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Research Article

Evaluation of Anticancer Activity of Methanol Extract of *Monstera deliciosa* in EAC Induced Swiss Albino Mice

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ABSTRACT

Aim of study: The purpose of the study was to evaluate the antitumor and antioxidant status of methanol extract of *Monstera deliciosa* (MEMD) on Ehrlich ascites carcinoma (EAC) treated mice.

Materials and methods: *In vitro* cytotoxicity assay has been evaluated by using the trypan blue and MTT assay method. The determination of *in vivo* antitumor activity was performed by using EAC cells inoculated mice groups (n=12). The groups were treated for 9 consecutive days with MEMD at the doses of 50 and 100 mg/kg b.w. respectively. After 24 h of last dose, half of the mice were sacrificed and the rest were kept alive for assessment of increase in life span. The antitumor potential of MEMD was assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight, hematological parameters and biochemical estimations. Furthermore, antioxidant parameters were assayed by estimating liver tissue enzymes.

Results**:** MEMD showed direct cytotoxicity on EAC cell line in a dose dependant manner. MEMD exhibited significant ($P< 0.05$) decrease in the tumor volume, viable cell count, tumor weight and elevated the life span of EAC tumor bearing mice. The hematological profile, biochemical estimations and tissue antioxidant assay were reverted to normal level in MEMD treated mice.

Conclusion: Experimental results revealed that MEMD possesses potent antitumor and antioxidant properties. Further research is going on to find out the active principle(s) of MEMD for better understanding of mechanism of its antitumor and antioxidant activity.

Keywords: *Monstera deliciosa*, MTT, EAC, Cytotoxicity, Trypan Blue.

INTRODUCTION

Cancer, the second leading cause of death worldwide next to cardiovascular diseases, is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, local tissue invasion and distant metastases.[1] It can be treated with surgery, radiation, chemotherapy, hormone therapy and biological therapy. Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells whereas the rest may damage healthy cells and tissue especially bone marrow, epithelial tissues, reticulo endothelial system and gonads.[2] Since medieval times, plants have been the source of medicines for the treatment of diseases. Regardless of the availability of a wealth of synthetic drugs, plants remain– even in the 21st century – an integral part of the health care in different countries, especially the developing ones. In the late 90's, the WHO stated that a big percentage of the world's population depends on plant based therapies to cover the needs of the primary health care (WHO 1999)[3]. The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious preparations drugs of natural origin have a share of 60% and 75% respectively[4]. It is worthy to mention the vivid current interest in discovery of natural drugs for cancer treatment and chemoprevention[5,6]. Huge number of plant species is screened and bioassayed for this purpose worldwide[7]. The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascetic form it has been used as a transplantable tumor model to investigate the antitumor effects of several substances[8].

The present study was carried out to evaluate the anticancer effect of the methanol extract of *Monstera deliciosa* against Ehrlich's Ascites Carcinoma (EAC) in Swiss albino mice. The *in vitro* antioxidant activities of this plant have been proven recently. The plant also traditionally used for so many diseases[9].

MATERIALS AND METHODS

Plant material

The leaves of *Monstera deliciosa* was collected from Majhitar village of East Sikkim and identified by

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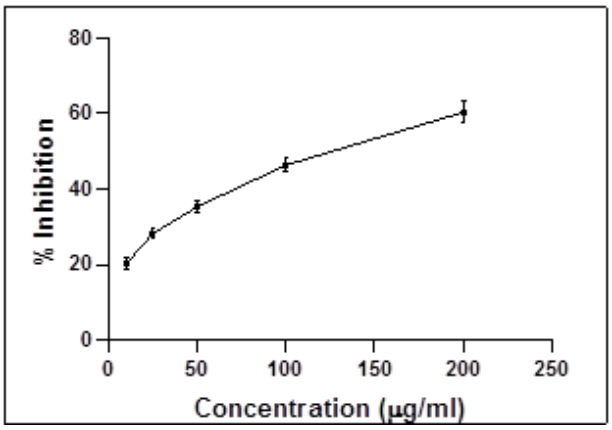

Figure 1: Cytotoxic effect of MEMD on *in vitro* EAC cell line by Trypan blue method. Values are Mean ± SEM; where n = 6.

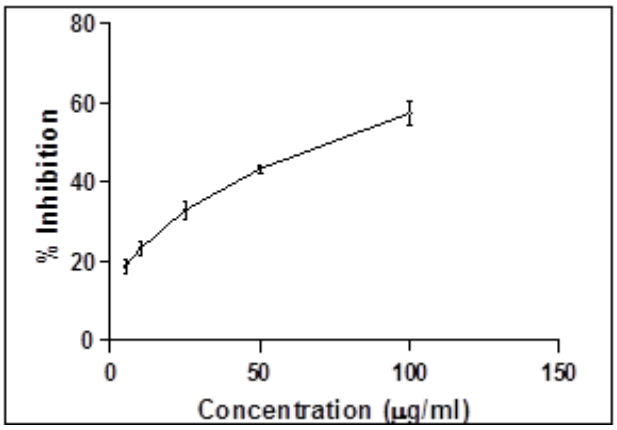

Figure 2. Cytotoxic effect of MEMD on *in vitro* EAC cell line by MTT assay. Values are Mean ± SEM; where n = 6.\

Table 1:

Parameters	EAC Control (2×10^6 cells/mouse)	EAC + MEMD (50 mg/kg)	EAC + MEMD (100 mg/kg)	EAC + 5-FU (20 mg/kg)
Tumor volume (ml)	2.1± 1.26	1.82± 0.13**	1.25± 0.10**	0.55± 0.05**
Tumor weight (g)	3.97± 0.36	2.67± 0.56**	2.02± 0.28**	0.51± 0.04**
Total cell	7.49×10^6±0.07	5.69×10^6± 0.27**	4.54×10^6± 0.09**	3.81×10^6± 0.09**
Viable cell	7.19×10^6±1.20	4.11×10^6± 0.27**	2.99×10^6± 0.08**	0.60×10^6± 0.08**
Nonviable cell	1.40×10^6±0.06	2.11×10^6± 0.15**	1.34×10^6± 0.05**	3.21×10^6± 0.09**
MST (days)	25	32**	39**	53**
% ILS	00	28.90	56.76	112.38

Statistical significance (p) calculated by one way ANOVA between EAC control group and the treated groups followed by Dunnett's test (** $p < 0.05$). Each point represents the mean ± SEM. (n = 6).

Table 2:

Parameters	Normal saline (5 ml/kg)	EAC control (2×10^6 cells/mouse)	EAC + MEMD (50 mg/kg)	EAC + MEMD (100 mg/kg)	EAC + 5-FU (20 mg/kg)
RBC (cell × $10^6/mm^3$)	5.00 ± 0.20	1.03 ±0.09	2.83 ±0.08*	3.03 ±0.25*	5.00 ±0.20*
WBC (cell × $10^3/mm^3$)	4.12 ± 0.32	6.84 ±1.13	3.70 ±0.62*	4.05 ±0.65*	4.89 ±0.33*
Hb. (g/dL)	10.48 ±0.38	4.00 ±0.14	5.9 ±0.12*	7 ±0.21*	10.00 ±0.46*

Statistical significance (p) calculated by one way ANOVA between EAC control group and the treated groups followed by Dunnett's test (* $p < 0.05$). Each point represents the mean ± SEM. (n = 6 mice per groups).

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Drugs and chemicals

5-fluorouracil from Sigma (St. Louis, MO); trichloroacetic acid (TCA) from Merck (Mumbai, India); thiobarbituric acid (TBA), nitroblue tetrazolium chloride (NBT) from Loba Chemie (Mumbai, India); 5,5′-dithio bis-2-nitro benzoic acid (DTNB), phenazonium methosulfate (PMS), nicotinamide adeninedinucleotide (NADH) and reduced glutathione (GSH) from SISCO (Mumbai, India). All the other reagents used were of analytical reagent grade obtained commercially.

Animals

Adult male Swiss Albino mice of about 8 weeks of age with an average body weight of 20–25 g were used for the experiment. The mice were grouped and housed in poly acrylic cages. The animals were maintained under standard laboratory conditions. The mice were acclimatized to laboratory conditions for 7 days before the commencement of the experiment.

Transplantation of Tumor cells

The transplantable tumor Ehrlich ascites carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained in Swiss albino mice by means of intra peritoneal transplantation of 2×10^6 cells/mouse after every 10 days. For *in vivo* and *In vitro* study the freshly

Table 3:

Parameters	Normal saline (5 ml/kg)	EAC control (2×10^6 cells/mouse)	EAC + MEMD (50 mg/kg)	EAC + MEMD (100 mg/kg)	EAC + 5-FU (20 mg/kg)
SGOT (IU/L)	9.84 ± 0.62	15.96 ± 0.86	13.23 ± 0.79*	10.14±0.35*	8.53 ± 0.33*
SGPT (IU/L)	13.45 ± 0.29	3.35 ± 0.73	7.05± 0.60*	15.73±0.62*	20.30 ± 0.56*
SALP (IU/L)	70.87 ± 1.70	300.1 ± 3.89	217.20±2.12*	177.8±1.53*	115.20 ± 4.46*
Protein (g/dL)	10.22 ± 0.14	3.63 ± 0.22	5.22 ± 0.17*	6.62± 0.27*	7.51 ± 0.11*

Statistical significance (p) calculated by one way ANOVA between EAC control group and the treated groups followed by Dunnett's test (* $p < 0.05$). Each point represents the mean ± SEM. (n = 3 mice per groups).

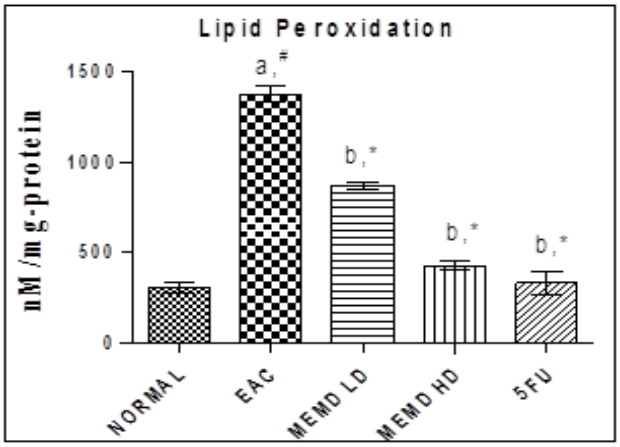

Figure 3: Effect of MEMD on EAC induces lipid peroxidation in mouse liver tissue. All data were expressed as mean ± SEM (n = 6). Where [a] EAC control group versus normal control group, [#]p<0.05. [b] All treated groups versus EAC control group, *p<0.05.

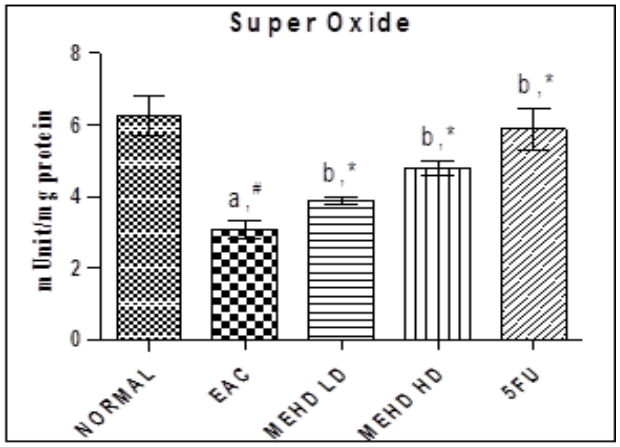

Figure 4: Effect of MEMD on EAC induces superoxide dismutase in mouse liver tissue. All data were expressed as mean ± SEM (n = 6). Where [a] EAC control group versus normal control group, [#]p<0.05. [b] All treated groups versus EAC control group, *p<0.05.

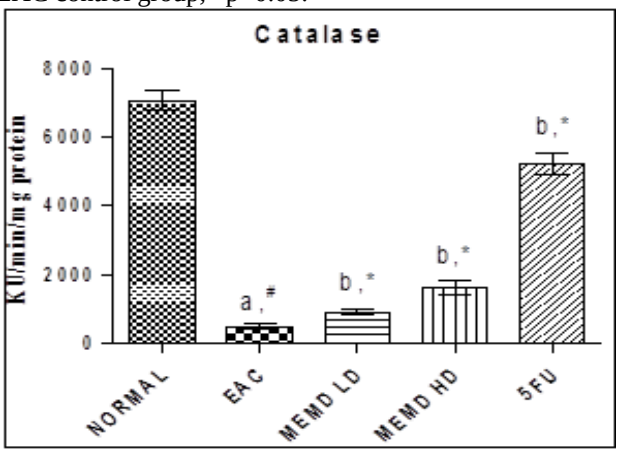

Figure 5: Effect of MEMD on EAC induces catalase in mouse liver tissue. All data were expressed as mean ± SEM (n = 6). Where [a] EAC control group versus normal control group, [#]p<0.05. [b] All treated groups versus EAC control group, *p<0.05.

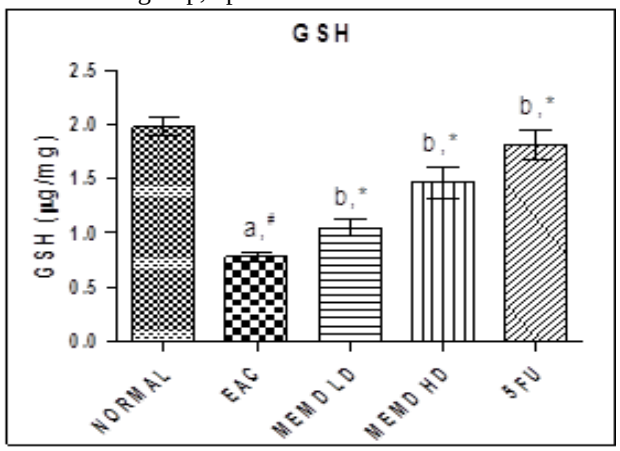

Figure 6: Effect of MEMD on EAC induces reduce glutathione in mouse liver tissue. All data were expressed as mean ± SEM (n = 6). Where [a] EAC control group versus normal control group, [#]p<0.05. [b] All treated groups versus EAC control group, *p<0.05.

drawn fluid was diluted with ice-c old sterile normal saline and the tumor cell count was adjusted to 2×10^6 cells/ml by sterile normal saline[10].

Acute toxicity and dose calculation

The acute oral toxicity of MEMD in Swiss albino mice was performed as per OECD guideline 425[11].

Assay for In vitro cytotoxicity

Trypan blue inclusion

In vitro short term cytotoxicity of MEMD was assayed by using EAC cell line described by Dolai et al 2012[12].

MTT assay

MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) is a wellknown tetrazolium salt was used to determine mitochondrial dehydrogenase activity in living cells was done by previously described method Muruganantham et al 2015[13].

Treatment schedule for assessment of in vivo antitumor potentiality

The Swiss albino mice (20–25 g) were divided into five groups (n=12). All the animals in each groups were being injected EAC cells ($2x10^6$ cells / animal, i.p.) Except group-I. This was considered as day "0". Group-I and group-II were served as normal saline control (5 ml/kg body weight, i.p.) and EAC control respectively. After 24 hr of EAC transplantation Group-III, Group-IV and Group-V were being injected MEMD 50 mg/kg b.w., and 100 mg/kg b.w, and standard drug 5-FU 20 mg/kg b.w. i.p for 9 consecutive days. After administration of last dose, half of the animals were sacrificed and all the haematological and biochemical parameter were done. The remaining six animals in each group were kept alive with food and water *ad libitum* to check the percentage increase in life span of the tumor host to determine the mean survival time (MST) [14].

Tumor volume, packed cell volume and tumor weight

Tumor volume, packed cell volume and tumor weight was measured previously described by Karmakar et al 2013[15].

Viable and non-viable tumor cell count

These viable and nonviable cells were counted by using the under-scribbled formula:

$$\text{Cell count} = \frac{(\text{number of cells X dilution factor})}{(\text{area X thickness of liquid film})}$$

Percentage increase life span (% ILS)

The effect of MEMD on tumor growth was monitored by recording the mortality of the experimental mice. The percentage increase in life span (% ILS) was calculated by the following formula:

$$\%\text{ILS} = \left[\left(\frac{\text{Mean survival time of treated group}}{\text{Mean survival time of control group}}\right) - 1\right] \times 100$$

Mean survival time = (Day of first death + Day of last death)/2[14].

Haematological parameters

Collected blood was used for the estimation of haemoglobin (Hb) content; red blood cell count (RBC) and WBC.

Biochemical parameters

Serum biochemical parameter like total proteins, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum bilirubin was done by previously described method Kumar et al 2015[16]. All the analysis was performed by using commercially available kits manufactured by the Span Diagnostics Ltd., Surat, India.

Tissue antioxidant assay parameters

The tissue antioxidant assay was performed with liver tissues and the evaluation was carried out by measuring the level thiobarbituric acid reactive substances (TBARS) in lipid peroxidation assay, the amounts of enzymatic Catalase, superoxide dismutase and non enzymatic antioxidant system such as reduced glutathione [14].

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by one-way ANOVA followed by Dunnett'spost *hoc* test of significance *p* values of <0.05 was considered as statistically significant using Graph Pad Prism software, version 5.0, San Diego, California.

RESULTS

Acute Toxicity Study

The methanol extract of *Monstera deliciosa* in mice was safe up to 2000 mg/kg body weight.

Assay for In vitro cytotoxicity

In the assay for *In vitro* cytotoxicity study, Trypan blue inclusion assay and MTT assay on MEMD showed direct cytotoxic effect on the EAC cell line in a dose dependent manner with IC_{50} value of 118 µg/ml and 72.5± 3.41 µg/ml respectively (Figure 1 and Figure 2).

Tumor growth and survival parameters

MEMD at a dose of 50 and 100 mg/kg body weight significantly decreased the tumor volume, tumor weight, and viable cell count in a dose dependent manner with compared to EAC control group ($p < 0.01$). The extract also increased the life span (ILS) and nonviable cell count at a dose of 50 and 100 mg/kg body weight (Table 1) EAC bearing mice.

Haematological parameters

Administration of MEMD significantly reduced WBC count in a dose dependent manner in respect to that of EAC control group. RBC count and haemoglobin content were found to be significantly restored to the normal levels. The result (Table 2) implies the protective role of MEMD on the haematological profile of EAC bearing mice.

Biochemical parameters

Biochemical parameters like SGPT, SGOT, SALP, indicates the elevated level of liver functional enzymes in serum in EAC treated group with respect to normal animals. The total protein was found to be significantly decreased in the EAC control group as compared with the normal saline group. Administration of MEMD at the dose of 50 mg/kg and 100 mg/kg in EAC bearing mice significantly increased the total protein content as compared with the EAC control (Table 3).

The level of lipid peroxide in liver tissue was significantly increased in EAC control mice when compared to normal control animals. After treatment MEMD (50 and 100 mg/kg body weight), MDA content of both liver and kidney were significantly reduced with compared to EAC control mice (Figure 3).

Figure 4 Illustrates the effects of MEMD on the antioxidant status of EAC bearing mice. Liver homogenates of EAC control group the SOD activity was reduced as compared to the normal controls. The dose dependent enhancement of SOD activity was observed in case of MEMD treatment group, as compared with EAC control group. The reduction in antioxidant enzyme

catalase activity in EAC control mice was improved in liver by the treatment with MEMD Figure 5.
The reduced GSH level of both liver and kidney were depleted in EAC control group. Treatment with MEMD significantly elevated reduced GSH level in a dose dependent manner (Figure 6).

DISCUSSION

Natural products provide one of the most important sources of promising leads for the development of novel chemotherapeutics in the areas of infectious diseases and cancer. Cragg and colleagues showed that, for the period 1989 to 1995, over 60% of the approved drugs developed for the treatment of cancerous and infectious diseases were of natural origin, including biological, natural and natural derived products [17,18]. Natural products exhibit a great variety of chemical structures, which underlines the important role of secondary metabolites as an important source for new leads. Ehrlich ascites carcinoma (EAC) is a widely used animal model, antitumor efficacy of various plant extracts representing one of its main application. Ehrlich ascitic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascitic fluid formation and accumulation[19]. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells[20].
Anticancer effect is quantified by attenuation of EAC-induced weight gain, decreasing in ascites volume and in viable cell count[19]. Reduction of the cancer systemic effects are other commonly used application of EAC in phytotherapy research; plant extracts improved the haematological parameters, oxidative stress markers in plasma and liver. The present study shows the Methanol extract at both the doses (50 and 100 mg/kg) significantly improved the MST (Mean survival time) in tumor bearing mice. No visible sign of toxicity and changes in vital functions were observed in any of treated animals. The prolongation of life span is a reliable criterion for judging efficacy of anticancer drugs and the extracts of this plant were able to meet this criterion. Myelosuppression and anemia (reduced haemoglobin) have been frequently observed in ascites carcinoma. Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number [20]. In this study, elevated WBC count, reduced haemoglobin and RBC count were observed in EAC control mice, and the intra peritonial administration of *H. salicifolia* restored haemoglobin content and maintained normal values of RBC and WBC, thus supporting its hematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy. The protein content was significantly reduced in EAC control group when compared to control animals. A significant reduction in protein content was noted in EAC control animals. The extract treated group significantly raised the protein content when compared to EAC control animals[15]. Lipid peroxidation mediated by free radicals is considered as a primary mechanism of cell membrane destruction and cell damage. Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of lipid peroxidation. MDA, the end product of lipid peroxidation, was reported to be higher in cancer tissues than in non diseased organ. Our findings indicate that TBARS levels in the tested cancerous tissues are higher than those in normal tissues. Moreover, it has been claimed that MDA acts as a tumor promoter and co-carcinogenic agent because of its high cytotoxicity and inhibitory action on protective enzymes [21].
Glutathione plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage to macromolecules, for example, lipid peroxidation *in vivo*. It was also reported that the presences of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially the liver, even when the site of the tumor does not interfere directly with organ function[16]. In our study, GSH levels in experimental mice were found to be significantly higher than that in the EAC control mice. SOD, CAT, and glutathione peroxides are involved in the clearance of superoxide and hydrogen peroxide (H_2O_2). SOD catalyses the diminution of superoxide into H_2O_2, which has to be eliminated by glutathione peroxidase and/or catalase. Further, it has been reported that a decrease in SOD activity in EAC bearing mice may be due to loss of Mn^{2+} containing SOD activity or due to neutralization of super oxide in EAC cells and the loss of mitochondria, leading to a decreases in total SOD activity in the liver. A small amount of catalase (CAT) in tumor cells was reported. The inhibition of SOD and CAT activities as a result of tumor growth were also reported. Similar findings were observed in our present study in EAC bearing mice. The administration of MEMD at two different doses significantly increased the SOD and CAT levels in a dose dependent manner. It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells and antitumor activity in experimental animals. Antitumor activity of these antioxidants is either through induction of apoptosis or by inhibition of neovascularisation. The implication of free radicals in tumors is well documented [15].
The free radical hypothesis supported the fact that the antioxidants effectively inhibit the tumor, and the observed properties may be attributed to the antioxidant and antitumor principles present in the plant extract.
Elevated levels of serum parameters i.e., SGPT, SGOT, SALP, serum bilirubin, serum protein are indicative of impaired liver functions due to cancer as observed in the EAC control group[21]. Biochemical measurements of these parameters showed that to some extent hepatotoxicity was associated after 9 days of inoculation with EAC. Treatment with the MEMD restored the elevated biochemical parameters more or less to normal range, indicating the

protection of the tumor cell induced hepatotoxicity by MEMD.

CONCLUSION

The presence of ceratin phytoconsttuents including phenolic compounds, alkaloids and flavonoid might have played a possible role in the mediation of anticancer activity of *Monstera deliciosa* extract. These results indicate the possibility of developing MEMD extract into a potential chemoptheraeutic agent. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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