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Research Article

ANTI CANCER ACTIVITY OF *PHYLLANTHUS AMARUS* IN AZASERINE INDUCED PANCREATIC CANCER OF WISTAR RATS

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ABSTRACT: Pancreatic cancer is a malignant neoplasm originating from transformed cells arising in tissues forming the pancreas. The most common type of pancreatic cancer is adeno-carcinoma. The present experiment was carried out to study histopathological changes occur in pancreas in different groups of azaserine induced pancreatic cancer in Wistar rats with and without the treatment of aqueous and alcoholic extract of *Phyllanthus amarus* at different doses. Histopathological examination of pancreas of untreated group of rats showed hyperplasia of pancreatic duct, necrosis, fatty changes, haemorrhages between pancreatic cells. The rats treated with *Phyllanthus amarus* extracts showed no pathological lesions.

Key words: Azaserine, Cancer, Histopathology, Pancreas, *Phyllanthus amarus.*

INTRODUCTION

Pancreatic cancer has been reported in various species of animals including dogs and cats. Eldredge *et al.* (2005) reported that 47% of dogs and 32% of cats over 10 years of age died of cancer.To date, only limited therapeutic options are available for the treatment of cancers.Azaserineis known carcinogenic agent and it damages DNA in pancreas, liver and kidneys (Lilja *et al.*, 1976, Sarma *et al.*, 1975). Lesions of pancreatic cancer would go undiagnosed most of the time. Knowledge of progression of pancreatic cancer will help in early diagnosis. Thus, this histopathological study of pancreas in azaserine induced pancreatic cancer would allow a better understanding for diagnosis and treatment of pancreatic cancer.

MATERIALS AND METHODS

All the protocols as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the care and use of Laboratory animals were followed and approved by the Institutional Animal Ethics Committee (IAEC) of Veterinary College, Anand.

The plant: *Phyllanthus amarus* (Family: Phyllanthaceae, English – Carry me seed, Hindi – Bhui aonla) is having reported traditional use

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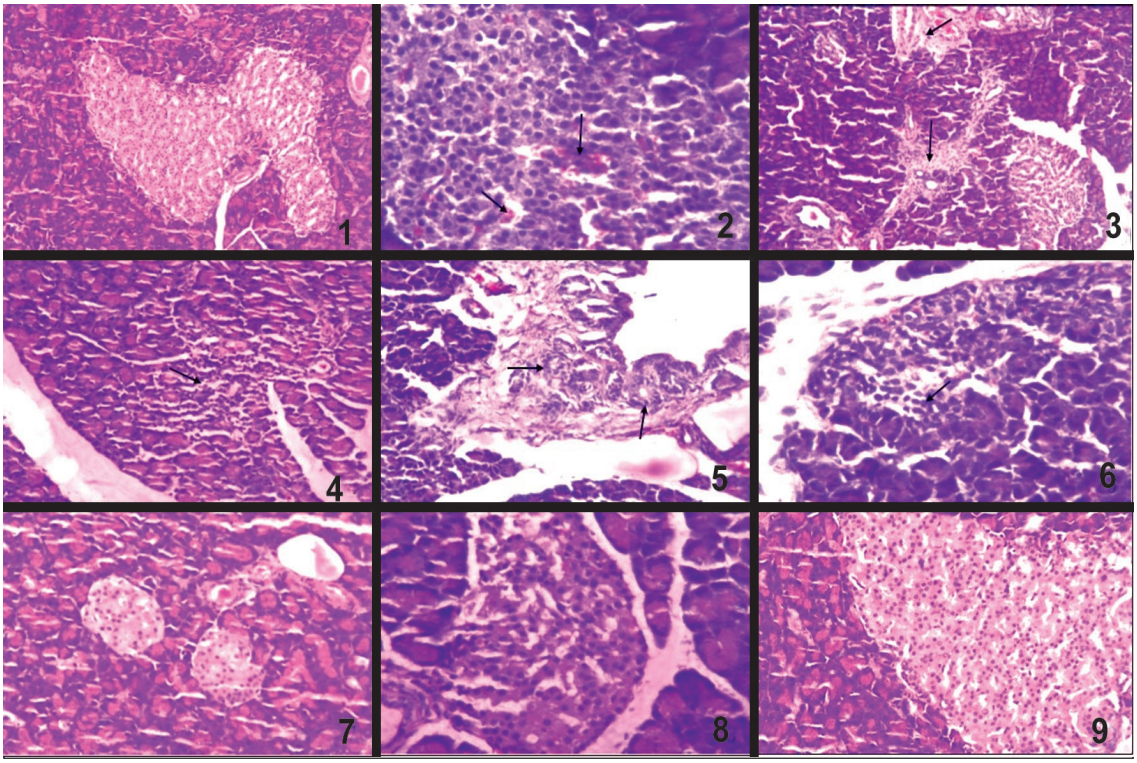

Fig. 1-9. 1. Section of pancreas of rat showing normal architecture of Group I rats. H & E Stain (×120) 2. Section of pancreas showing hemorrhages between acinar cells in Group II rats. (Arrow). H & E Stain (×480) 3.Section of pancreas showing fibrous tissue proliferation in Group II rats. (Arrow). H & E Stain (×240) 4. Section of pancreas showing inflammatory condition with mononuclear cell infiltration and parenchymal necrosis (arrow) in Group II rats. H & E Stain (×240) 5. Section of pancreas showing hyperplasia of pancreatic duct in rat. (Arrow). H & E Stain (×240) 6. Section of pancreas showing lymphocytic infiltration in pancreatic cells in group II rat. (Arrow). H & E Stain (×480). 7. Section of pancreas showing normal architecture of pancreas in group IV rat treated with aqueous extract. H & E Stain (×480). 8. Section of pancreas showing normal architecture of pancreas in group VI rat treated with methanolic extract. H & E Stain (×480). 9. Section of pancreas showing normal architecture in group VII and VIII group kept as extract control. H & E Stain (×240).

in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds. Further, these are used in the treatment of kidney problems, urinary bladder disturbances, pain, gonorrhea, diabetes and chronic dysentery. Topically, it is used for several skin problems ranging from skin ulcers, sores, swelling and itchiness, wounds, bruises, scabies, ulcers and sores, edematous swellings, tubercular ulcers, ringworm, scabby and crusty lesions (Verma *et al*., 2014)

Procedure: Eighty rats weighing 200-300 g were selected for study.Rats were selected randomly and divided in to 8 groups (Group – I, II, III, IV, V, VI, VII and VIII). All Groups had ten animals each. All the rats were numbered group wise and individually.

Group I served as normal control consisted of healthy animals. Pancreatic cancer was induced in group II animals using Azaserine as cancer inducing agent. Group II animals were kept untreated. The rats in group III-VI received azaserine (5 mg/kg body weight) injection once in a week intra peritoneally (IP) for three weeks. Aqueous and ethanolic extracts of *Phyllanthus amarus* leaf and stem were dispersed in water and administered to animals. Group III animals were administered aqueous extract of *Phyllanthus amarus* at dose of 200 mg/kg body weight and group IV animals were administered aqueous extract of *Phyllanthus amarus* at dose of 400 mg/kg body weight. Group V animals were administered ethanolic extract of *Phyllanthus amarus* at dose of 200 mg/kg body weight and group VI animals were administered extract of *Phyllanthus amarus* at dose rate of 400 mg/kg body weight. Animals of Group VII and VIII were administered aqueous and alcoholic extract at dose rate of 400 mg/kg as extract control. The extracts were administered to rats directly in oesophagus by using rat oral feeding needle with 2 ml BD syringe for 21 days.

Pancreatic cancer was induced in ten Wistar rats. Azaserine was used to induce pancreatic cancer (Sigma Chemical Company, St. Louis, MO, USA). All rats were sacrificed at the end of study.All sacrificed animals were subjected to post mortem examination to determine the presence/absence of gross lesions. Detailed post mortem lesions from all the animals were recorded. Tissue samples *viz.*, pancreas were collected from ten rats and preserved in 10 % formalin solution for histopathological examination. The formalin fixed tissues were processed by paraffin wax embedding method of tissue sectioning. Sections from all the tissues were cut at 5-6 microns thickness with automatic section cutting machine (Leica Automatic Microtome Machine, Germany) and were stained with Haematoxylin and Eosin (H & E) stains (Luna 1968). The H & E stained slides were observed under microscope and lesions were recorded.

RESULTS AND DISCUSSION

Pancreas was examined for gross abnormalities during necropsy in all eight groups of Wistar rats. In group I, pancreas was found to be normal. The colour of pancreas in group II rats was severely pale. Rats from group III, IV, V, VI, VII and VIII showed no gross lesions.

The histo-pathological section of pancreas kept as pancreatic cancer control (group II) showed haemorrhages between acinar cells (Fig. 2). Proliferation of fibrous tissue (Fig. 3) was also observed in rats of group II which were treated with Azaserine. Also, inflammatory condition with mononuclear cells infiltration along with parenchymal necrosis (Fig. 4) was found in group II rats. Hyperplasia of pancreatic duct (Fig. 5) was found in Azaserine treated group II rats. In group II rats lymphocytic infiltration in acinar cells (Fig. 6) was found. In Azaserine treated (group II), all rats showed proliferative changes in pancreas section when compared to normal control. This observations were found similar to Revathi *et al* (2012). In group III, IV (Fig. 7), V, VI (Fig. 8), VII and VIII (Fig. 9) rats, which were treated with

Phyllanthus amarus extracts, no pathological lesions were found.

Phyllanthus amarus consists of many principles having anticancer properties including both flavanoids and phyllanthin (Animul *et al*., 2011). These principles in *Phyllanthus amarus* may be responsible for reduction of lesions in pancreas. No toxic effect of aqueous and ethanolic extracts upto a dose of 400mg/Kg body weight was found.

CONCLUSION

Azaserine damages pancreatic cells. Supplementation of *Phyllanthus anarus* extract significantly reduces the histopathological lesions. Extract can be used for prevention of pancreatic cancer.

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