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Histopathological study on effect of *Acorus calamus* L. rhizome on liver and kidney of DAL induced Swiss albino mice

[1]Santhy, K.S. and [2]Sreejaya,S.B.

[1]Faculty, [2]Research Scholar, Department of Zoology,
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore – 641043.

ABSTRACT

Ethnobotanical alternates for synthetic allopathic drugs are soon gaining importance in the treatment of diseases due to their fewer side effects. *Acorus calamus* has been widely used globally due its richness in antioxidant phytoconstituents in the treatments yielding beneficial functions. The methanol extract obtained by soxhlet extraction was screened for acute toxicity studies on DAL induced cells. Accordingly 100 and 200 mg/kg body weight were taken as low and high dose of MEAC for the histopathological studies on liver and kidney.In the present study the damage induced by DAL cells in the liver sections were partially reversed by the *A. calamus* extract treatment. The kidney histopathology micrograph of DAL induced mice showed marked tubular and glomerular damage. This damage is regenerated by the treatment with methanol extract of *A.calamus*. The protective effect of *A.calamus* against liver and renal cell injury induced by DAL cells might be attributed to its antioxidant action.

INTRODUCTION

Medicinal plants, either as an extract, pure compound or as a derivative, offer unlimited opportunities for the discovery of new drugs. Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 8% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors.

It has been estimated that only 6-8 % of the world's flora (approximately 250000 plants) and less than 10% of the organic constituents are known and have been investigated chemically, and 90% remains for discovery and investigations (1).

They have various effects on living systems. Some are sedatives, analgesics, antipyretics, antibacterials, antivirals, antiprotozoals, cardioprotectives, hepatoprotective and nephroprotective agents (2). One of the problems of using plants as medicines is that in many cases no definite doses are prescribed, often resulting in overdose. Many indigenous plants have been in the use of man since time immemorial for curing various ailments without the actual knowledge of their toxic potential (3).

Acorus calamus is an important herb found in marshy land, shallow water and pond edge on the northern emperate, subtropical and warm regions of Indian subcontinent (4). It has been known for its beneficial and nedicinal value in Asia for a long time. In different systems, it is used in different ways: In Agni Purana, this plant s used in the treatment of epilepsy and in rheumatoid arthritis. In Siddha system, fresh root of *A. calamus* is used n the treatment of bronchial asthma. In the Unani system, it is used in the treatment of flatulent colic, carminative, metic, stimulant and as a bitter tonic. In Birhor system, it is used for the medicinal benefit in the alopecia, root as massage to relieve teething problems in children and in treatment of malaria and cancer. In Ayurvedic medicine, *A. calamus* is an important herb and is valued as a "rejuvenator" for the brain and nervous system and as a remedy or digestive disorders.

However, in spite of the extensive use of this herb, there is insufficient scientific evidence validating their fficacy and safety. There are possibilities of toxic effect present due to long term use and unpredictable amounts f the substance that produces the therapeutic effect especially hepatotoxic and nephrotoxic effects [5] as the liver nd kidney are the two most important organs for detoxification process in the body. Therefore, the aim of this tudy was to investigate the hepatotoxic and nephrotoxic effects of methanol extracts of A.calamus on liver and idney tissue in DAL induced Swiss albino rats.

MATERIALS AND METHODS

Collection and identification of the plant

Acorus calamus, the plant used for the present study was collected from Alappuzha district of Kerala, India. dentification of the plant was done in the Department of Botany, Sanadhana Dharma College, Alappuzha. A oucher specimen is preserved as herbarium and submitted to the Department of Zoology, Avinashilingam Institute r Home Science and Higher Education for Women, Coimbatore.

reparation of Rhizome powder

The plant *Acorus calamus* with rhizome washed thoroughly to remove soil particles and adhered debris sing sterile distilled water. Fresh rhizomes used for extraction were shade dried and powered using a mechanical inder. Fine powder was obtained by sieving.

The powder was collected in two clean air tight containers. Powdered plant material in one container was ed for determining the physico- chemical parameters and the other was used for methanolic extraction.

oxhlet extraction

10 gm of the powder was weighed using an electrical balance (Denver 210) and made in to 8 packets using rohaze filter paper (10 A grade SD's). The powder was subjected to sequential soxhlet extraction using different lvents like petroleum ether, chloroform, ethanol, methanol and water to get respective extracts. All the extracts ere filtered and evaporated to dryness and percentage yields of the extracts were determined. From this the aximum percentage yield was noted and that extract alone was stored in a refrigerator and used for the further alysis.

xperimental animals

Healthy Swiss albino mice, Mus musculus (20 ± 5 gm) were used for the study. The animals were obtained m Amala Cancer Institute, Trissur, Kerala and brought to the laboratory. Animals were kept in polypropylene ges with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water s provided ad libitum. The animals were acclimatized to laboratory condition for about one week before mmencement of the experiment. The experiments were performed after the approval from the institution of imal Ethical Committee and in accordance with the recommendation for the proper care and use of the oratory animals.

Acute toxicity study

Swiss albino mice starved overnight, were divided in to five groups. Group I - IV animals were orally fed with MEAC in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 gm/kg body weight, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24 h, 48 h and 72 h for any lethality or death. One - tenth and one - fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the *in vivo* experiment (6).

Histopathological studies

The animals were anesthetized with diethylether and then sacrificed by cervical dislocation. Tissues like liver and kidney were removed from the mouse body and tissues were transferred to ice cooled containers. Wiped thoroughly using blotting paper to remove blood and other body fluids then they were washed in normal saline. Kept the tissues in formalin (10%), fixed and soaked in paraffin, cut at 2-3μm thin, and the slices were stained using hematoxylin and eosin. Tissue slices were photographed using optical microscopy under polarized light.

RESULTS

The results of acute toxicity study of MEAC are presented in Table I. No mortality or change in body weight was observed in mice at a dose level of MEAC 50 mg/kg and 500 mg/kg body weight. Some clinical signs such as tremors, pilo erection and abdominal breathing were observed after the oral dosing of 1000 and 2000 mg/kg but no mortality or change in body weight was observed.

These observations indicated that the calculated LD 50 value (Dixons likelihood method) for the oral doses of the MEAC was found to be more than 2000 mg/kg body weight, accordingly 100 and 200 mg/kg body weight were taken as low and high dose of MEAC for the experiment.

Table I: Clinical signs of toxicity observed during acute oral toxicity study of MEAC

SI.No	Dose (mg/kg b.wt)	Latency	Symptoms
1	50	-	None
2	500	-	None
3	1000	-	Piloerection, abdominal breathing
4	1500	-	Tremor, Piloerection, abdominal breathing
5	2000	-	Tremor, Piloerection, abdominal breathing

Latency - Time of death after the dose.

Fig.1 shows the histopathological observation of liver section of control and experimental animals. Control animals showed normal histological appearance with central vein and unremarkable sinusoids and portal tracts whereas DAL induced mice showed loss of normal architecture. Dissolution of hepatic cords, congested branches of portal vein, destruction of bile ductules and epithelium with inflammatory cells at portal areas were obsedrved. Lobules showed neutrophilic satellitosis with apoptosis. However the treatment groups especially the standard drug showed almost normal hepatocellular architecture. MEAC treated sections of liver tissue revealed manifestation of mild hepatic damage with preserved architecture. There was no fibrosis submassive necrosis and carcinoma.

The kidney of normal animals showed normal zonal variation extending from centre to medulla with normal glomeruli. Tubules were normal. Medulla showed collecting duct, but in case of DAL induced mice, alterations in

glomerular region was observed and suppuration with collection of foamy macrophages. The animals treated with standard drug, MEAC 100 and 200 mg/Kg exhibited regeneration of glomerular region with slightly dilated tubules. No acute tubular necrosis, malignancy or kidney infarct seen. Renal medulla was found to be normal (Fig.2).

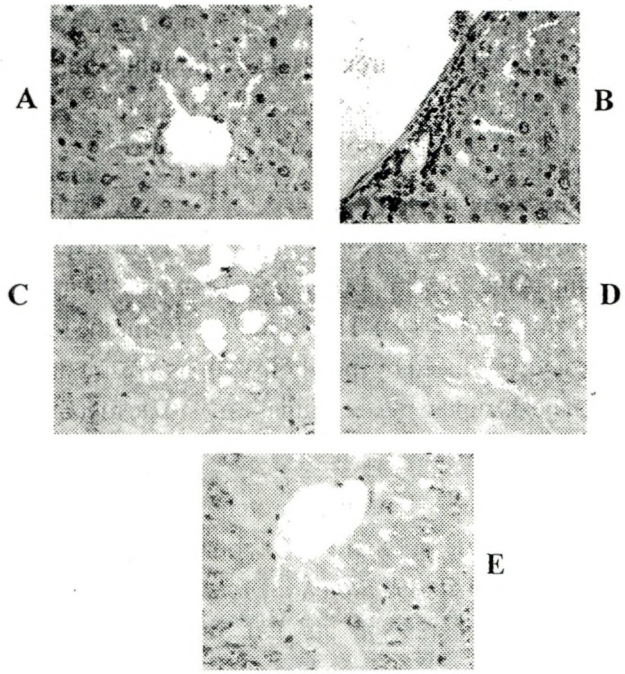

Fig. 1 (A) Histology of control mice liver shows the normal hepatocytes, nucleus, vacuoles, central vein and sinusoids. (B) AL control mice shows deformation of central vein, loss of architecture. Well developed hepatocytes with prominent nucleus d maintained sinusoidal space after *A.calamus* treatments (C & D) and 5-fu (E) administered.

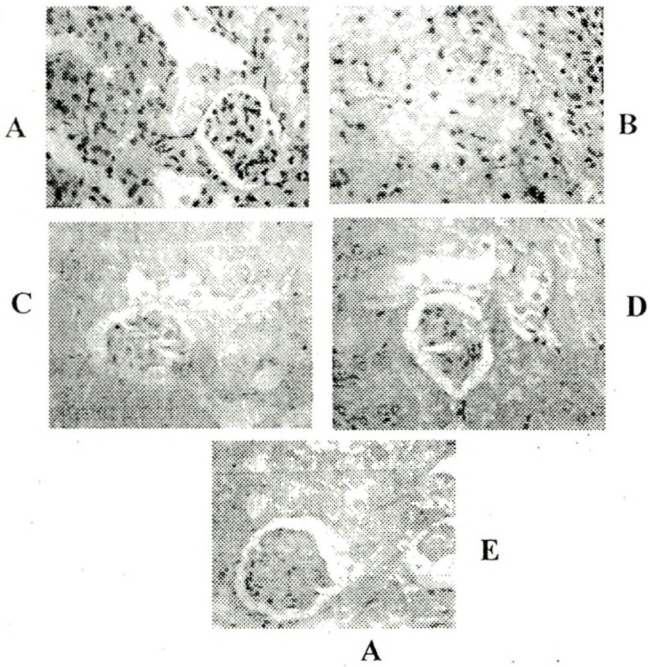

Fig. 2 (A) control mice kidney architecture, (B) DAL control kidney architecture, it shows some alteration in meruli region. Regeneration of glomeruli region after *A.calamus* treatments (C & D) and 5-fu (E) administered.

DISCUSSION

The administration of herbal preparations without any standard dosage, coupled with a scarcity of adequate scientific studies on their safety, has raised concerns regarding their toxicity [7]. Recently studies on the teratological effects of *Asparagus* racemosus in rats which have been advocated in indigenous system of medicine during pregnancy and lactation [8], indicated that herbal drugs are not safe as thought otherwise. In oral acute toxicity study, as high dose of MEAC at 1000 mg/kg did not show any observable toxic effects in the mice in terms of any deaths or abnormal symptoms which points to its being nontoxic and safe in mice.

For centuries, many herbs have been used as natural remedies for the prevention and treatment of liver and kidney diseases. Liver is the largest organ in the body of vertebrates. The liver is particularly susceptible to chemical injury because of its anatomical relationship to the most important portal of entry, the gastrointestinal tract; and the high concentration of xenobiotic - metabolizing enzymes (9). The Kidney performs the functions of getting rid of the body's waste materials that are either ingested, produced by metabolism or as a result of detoxification of the liver. This, and other functions of the kidney, can be disrupted by accumulation of toxic metabolites or chemicals leading to renal diseases (10).

Various herbs and herbal products are believed to have histoprotective / nephroprotective effects and widely used in clinical practice in many parts of the world, for example Silybum marianum attenuated nephrotoxicity induced by gentamicin in dogs (11). Aqueous extract of *Kalanchoe pinnata* leaves significantly protects rat kidneys from gentamicin-induced histopathological changes in rats (12). Similarly in the present study the damage induced by DAL cells in the liver sections was partially reversed by the *A. calamus* extract treatment. The kidney histopathology micrograph of DAL induced mice showed marked tubular and glomerular damage. This damage is regenerated by the treatment with methanol extract of A.calamus. The protective effect of *A.calamus* against liver renal cell injury induced by DAL cells might be attributed to its antioxidant action .

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