Research Article

Effect of Ethanolic Extract of *C. senna* leaves (EECSL) on Marker Enzymes of Ehrlich Ascites Carcinoma (EAC) Induced Mice.

Amutha Priya R*, Suganthi B[1]

*Ph.D Scholar, Department of Biochemistry, [1]Associate Professor, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India.

***Corresponding author's E-mail:** amuthabiochem@gmail.com

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ABSTRACT

Cancer is a global challenge as it is a largest cause of death around the world. A treatment with synthetic chemotherapeutic drugs for cancer causes severe side effects which appear to be dose-limiting factors. Plants are the effective source of anticancer agents and over 60% anticancer agents are derived from plants. *Cassia* species have more important attention worldwide and are reported to have various pharmacological activities such as antitumor, antioxidant, hepatoprotective and hypoglycemic activity. The current study aims at investigating the effect of ethanolic extract of *C. senna* leaves (EECSL) on marker enzymes of Ehrlich Ascites Carcinoma (EAC) induced mice. Adult male swiss albino mice were used as experimental model and were divided into six groups of six each. EAC cells were injected with into all groups of mice except Group I(Untreated control) animals by intraperitoneal inoculation of 10^6 cells/mouse. After 24 hrs of the tumor cell induction, treatment with EECSL and standard anticancer drug (methotrexate) was started and was continued for 14 days. The elevated activities of GGT(gamma glutamyl transferase, AST(Aspartate transaminase), ALT(Alanine transaminase) and ALP(Alkaline phosphatase) and the increased nitric oxide level in the serum of tumor bearing animals were significantly reduced after administration with EECSL as compared with the tumor control groups. This reduction rate was comparable with the standard drug methotraxate.

Keywords: Ethanolic extract of *C. senna* leaves, ehrlich ascites carcinoma, gamma glutamyl transferase, aspartate transaminase, alanine transaminase, alkaline phosphatase, nitric oxide.

INTRODUCTION

Cancer is a global challenge as this disease remains the second largest cause of death around the world, with some predictions that it will move into the top rank in future. Cancer accounts for one out of every eight deaths annually[1]. For patients diagnosed with distinct diseases, the probability of dying of cancer was much higher than the probability of dying of other causes[2].

In 2012, there were an estimated 14.1 million cases around the world, of these 7.4 million cases were men and 6.4 million women. This number is expected to increase to 24 million by 2035[3]. Synthetic chemotherapeutic agents are used to stop the cancer growth. However, synthetic agents do not distinguish between a cancer and normal cell, and eliminate not only the fast-growing cancer cells but also other fast-growing cells in the body, including hair and blood cells[4].

Synthetic chemotherapeutic drugs for cancer such as 5-fluorouracil derivatives, cisplatin and mitomycin have been used extensively for the treatment of certain types of cancer. However, with these treatments, severe gastrointestinal toxicity with diarrhea, mucosis and hematological toxicity with leucopenia and immune suppression appear to be dose-limiting factors[5]. The inhibition of tumor cell growth without side effects is recognized as an important target for cancer therapy[6].

Plants are the effective source of anticancer agents and over 60% anticancer agents are derived from plants[7]. Since ancient times, natural products, herbs and spices have been used for preventing several diseases, including cancer. Till date large numbers of natural products have been screened for their anticancer potential through various experimental models. This has resulted in the discovery of 30 effective anticancer drugs[8].

Plants belonging to the genus, *Cassia* are considered as leguminous plants (Family - Fabaceae) and due to their medicinal, agricultural and economic value this species have more important attention worldwide[9]. This large genus is widely distributed in several parts of the world, including India, Mauritius, China, East Africa, South Africa, America, Mexico and Brazil[10].

Cassia species are reported to have various pharmacological activities such as antitumor[11], antioxidant[12, 13], hepatoprotective[14,15] and hypoglycemic activity[16,17]. *C. senna* leaves have been investigated for the presence of secondary metabolites and evaluated for the biological activities of the crude extracts with special emphasis to the antimicrobial, cytotoxic and thrombolytic activities[18]. With this background, the present study was designed to determine the effect of ethanolic extract of *C. senna* leaves (EECSL) on marker enzymes of Ehrlich Ascites Carcinoma (EAC) induced mice.

MATERIALS AND METHODS

Collection of the plant sample

The fresh plat of *C. senna* was collected from Madurai district, Tamilnadu. The plant was identified and authenticated in Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2012). The leaves of the plant were shade dried and were coarsely powdered.

Preparation of ethanolic extract

Leaf powder of *C. senna* (10 gm) was taken in 100 ml of ethanol and macerated in stopper flask for 48 hours, shaking frequently at room temperature. Next day the mixture was filtered by using Whattmann no.1 filter paper and it was dried on water bath until the constant weight with dry mass was obtained[19].

Standard anticancer drug

Methotrexate (4-amino-4-deoxy-10-methylfolic acid) was used as standard anticancer drug and it was dissolved in saline solution. Methotrexate (MTX) is used as a chemotherapeutic agent used to treat many cancer types[20].

Experimental animals

Adult male swiss albino mice weighing approximately 20-25g were used as experimental model. The mice were acclimatized to laboratory conditions for 10 days before the commencement of the experiments. The mice were divided into six groups of six each. Institutional Animal ethical clearance (RegNo.623/02/b/CPCSEA19.06.2002-AUW.IAEC.2013-14.BC:07) was obtained before starting the experiment.

Cell lines

Ehrlich ascites carcinoma (EAC) cells were procured from Amala Cancer Research centre, Thrissur, Kerala and were propagated in Swiss albino mice by intraperitoneal transplantation of $1X10^6$ cells in 100µl of PBS.

Tumour induction in experimental animals

After 10 to 15 days of transplantation, the cells were drawn from the intraperitoneal cavity of the mice and were injected into experimental groups of mice intraperitoneally at concentration of $1X10^6$ cells/mouse. After 24 hour of the tumor cell induction treatment with EECSL and standard anticancer drug (methotrexate) was started. Group I and Group II animals received saline only. Group III was administered with the standard drug and the remaining groups (IV, V and VI) were treated with various concentrations (100-300mg/kg b.wt) of EECSL of *C. senna* leaves for 14 days. After the experimental period, the animals were sacrificed by cervical decapitation, the blood was collected and the serum was separated out for the biochemical analysis.

Assessment of Tumour markers in serum

Estimation of gamma glutamyl (GGT) transferase activity in serum

The Gamma glutamyl transferase was activity estimated by the method of Persijn and van der Slik, (1978)[21] using kit procured from Span Diagnostics Limited, Sachin, India. Working reagent was prepared by dissolving substrate tablet (L-γ-glutamyl-3-carboxy-4-nitroanilide) in 2.2ml of buffer and that working reagent (1.0ml) was incubated at assay temperature (37°C) for one minute and 0.1ml of serum sample was added. The contents were mixed well and the initial absorbance was read at 405nm in a spectrophotometer (Genesys 10-S, USA) after one minute and the absorbance reading was repeated after every 1, 2 and 3 minutes. The mean absorbance change per minute was calculated (DA/minute) and enzyme activity is expressed as IU/L.

Estimation of nitric oxide level in serum in serum

The level of nitric oxide (NO) was measured by the method of Green *et al*., (1982)[22]. Nitrite was estimated by Griess reaction. 600 mL of water/standards (sulfanilamide) /serum filtrates were placed in glass tubes. The reaction was started by adding two granules of Cu-coated cadmium. These were put on a shaker for 5 min. Addition of equal volume of glycine buffer is omitted. From the above tubes 500 ml of sample were placed into fresh glass tubes. To it 250 ml sulfanilamide solution were mixed in, followed by 250 ml of N-Naphthylethylene diamine dihydrochloride solution. Tubes were incubated for 10 min at room temperature for a pink colour development and absorbance was read at 545 nm within 60 min.

Estimation of liver marker enzyme activities in serum

The marker enzymes for hepatic damage namely AST, ALT and ALP were assayed using kits procured from Span Diagnostics Limited, Sachin, India.

Estimation of aspartate transaminase (AST) activity in serum

AST and ALT activities were determined by the method of Bergmeyer *et al.*, (1978)[23]. Working reagent was prepared by mixing four parts of reagent 1(Buffer- 80 mmol/l Tris with pH 7.8, 240 mmol/L L-aspartate (AST) or L-alanine(ALT)), with one part of reagent 2(Substrate- 12 mmol/L 2-oxoglutarate, 0.18 mmol/L NADH). Then 1000µl of working reagent was added to 100µl of serum. The tubes were mixed well and the absorbance was read after 60 seconds and the change in absorbance was measured for 2 minutes at 340nm in a spectrophotometer (Genesys 10-S, USA). AST and ALT activities were expressed as IU/L.

Estimation of alkaline phosphatase (ALP) activity in serum

ALP activity was assayed by the method of Schlebusch *et al.*, (1974)[24]. Working reagent was prepared by mixing one vial of p-nitrophenyl phosphate substrate with 5.0ml

buffer. To 20µl of serum, 1.0ml of working reagent was mixed and after one minute, the increase in absorbance was measured at 415nm in a spectrophotometer (Genesys 10-S, USA). The ALP activity was expressed as IU/L.

RESULTS AND DISCUSSION

Tumour Markers in serum

Gamma glutamyl transferase (GGT) activity

Data presented in Figure 1 indicated that the serum of tumor bearing animals showed more than threefold significant ($p<0.05$) increase in GGT activity. GGT, an enzyme involved in cellular glutathione homeostasis which is often increased in tumor conditions. This membrane bound enzyme GGT is expressed highly in embryo livers and decreases rapidly to lowest levels after birth. GGT is highly re-expressed during the development of (HCC) Hepatocellular carcinoma[25]. In the present study this elevated level was significantly ($p<0.05$) reduced after administration with EECSL and the reduction rate was comparable with that of standard drug methotrexate.

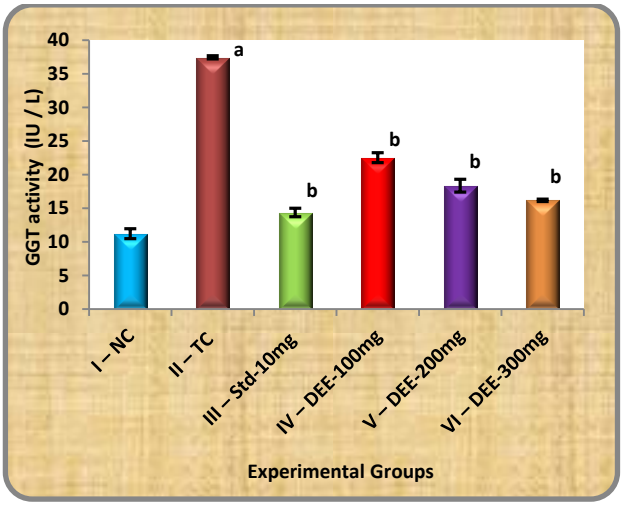

Figure 1: Effect of EECSL on Gamma glutamyl transferase activity in serum of control and experimental animals

Values are mean ± SD(n=6)

a – GpII Vs GpIII, GpIV, GpV, GpVI

a, b - statistically significant ($p<0.05$)

Many researchers have reported GGT activity in cancer condition. The findings of Corti *et al*., (2009)[26] revealed that GGT activity was able to promote iron-dependent DNA oxidative damage, thus potentially representing an important mechanism in initiation/progression of neoplastic transformation. Pro-oxidant activity of GGT can promote oxidative DNA damage, thus contributing to cancer genomic instability thereby suggesting a potential role for membrane-bound gamma-glutamyltransferase (GGT) in tumor progression.

Treatment with ethanolic leaf extract of *C. fistula* significantly reversed the alteration of GGT to normal levels, possibly by maintaining the hepatocellular membrane integrity which is an indicator of possible hepatoprotective property[27]. Usha *et al*., (2007)[28] reported that the increased activity of GGT in experimental animals after liver damage with carbon tetrachloride was near to normal value when treated with aqueous extract of the root sample of *C. occidentalis* which proved the hepatoprotective effect.

Nitric oxide (NO) level

As shown in Figure 2 the serum NO level of control animals was significantly ($p<0.05$) elevated (27.36 ± 0.1µmol) after 14 days of tumour challenge whereas administration of EECSL at high dosage level (300mg/kg b.wt.) significantly ($p<0.05$) reduced the NO level to 15.32 ± 0.13µmol which was nearer to normal level (10.52 ± 0.39 µmol) and also comparable with that of methotrexate, standard drug (13.56±0.33) treated animals.

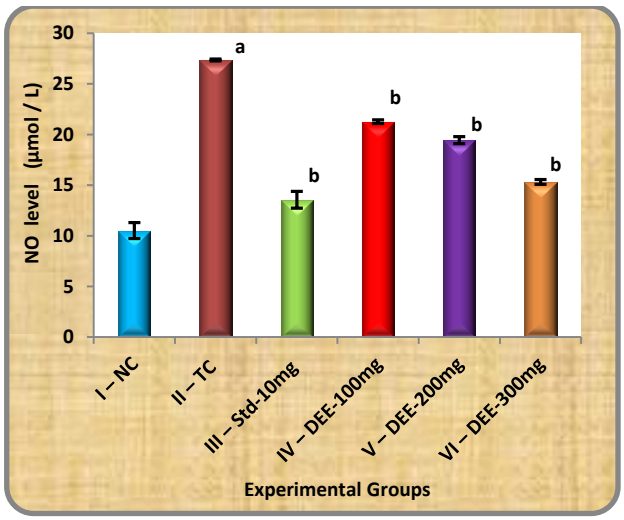

Figure 2: Effect of DEE on NO level in serum of control and experimental animals

Values are mean ± SD(n=6)

a – GpII Vs GpIII, GpIV, GpV, GpVI

a, b - statistically significant ($p<0.05$)

Nitric oxide (NO) is an important regulator of tumor growth and involved in various pathophysiological process includes inflammation and carcinogenesis[29]. Investigation of human cancers, including tumours of the central nervous system, stomach and cervix revealed high levels of expression of nitric oxide synthase(NOS) and nitric oxide in some tumours compared with normal tissue[30].

Angiogenesis, a crucial step in the growth and metastasis of cancers, is initiated with vasodilation that is mediated by nitric oxide (NO). To use antiangiogenesis approach successfully as an anticancer therapy, it is essential to identify the agents that can demote proangiogenic factors like NO. The results of Thejass and Kuttan, (2007)[31] clearly demonstrated that two natural isothiocyanates occurring in *Brassica nigra*, *Lepidum sativum*, *Wasabia japonica*, *Raphanus sativus*, and *Synapis* spp (Boggards *et*

al., 1990)[32] inhibited tumour-specific angiogenesis at non-toxic concentrations in B16F-10 melanoma cell-induced C57BL/6 mice by downregulating NO and they also indicated the decreased tumour-directed capillary formation in treated mice. This is in accordance with our results where nitric oxide level was being downregulated by EECSL that may indicates its antiangiogenic effect and also suggested that EECSL could be a novel anticancer therapy.

Liver marker enzymes in serum

From the Figure 3, it is evident that the activities of liver marker enzymes such as AST, ALT and ALP in serum were significantly ($p<0.05$) increased in EAC group as compared to those of normal group. Abu-Sienna *et al*., (2003)[33] suggested that, the consumption of free amino acid for building the proteins of rapidly dividing tumor cells might result in the disturbance of the enzyme activity in the liver. The increased activities of AST, ALT and ALP in serum are indicative of cellular leakage and loss of functional integrity of liver cell membrane[34].

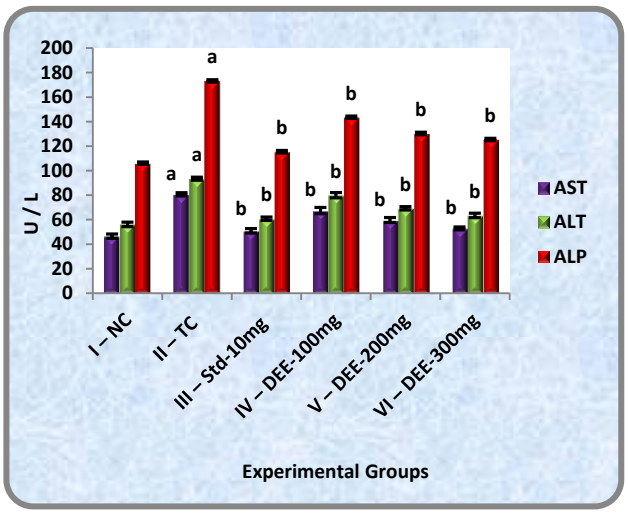

Figure 3: Effect of EECSL on AST, ALT and ALP activities in serum of control and experimental animals

Values are mean ± SD(n=6)

a – GpII Vs GpIII, GpIV, GpV, GpVI

a, b - statistically significant ($p<0.05$)

Many researchers have reported elevated activities of liver marker enzymes in cancer condition. Tissue damage is the sensitive feature in the cancerous conditions so any deterioration or destruction of the membrane can lead to the leakage of the enzymes from the tissues. Hence elevation of these liver specific enzymes observed in breast cancer condition may be due to the progression of tumor growth[35]. Serum AST and ALT levels increased as a result of metabolic changes in the liver such as liver cancer and hepatitis[36]. As a marker for liver metastases in breast cancer patients and also as a marker for hepatotoxicity, AST and ALT were found to be increased[37]. This is in agreement with our present findings where the elevated activities of liver specific enzymes might be due to the progression of tumor growth and metastasis of ascites tumour to liver. With the administration of DEE to the EAC induced mice, the activities of the above enzymes were significantly ($p<0.05$) reduced as compared to EAC group.

In a previous study reported by Shanmuga sundaram *et al*., (2011)[38], it was found that the ethanol extract of *S. auriculata* leaf regulated the activity of AST, ALP and ALP in liver of rats intoxicated with alloxan. The reports given by Asirvatham and Christina, (2012)[39] demonstrated that altered levels of liver enzymes namely ALT, AST and ALP of DAL control group were restored as that of the normal group on treatment with ethanol and aqueous extracts of *Drosera indica* and the cancer induced metabolic changes were also normalized. The daily oral treatment of aqueous extract of *Terminalia chebula* to liver cancer bearing rats demonstrated a significant decline in AST, ALT, ALP and GGT and those results have confirmed the efficacy of that extract as an effective chemotherapeutic agent[40].

CONCLUSION

In conclusion, it could be stated that in case of tumour markers, the GGT activity and the NO level were significantly ($p<0.05$) reduced with the treatment of EECSL as compared with the tumour control. The reversion of GGT activity into near normal may possibly due to the ability of EECSL in reducing the hepatic damage caused by ascites tumour. The downregulation of nitric oxide level by DEE may indicate its antiangiogenic effect. The activities of liver marker enzymes, namely AST, ALT and ALP were significantly ($p<0.05$) reduced in EECSL treated mice as compared with the tumor control group. The significant ($p<0.05$) recovery of the elevated hapato-specific enzyme activities into near normal may indicate the preventive role of EECSL on liver damage caused by ascites tumour. Hence, the research outcome of the present study revealed that EECSL exhibited significant effect on marker enzymes of ehrlich ascites carcinoma (EAC) induced mice which might be due to the antitumour activity of active principles present in EECSL.

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