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Biochemical Changes in the Serum of Experimental Animals Treated with *Acorus calamus* Rhizome

S. B. Sreejaya, D. Archana and K. S. Santhy*

Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore 641 043, Tamil Nadu, India

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ABSTRACT The present study was undertaken to examine the effect of *Acorus calamus* rhizome on serum biochemical parameters in experimental animals. Tumor was induced in mice intraperitoneally using Dalton's ascites lymphoma cells. Methanol extract of *Acorus calamus* (MEAC) was administered to the mice at the dose of 100 and 200 mg/kg/day. The effect of the extract on serum enzyme levels was evaluated by analyzing the serum hematology and clinical biochemistry of experimental animals. Inoculation of Dalton's ascites lymphoma cells caused significant decrease in the serum levels of alkaline phosphatise (ALP), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in the treated mice when compared with the control group. Urea, uric acid, creatinine and triglyceride levels were controlled significantly. The results clearly indicated that the rhizome extract has the ability to retain the altered biochemical parameters as normal in induced mice supporting its potent anticancer and hepatoprotective effects.

INTRODUCTION

Acorus calamus is a plant, rich in alkaloids, phenols and flavonoids and a potential source of compounds possessing beneficial biological activities. It has a long history of medicinal, cultural and ritual use and hence was spread outside its indigenous areas in Asia and now found across Australia, Europe and North America. Historical ethno botanical review of *A. calamus* dates back possibly to the time of Moses in the Old Testament of the Holy Bible and in early Greek and Roman medicine (Motley 1994).

The rhizomes are considered to possess anti spasmodic, carminative, anti-helminthic, sedative and stimulant properties and also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers, and glandular and abdominal tumors. In the Ayurveda system of medicine the powder of rhizome is being used to produce therapeutic emesis (Vamana), one of the Panchakarma specialized therapeutic procedures of Ayurveda. Recently, new sesquiterpene compounds were isolated from the rhizomes, which would be useful for the biosynthetic pathways of various diseases (Li et al. 2017).

With the upsurge of interests in medicinal plants, there is need for thorough scientific investigations on their efficacy and potential toxicity. Traditional herbal medicines are perceived by the public as (relatively) safe. But nowadays due to various factors such as adulteration, contamination, plant misidentification and use of substitutes, there is a rise in interactions that indicate toxicity of herbs (Zhang et al. 2012). Therefore, it is important to emphasize the traditional use of any plant for medicinal purposes and guarantee the safety of such plant.

Nowadays, the treatment of many diseases including cancer owes much to plants-derived drugs. The results of anticancer studies using *in vivo* models demonstrated the effect of plant extracts to stimulate both the humoral and cell-mediated components of the immune response in the experimental mice, suggesting the therapeutic usefulness of the medicinal plant in a variety of ailments (Geetha et al. 2017). Regarding the toxicity evaluation of drugs and plant extracts, liver and kidney function analysis is very important, as they are both necessary for the survival of an organism.

Address for correspondence:
Dr. K.S. Santhy
Associate Professor
Department of Zoology,
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore 641 043, Tamil Nadu, India
Mobile: 09444439960,
*E-mail:*santhyanandan@gmail.com

Liver is regarded as the central metabolic organ in the body with an important role in homeostasis (Badole and Bodhankar 2010). Liver diseases have become one of the major causes of morbidity and mortality all over world. It was reported that the presence of tumor in the humans or experimental animals is known to affect many functions of the vital organs especially in liver and kidney, even when the site of the tumor does not interfere directly with organ functions.

Biochemical analyses of blood serum are very useful to get insights into the metabolic and health status of animals. During this study, it is very useful to compare the values obtained from tumor-inoculated animals with normal values in healthy animal. The possible alteration in hepatic and renal functions influenced by the rhizome extract is evaluated by analyzing the serum hematology and clinical biochemistry. In the present study, the liver functioning and kidney functioning were assessed to check the level of different components. The liver function tests measured the levels of important chemicals including ALP, SGOT and SGPT. Kidney function test is used for a variety of tests including blood urea, nitrogen, creatinine and uric acid in the blood.

METHODOLOGY

Plant Material

The fresh plant of *Acorus calamus* was collected from Alappuzha District, Kerala, India. Identification of the plant was done in the Department of Botany, Sanatana Dharma College, Alappuzha. A voucher specimen (10001) is preserved as herbarium and submitted to the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

Preparation of the Extracts

Fresh rhizomes used for extraction were shade dried and powdered using a mechanical grinder. Thirty gram of fine powder was subjected to sequential soxhlet extraction using methanol (300 ml) as the solvent. After extraction the solvents were evaporated using rotary evaporator and were stored at -20^{o}C until use.

Experimental Animals

Healthy Swiss albino mice, *Mus musculus* (20 ± 5g) were used for the study. The animals were obtained from Amala Cancer Institute, Kerala. Animals were kept in polypropylene cages with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided *ad libitum.* The animals were acclimatized to laboratory condition for about one week before commencement of the experiment. The experiments were performed after the approval from the Institution of Animal Ethical Committee (IAEC No: KMCRET/Ph.D./2012 -13) and in accordance with the recommendation for the proper care and use of the laboratory animals.

Experimental Design

Animals were divided into five groups comprising six animals in each group. One group served as the control while the remaining four groups were injected with Dalton's ascites lymphoma (DAL) (1x10^{6}cells/mouse) to induce tumor. The treatments were given intraperitonialy at 24 hours after the tumor inoculation and continued for 14 consecutive days.

The designation of the animal groups and treatment details were as follows:

Group I: Normal control
Group II: DAL control
Group III: DAL + Standard drug (5-FU: 10mg/kg)
Group IV: DAL + MEAC (100mg /kg)
Group V: DAL+MEAC (200mg/kg)

Biochemical Estimation

After the treatment period the animals were anaesthetized by ketamine hydrochloride and the blood was collected from retro-orbital sinus by using capillary tube into a centrifugation tube without EDTA for serum biochemical parameters. It was allowed to clot at room temperature and serum was separated by centrifuged at 10000 rpm for 10 minutes and utilized for the estimation of various biochemical parameters like ALP, SGOT, SGPT, creatinine, urea, uric acid, cholesterol, HDL, total protein and triglycerides.

Statistical Analysis

The statistical analysis was performed using one way analysis of variance (ANOVA) fol-

lowed by Dunnett's test using SAS (Version 9.1) software. Values were expressed as mean ± S.E.M.

RESULTS

ALP activity was significantly increased in DAL bearing mice compared to normal control animals as depicted in Table 1. The MEAC dose at 100 and 200mg/kg significantly ($p < 0.01$) decreased the level in blood serum when compared to DAL bearing mice. The data pertaining to SGOT and SGPT was found to be decreased significantly in all the treatment groups when compared with DAL control group. The lower dose of MEAC (100 mg/kg) was found to be more significant than higher dose.

The inoculation of DAL cells caused significant decrease in the level of cholesterol and HDL and significant increase in the level of total protein, urea, uric acid, creatinine and triglyceride, when compared to the normal control group as evidenced in Table 2. The treatment with rhizome extract reversed these changes towards the normal control group. Most of the values were found to be significant. The treatment with the standard drug, 5-Fu also gave similar results.

DISCUSSION

Biochemical parameters are the primary markers of hepatotoxicity and nephrotoxicity. Analysis of these parameters indicates any adverse effects on liver and kidney functions. In the present study biochemical examination of DAL inoculated mice showed marked elevation in ALP indicating the hepatotoxic effect of the tumor. ALP, SGOT and SGPT are considered as liver toxicity markers. An increase in the activities of ALP level in DAL bearing mice indicated that tumor inoculation might induce hepatic dysfunction. The ALP level will rise with intrahepatic cholestasis and infiltration disease was reported earlier by Harada et al. (1986).

Administration with MEAC exerted a protective effect by reversal of these enzymes more or less to normal. This indicates the protective effect of MEAC in DAL induced hepatotoxicity, supporting its potent anticancer and hepatoprotective effect. In a recent study, Kariuki et al. (2017) used haematological and biochemical parameters to assess the effect of *Allium sativum* extracts on blood, liver and kidney related functions. The dichloromethane-methanolic extract of *A. sativum* caused a significant increase in the levels of liver function profiles across the different doses tested (100, 500 and 2000 mg/kg.bw). The data generated from the investigation helps reduce the confusion among the common people about the indiscriminate use of *Allium sativum* in treatment of various diseases.

Abirami and Kowsalya (2012) reported similar effects in *Ulva faciata* extract against the cancer bearing animals. In another study, Meireles et al. (2016) reported toxicity evaluation and anticancer efficacy of *Croton polyandrous* oil against Ehrlich ascites carcinoma cells using serum biochemical parameters as a measure of hepatotoxicity evaluation.

SGOT and SGPT are associated with the functioning of liver parenchymal cells. It is raised in acute liver damage (Gaze 2007). In the present investigation SGOT in MEAC 200mg/kg dosage was significantly restored to normal level whereas no significant changes with respect to SGPT were observed. These results were in accordance with the findings of Sakthivel et al. (2012). In correlation with this, Palani et al. (2009) reported the anti-hepatotoxic and antioxidant activities of *A. calamus* in the ethanolic extract.

The elevation in serum urea, uric acid, creatinine and triglyceride levels in DAL induced mice

Table1: Effect of MEAC on serum biochemical parameters

Parameters	*ALP (IU/L)*	*SGOT (IU/L)*	*SGPT (IU/L)*
Normal control	90.47 ± 2.48	35.26 ± 0.65	26.17 ± 0.99
DAL control	106.25 ± 5.98[a]	70.95 ± 0.35[a]	56.50 ± 0.49[a]
Positive control (5-Fu)	91.57 ± 2.32[b]	37.45 ± 1.08[b]	28.29 ± 0.78[b]
MEAC (100mg/kg)	93.96 ± 1.01[b]	40.82 ± 0.44[b]	25.90 ± 0.77[b]
MEAC (200mg/kg)	97.61 ± 1.46[b]	42.05 ± 1.29[b]	22.90 ± 1.29[b]
SEd	1.833	0.488	0.520
CD ($p<0.01$)	5.110	1.359	1.449

Values are expressed as mean ± SD of six samples in each group
a: $p < 0.01$ normal control vs DAL control b: $p < 0.01$ DAL control vs treatment groups

Table 2: Effect of MEAC on serum protein and lipid level

Parameters	*Total Protein (gm/dl)*	*Urea (mg/dl)*	*Uric acid (mg/dl)*	*Creatinine (mg/dl)*	*Cholesterol (mg/dl)*	*HDL (mg/dl)*	*TGL (mg/dl)*
Normalcontrol	6.28 ± 0.56	54.45 ± 1.48	3.75 ± 0.35	1.05 ± 0.10	142.78 ± 2.92	53.38 ± 1.08	132.73 ± 11.93
DALcontrol	12.94 ± 0.91[a]	81.23 ± 2.93[a]	5.54 ± 0.62[a]	1.19 ± 0.18	178.90 ± 7.16[a]	25.26 ± 1.32[a]	186.89 ± 7.71[a]
PositiveControl (5-Fu)	6.73 ± 0.69[b]	71.13 ± 2.13[b]	4.19 ± 0.45[b]	1.03 ± 0.09	149.68 ± 8.93[b]	32.02 ± 0.73[b]	141.07 ± 0.86[b]
MEAC(100mg/kg)	6.82 ± 0.93[b]	72.23 ± 4.15[b]	5.01 ± 0.41[b]	1.05 ± 0.04	175.83 ± 13.11	46.88 ± 0.83[b]	146.53 ± 1.42[b]
MEAC(200mg/kg)	6.10 ± 0.64[b]	70.09 ± 3.93[b]	4.24 ± 0.62[b]	1.02 ± 0.04	167.92 ± 9.47[b]	41.28 ± 0.64[b]	162.14 ± 0.73[b]
SEd	0.439	1.787	0.290	0.059	5.170	0.550	3.697
CD (p<0.01)	1.223	4.983	0.809	0.166	14.413	1.553	10.307

Values are expressed by mean ± SD of six samples in each group
a: p<0.01 normal control *vs* DAL control; b: p<0.01 DAL control *vs* treatment groups

is considered as a significant marker of renal dysfunction and it may be related to metabolic disturbances in liver function. Urea is an endogenous product of protein and amino acid catabolism. It is formed in the liver from ammonia, which is a deamination product of amino acids. In animals, it is formed during normal physiological processes that occur primarily in the liver for removal of nitrogen from the body.

Uric acid is regarded as a marker of oxidative stress and as an end product of purine metabolism. At its elevated level, it can act as a pro oxidant. The rapid destruction of tumor cells leads to a release of their intracellular content in to the circulation with a marked rise in potassium and phosphate. The increased nucleotide release and turnover results in increased synthesis of uric acid. MEAC treatment groups in the present study were found to be within the normal range, indicating MEAC showed significant effect on the tumor cells.

The significantly elevated levels of creatinine and cholesterol in tumor-inoculated animals indicated the liver damage and the loss of functional integrity of cell membrane. It was reported that intracellular alterations in cholesterol were accompanied by specific changes of cholesterol in plasma. Zahan et al. (2011) reported similar findings in *Alangium salvifolium* flower against ehrlich ascites carcinoma bearing mice.

The elevation in serum triglycerides in DAL has been attributed to an inhibition of the lipase enzyme activity of both the hepatic triglycerides and plasma lipoproteins (Goldberg et al. 1982). The major function attributed to HDL is to maintain normal cell cholesterol homeostasis by removing excess of cholesterol from intracellular pools and is markedly reduced in cancer patients. Low serum High Density Lipoprotein-Cholesterol (HDLC) is an important component of metabolic syndrome and has recently been related to increase the breast cancer risk in women (Furberg et al. 2005).

Similar results were shown in DAL bearing mice. The administration of MEAC significantly reduces the elevated levels of these parameters and increased protein synthesis in DAL induced mice. The improvement of these altered parameters could be attributed to its hepatoprotective action resulting in alleviation of altered metabolic status in tumor-induced animals resulting in membrane stability.

CONCLUSION

It was observed that the methanol extract of *A. calamus* rhizome significantly protects mice liver from DAL induced hepatotoxic effects. This extract also normalized the DAL induced increases in plasma creatinine and blood urea. Both biochemical findings and serum hematology showed that the extract is relatively safe, as it was found to have no adverse effects on the functions of the liver and kidney

RECOMMENDATIONS

The present investigation on *Acorus calamus* increases the confidence in their safety to humans, particularly for use in the development of pharmaceuticals. This may be a crucial part of the assessment of nontoxic effects of the plant extract intended to be used in animals or humans. However, detailed investigation on the bioactive compounds of the extract responsible for these effects should be undertaken in order to confirm and clarify the mechanism behind the activity.

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