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TO STUDY THE EFFECT OF WATER EXTRACT OF PLANT *CASSIA FISTULA* AS AN ANTIINFLAMMATORY AGENT ON *CHANNA PUNCTATUS* FISH

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ABSTRACT

The present work deals with evaluation of anti-inflammatory response of water extract of *cassia fistula* leaves on *channa punctatus* fishes. For that, fishes were exposed to 0.15 gm/L^{-1} (96 hr) sub-lethal concentration of a dride *Parthenium* flower extract in aquatic medium of aged tap water for 30 days with a regular interval of 7 days. In gonads, exposure dependent histological damage has been observed in terms of vacuolization, condensation of spermatogonic cells, distortion of tubular epithelium and shrinkage of interstitial cells. Gross histoanatomy of ovarian tissue reveals epithelial lesions, stormal haemorrhage, increased interstitium and shrinkage of yolk vesicles towards periphery. These findings are quite suggestive of reproductive impairments leading to delayed gonadial maturity and adversely affecting processes of sperm production and ovulation and thus, the fish production. In the next set of experiment, (0.15 gm/L^{-1}) concentrations of water extract of *cassia fistula* leaves were run simultaneously with the same concentration of a *Parthenium* flower extract (0.15 gm/L^{-1}) in aquatic medium of aged tap water for 30 days with a regular interval of 7 days. In mixed treatment, *cassia fistula* leaves extract significantly reduce the histological damage observed due to sub-lethal concentration of a *Parthenium* flower extract and help for healthy reproduction. In the next set of experiment, fishes were exposed to 0.15 gm/L^{-1} concentrations of water extract of *cassia fistula* leaves and that better histological changes were observed as compared to previous set of experiment i.e. control. Results suggested that under present experimental conditions; water extract of *cassia fistula* leaves exhibit reproductive activity and it may show anti-inflammatory activity in this fish model, which could further contribute to study its benefit in humans.

Key words: C*assia fistula*, *Channa punctatus*, Antiinflammatory.

INTRODUCTION

Cassia fistula is the deciduous plant found all over India. Basically, it is the sub

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tropical plant. It is known as golden shower because its flower is in golden colour. This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections. It is used by Malaialis tribe in India to treat nasal infection. The pulp of the ripe fruits has a mild, pleasant purgative action and is also used as an anti-fungal drug. Indian people are using the leaves to treat inflammation, the flowers as a purgative, the fruit as anti-inflammatory, antipyretic, abortifacient, demulcent, purgative, refrigerant; the plant is good for chest complaints, eye ailments, flu, heart and liver ailments and rheumatism. It is useful in treating haematemesis, pruritus, eucoderma and diabetes. Besides its pharmacological uses, its extract is also recommended for pest and disease control. *Cassia fistula* exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. The whole plant is used to treat diarrhea; seeds are used to treat skin diseases, flowers and fruits are used to treat skin diseases, fever, abdominal pain and leprosy by traditional people.

Parthenium is the an unwanted weed, which grows all over India in any climatic condition and soil. Mostly it grows near the small ponds. This may create the inflammation in the fish. Therefore, in the present study, we use dose of water extract of *Parthenium* for the induced inflammation on the gonad of *Channa puctatus*, as healthy gonad of the fish are an important determinant of its breeding potential. Thus, any toxicological factor adversely affecting the histoanatomy of gonad. Keeping this in mind, present study was undertaken to assess the antinflammatory activity of water extract of *cassia fistula* plant on induced gonad of *Channa puctatus*.

EXPERIMENTAL

Materials and methods

Live specimen of *Channa punctatus* were purchased from the market and brought to the laboratory in the wide mouthed plastic container. After thorough washing in the tap water, these were put in deep treatment in 2% $KMnO_4$ solution. Apparently healthy fishes (10 ± 1 cm and 30-40 g) were acclimatized to the laboratory conditions for 30 days in aged tap water. They were fed twice a day with pieces of earthworms, eggs and special floating type fish food marketed under the trade name “Tokyo’ at regular interval of 12 hours.

For this purpose, four aquarium were set, each having 8 healthy and live specimens of fishes. These four aquariums were classified as follows -

Table 1: The experimental setup for the 4 aquariums

S. No.	Time interval	Aqarium 1 (Control)	Aqarium 2 (Induced)	Aqarium 3 (Induced + Treated)	Aqarium 4 (Treated)
1.	07.01.2011	Bring the fishes	Bring the fishes	Bring the fishes	Bring the fishes
2.	07.01.2011	As it is	As it is	As it is	As it is
3.	14.01.2011	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight
4.	14.01.2011	As it is	Induced with *Parthenium* extract (0.15 g/L^{-1})	Induced with *Parthenium* extract (0.15 g/L^{-1}) and treated with *Cassia fistula* extract (0.15 g/L^{-1})	Treated with *Cassia fistula* extract (0.15 g/L^{-1})
5.	21.01.2011	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight
6.	21.01.2011	As it is	Induced with *Parthenium* extract (0.15 g/L^{-1})	Induced with *Parthenium* extract (0.15 g/L^{-1}) and treated with cassia fistula extract (0.15 g/L^{-1})	Treated with *Cassia fistula* extract (0.15 g/L^{-1})
6.	28.01.2011	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight
7.	28.01.2011	As it is	Induced with *Parthenium* extract (0.15 g/L^{-1})	Induced with *Parthenium* extract (0.15 g/L^{-1}) and treated with cassia fistula extract (0.15 g/L^{-1})	Treated with *Cassia fistula* extract (0.15 g/L^{-1})
8.	04.02.2011	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight	Sacrified two fishes out of eight
9.	04.02.2011	As it is	Induced with *Parthenium* extract (0.15 g/L^{-1})	Induced with *Parthenium* extract (0.15 g/L^{-1}) and treated with cassia fistula extract (0.15 g/L^{-1})	Treated with *Cassia fistula* extract (0.15 g/L^{-1})

Fishes were classified into four groups as mentioned in the Table 1. These were induced by extract of *Parthenium hysterophorus* flowers and screened against gonads of C*hanna punctatus* fish with special reference to their impact on abnormalities related to body inflammation.

Fishes were exposed to sub-lethal concentration of the toxicant *Parthenium hysterophorus* flowers extract (0.15 g/L^{-1}) (96 hr) for 30 days of exposure period with regular interval of 7 days. The induced fishes were also treated with the water extract of *cassia fistula* leaves similar numbers of fishes were maintained in controlled medium. For all the purposes, 30 liter water was taken in each aquarium. At the end of the exposure periods, both control and treated fishes were taken out of aquaria and promptly anaesthetized with wild dose of formalin. The fishes were dissected in Ringer's saline solution; the testis and ovary were quickly taken out, sliced and initially fixed in Bouin's fluid. After washing several times with 70% alcohol, tissue slices were cleared in Cedar wood oil and processed for paraffin embedding. Tissue sections of 4 μm thickness were cut, passed through descending and ascending series of graded alcohol and stained with haematoxylin and eosin for photomicrography. Significant findings were recorded by NIKONHFX-DX trinocular microscope and NIKON-FX-35-DX (12 megapixel) camera with automatic time recorder. The changes in exposed sections of gonads (testis and ovary) were compared with those of the control sections.

RESULTS AND DISCUSSION

Histopathological observations

Testes are paired, elongated and situated on ventral side of the kidney in the posterior region of the abdominal cavity. Histologically, the testes are composed of a large number of seminiferous tubules, which are closely bound together by a thin layer of connective tissue. The tubules open into a spermatic duct, which is generally lined by secretory epithelium. The spaces between the lobules are filled with connective tissue, blood capillaries and interstitial cells (Leydig cells). During the growth period, the resting germ cells become active and various stages of spermatogenesis are seen such as; primary spermatocytes, secondary spermatocytes, spermatids and the sperms.

Fishes were exposed to sub-lethal concentration of the toxicant *Parthenium hysterophorus* flower water extract for different exposure periods (Table 1) showed considerable degree of alteration in the histoanatomy of the gonads. These changes were profound and the degree of changes in histoanatomy showed variation during different exposure periods. The seminiferous tubules are generally of varying shapes and sizes. Each

tubule has a definite, thin fibrous wall (Fig. 1). The testis of *Channa punctatus* has shown significant changes on exposure to test *Parthenium hysterophorus* flowers extract. After 7 and 15 days of initial exposure period, no significant changes were observed. But after 30 days of exposure period, there is appearance of a large number of intertubular vacuoles were observed (Fig. 2).

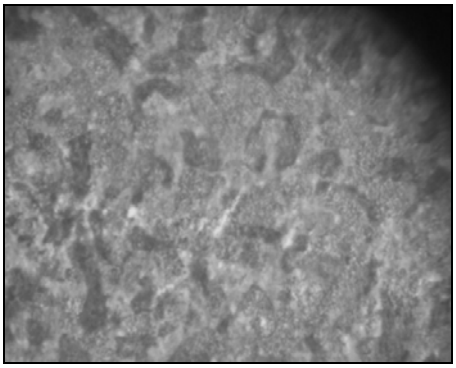


Fig. 1: Section of testis of control fish showing seminiferous tubules

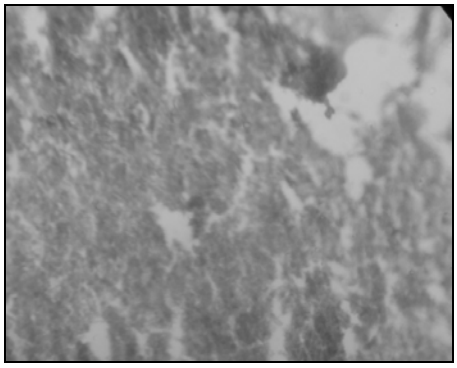


Fig. 2: Section of testis of fish treated with toxicant *Parthenium* extract showing intertubular vacuoles

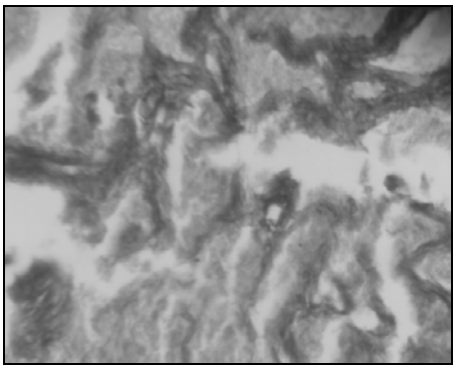


Fig. 3: Section of testis of fish treated with Toxicant *Parthenium* extract showing intertubular vacuoles

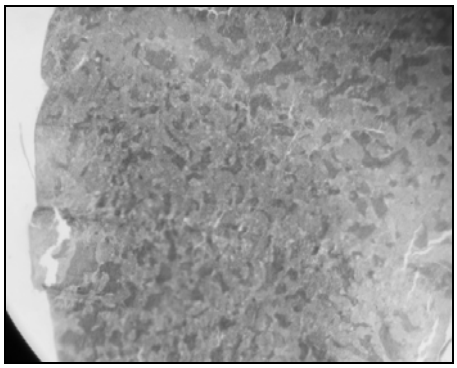


Fig. 4: Section of testis of fish treated with *Parthenium* extract along with *cassia fistula* extract showing recovery of intertubular vacuoles

In aquarium No. 3, fishes were exposed to sub-lethal concentration of the toxicant *Parthenium hysterophorus* flowers extract along with water extract of *Cassia fistula* leaves for different exposure periods. After 30 days of exposure period, the testes show the recovery of vacuolization of tubular cells and distortion of seminiferous cells and tubular cells.

In aquarium No. 4, fishes were exposed to only water extract of *Cassia fistula* leaves for different exposure periods. After 30 days of exposure period, the testes show similarity to those of control fish.

The ovary

The wall of the ovary is fairly thick during the non-breeding season. It consists of three layers : (i) an outermost, thin Peritoneum (ii) thick, Tunica albuginea and (iii) the germinal epithelium that project in to ovocoel in the form of lamellae, which are seats for the development of oocyte.

In the months of December, January and February phase ovaries are small, thin thread like translucent, pale white in color, having nests of oogonia.

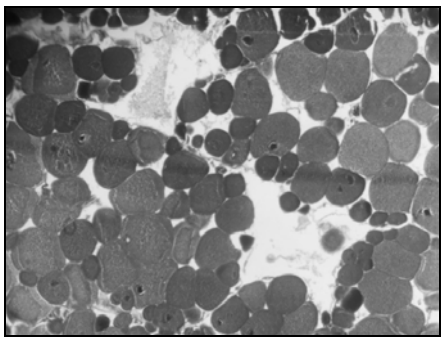


Fig. 1: Section of ovary of fish treated with *Parthenium* extract showing nucleolu condensation of crescent shaped dark granules at one side

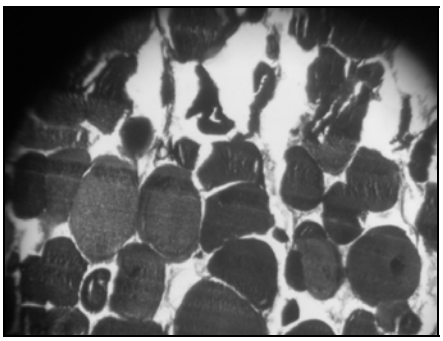
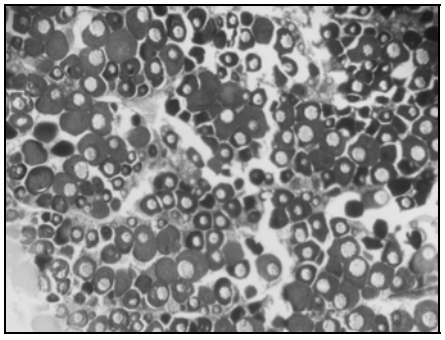


Fig. 2: Section of ovary of fish treated with *Parthenium* extract showing vacuolisation



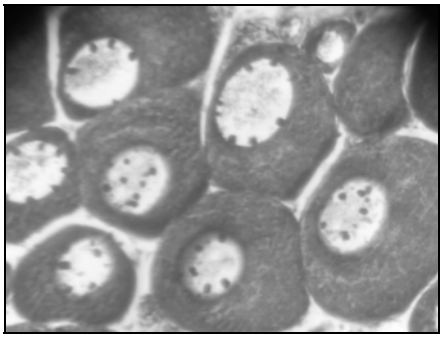


Fig. 3 and 4: Sections of ovary of fish treated with *Parthenium* extract along with *Cassia fistula* leaves extract showing recovery of developmental stages than control

In aquarium No. 3, fishes were exposed to sub-lethal concentration of the toxicant *Parthenium hysterophorus* flowers extract along with water extract of *Cassia fistula* leaves for different exposure periods. After 30 days of exposure period, the ovaries show the recovery of developmental stages such as oogonic stage, early perinuclear stage (nucleoli arranged peripherally) as shown in Fig. 4. Cytoplasm and nucleus shows normal position.

In aquarium No. 4, fishes were exposed to only water extract of *cassia fistula* leaves for different exposure periods. After 30 days of exposure period, the ovaries shows similarity to those of control fish. To healthy reproduction as gonad and ovary show remarkable growth in their vacuoles and oogonia, respectively in treated animals speaks much about their reproductive visors, when compared to the genital growth of induced animals; the animals of group 2 and 3 have an advantage in the above context. These experiments show the maintenance of body temperature of test animals, which creates a conducive environment for healthy growth of the gonads in both; male and females test animals.

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