposed to AF3 of I.C. Showing total loss of cellularity, fibrosis and lipidosis (×400)

The neurotoxicity of *I. carnea* on brain tissue of the fish *H. fossilis* results in severe dysfunction of the Central nervous system, which interfered with normal behaviour.

The behavioural abnormality observed in the present experiment due to exposure of phytochemicals in the leaves of *I. carnea* cause sequential changes in liver of goat, sheep and calves<sup>9</sup> and also caused normochromic anemia<sup>10</sup>. In Brazil Armien et.al<sup>11</sup> reported intoxication in goat characterized by cytoplasmic vacuolation in neurons of central nervous system (CNS) and autonomic nervous system (ANS). Bhattacharya, et.al.<sup>5</sup> also reported adverse effects of *I. carnea* leaf extract on the nervous and endocrine systems of rats including brain.

It can be concluded that *I. carnea* is a potent neurotoxic agent. It has tremendous scope for use as an herbal insecticide since it affects central nervous system of animals.

## Conclusion

In conclusion, *Ipomea Carnea* is a potent neurotoxic agent, it has tremendous scope for use as a herbal insecticide, since it affects C.N.S of the animal.

## Acknowledgement

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