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Antitumour activity of Microencapsulated Paprika Oleoresin *In vivo* on Ehrlich ascites carcinoma in mice

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ABSTRACT

Paprika oleoresin, obtained from *capsicum* species is used as a natural colourant and flavour enhancer in food and pharmaceuticals and is shown to be effective free radical scavengers including potential antioxidative and anticancer properties. The current investigation aims to evaluate the anticancer activity of developed MPO (Microencapsulated Paprika Oleoresin) on EAC tumour model. Male Swiss albino mice were divided into 6 groups (n=12). Group I (normal) animals received normal saline solution orally, all the other groups (Group II to VI) were injected with EAC cells (0.2 ml of 2 $x10^6$ cells/mouse) intraperitoneally. MPO at different doses such as 0.5, 2.5 and 5 mg. $kg^{-1}.mouse^{-1}.day^{-1}$ and standard drug 20mg of 5 Flurouracil $kg^{-1}.mouse^{-1}.day^{-1}$ were administered orally through gavage for 14 days to group III, IV, V and VI respectively. Anti tumor effect of MPO was assessed by observing the changes with respect to body weight, packed cell volume, viable and non-viable tumor cell count, median survival time and percentage increase in life span (% ILS). Ingestion of 5 mg of MPO produced desirable changes in cancer induced mice and it was comparable with the EAC mice treated with 5FU 20 mg/kg body weight. In addition, MPO facilitated to reverse the changes by tumor growth proving its health benefits.

Introduction

Cancer is one of the leading causes of adult deaths worldwide. In India, around 5, 55, 000 people died of cancer in 2010, according to estimates published in *The Lancet* (Dikshit *et al* 2012). The absolute number of cancer deaths in India is projected to increase because of the population growth and increasing life expectancy. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or in combination) to block the development of cancer in humans (Karthigayan *et al* 2007). Conventional radiotherapy and chemotherapy can induce apoptosis in some cancer cells but will also damage some surrounding healthy cells.

Spices are emerging as a possible therapeutic agents to prevent and treat diseases- even cancer. Among the spices, chillies despite their fiery “hotness” are one of very popular spices known for medicinal and health benefiting properties. Red chilli’s pungent nature has captured the attention of physicians, researchers and connoisseurs throughout history. The fruit's degree of pungency, determines its primary use as a potent medicine, a functional food or an accessory spice. Capsaicin, the "hot" ingredient in red chilli, caused cancer cells to commit suicide by a process called apoptosis. Apoptosis is a normal cellular event in many tissues that maintains a balance between newer replacement cells and aged or worn cells. In contrast, cancer cells seek to be immortal and often dodge apoptosis by mutating or deregulating the genes that participate in programmed cell death. Capsaicin is a potent anticancer agent, induces apoptosis in cancer cells and produces no significant damage to normal pancreatic cells, indicating its potential use as a novel agent for the prevention and treatment of pancreatic cancer (Srivastava, 2006). Several investigators have reported the ability of capsaicin to inhibit events associated with the promotion of cancer (Ito *et al* 2004).

Oleoresin is the true essence of spice and can replace whole or ground spices without impairing their flavour and aroma (Sivaraman *et al* 2001).Essential oils, oleoresins and even aqueous extracts of spices possess antioxidative properties. The problems encountered in using oleoresin are that, it is viscous, sticky, non uniformity in mixing, difference in flavour profiles and shorter storage life (Krishnan, 1981). Microencapsulation is one of the quality preservation techniques of sensitive substances and a method for production of materials with valuable properties in food and pharmaceutical industries. Microencapsulation technique entraps the sensitive ingredients within a coating to protect them from environmental factors such as moisture, air or light (Onwulata *et al* 1998). Paprika oleoresin of 1,00,000 CU is microencapsulated using gum arabic as wall material at three different concentrations with a pilot model spray drier. The developed microencapsulated paprika oleoresin (MPO) retained the native antioxidants and free radical scavenging property. Hence the present study aims to investigate the antitumor property of MPO *in vivo* with animal models using Swiss albino mice.

Materials and Methods

Selection of animals

Healthy adult male Swiss albino mice, weighing about 20-30g were procured from Nandha college of Pharmacy, Erode, Tamil Nadu. Animals were housed in microlon boxes and acclimatized to the laboratory condition. The animals were maintained on commercial standard pellet diet and water ad libitum. The study protocol was reviewed and approved by the Institutional Animal Ethical Committee (Regd.No:688/02/C–CPCSEA).

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Selection of tumor cells

Ehrlich ascites carcinoma (EAC) a hyperdiploid subline, was obtained from Amala Cancer Research Center, Thrissur, Kerala. They were maintained by weekly intraperitoneal (IP) inoculation of 10^6 cells/mouse. Ascites tumor cells, obtained from donor mouse bearing 6- to 8- day old Ascites tumours, were diluted with buffer solution saline (BSS) and transplanted to mice using sterile disposable syringe under aseptic conditions. Preparation of microencapsulated paprika oleoresin for oral administration

Of the developed Microencapsulated Paprika Oleoresin, I (5 per cent), II (10 per cent) and III (15 per cent), the one with 10 per cent core material had better quality characteristics. Considering this, MPO type II was chosen to test its antitumor activity. Wyk *et al* (1995) tested the efficiency of various concentrations of chilli extract on the growth of human buccal mucosa fibroplast cell line and concluded that the cancer cells death occurred after 6 days with 400-500 µg/ml of chilli powder extract (1.8 to 6.9 and 2.3 to 8.7 µM capsaicin per concentration). According to Mori *et al* (2006) capsaicin inhibited the clonal proliferation of prostate cancer cell lines in a dose-dependent manner. Complete inhibition was observed at a concentration of 5 x 10^{-4} mol/L. Based on the literature cited three different concentrations of MPO viz., 50, 250 and 500 µM were selected for administration. The developed powder is readily soluble in water. The selected different concentration of the powder was weighed and dissolved in water and used for administration.

Effect of MPO on in-vivo antitumour activity

Swiss albino mice were divided into six groups with 12 animals in each group. Male Swiss albino mice were divided into 6 groups (n=12). Group I (normal) animals received normal saline solution orally, all the other groups (Group II to VI) were injected with EAC cells (0.2 ml of 2 x106 cells/mouse) intraperitoneally (Gupta et al 2004). From the first day normal saline 5mL.kg^{-1}.mouse^{-1}.day^{-1}and propylene glycol 5mL.kg^{-1}.mouse^{-1}.day^{-1} were administered to normal and EAC control groups respectively. Similarly, MPO at different dose as 0.5, 2.5 and 5 mg. kg^{-1}.mouse^{-1}.day^{-1} and standard drug 5 Flurouracil 20mg kg^{-1}. mouse^{-1}.day^{-1} were administered to group III, IV, V and VI respectively. All the treatments were given orally through gavage for 14 days. After administering the last dose of MPO, six mice from each group were fasted overnight, anesthetized, and sacrificed by cervical decapitation. Blood and peritoneal fluid were collected in heparinized tubes and stored at –20°C for further analysis. The remaining animals were left to calculate the mean survival time.

Group I	-	normal
Group II	-	Cancer control, EAC cells (2 x10^6 cells/mouse)
Group III	-	EAC cells treated with 0.5mg of MPO/kg body weight
Group IV	-	EAC cells treated with 2.5mg of MPO/kg body weight
Group V	-	EAC cells treated with 5mg of MPO/kg body weight
Group VI	-	EAC cells treated with standard drug (20 mg of 5- FU/kg body weight)

Anti tumor effect of MPO was assessed by observing the changes with respect to body weight, packed cell volume, viable and non-viable tumor cell count, median survival time and percentage increase in life span (% ILS).

Median Survival Time (MST) and body weight

MST of each group containing six mice were monitored by recording the mortality condition daily for a period of 5 weeks and percentage of ILS was calculated using the following equation (Gupta *et al* 2000). Body weight is directly proportional to the tumor development and it increases with the growth of tumor.

MST= (Day of first death + Day of last death)/2

$$\% \text{ ILS} = \left(\frac{MST \text{ of treted group}}{MST \text{ of control}} - 1\right) \times 100$$

Hematological indices

In order to detect the influence of MPO on the hematological status of EAC bearing mice, a comparison was made among the six groups of mice on 14th day of inoculation. Blood was collected from freely flawing tail Vern and used for the estimation of hemoglobin (Hb) content, Red Blood Cell count (RBC) and White Blood Cell count (WBC) (Wintrobe *et al* 1961). Differential WBC leukocyte count was carried out from Leishman stained blood smears of normal, EAC control and MPO treated and standard drug treated groups respectively.

Tumor volume, viable and non-viable tumor cell count

The ascitic fluid from the mice was collected from the peritoneal cavity. The tumor volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min (Kumarappan *et al* 2007). The viable and non viable tumor cell counts were carried out with Neubauer hemocytometer. The cells were then stained with trypan blue (0.4 % in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non viable. These viable and non-viable cells were counted (Shapiro, 1988).

Cell Count = (No. of cells x Dilution)/ (Area x Thickness of liquid film)

Estimation of biochemical indices

The liver and kidney were excised out from the sacrificed animals rinsed in ice cold normal saline followed by cold 0.15M Tris –HCl buffer (pH 7.4), dried and weighted. A 10% w/v homogenate was prepared in 0.15 M Tris-Hcl buffer, and portion utilized for the estimation of lipid peroxidation, and a second portion after precipitating proteins with TCA was used for the estimation of glutathione (Ohkawa *et al* 1979). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of biochemical parameters namely SOD, Catalase, reduced glutathione, lipid peroxide, ALT or SGPT and AST or SGOT.

Statistical analysis

Biochemical indices of normal control, EAC control and experimental groups of mice were expressed as mean ± S.D. Data within the groups were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). A value of P>0.05 was considered statistically significant.

Results and Discussion

Effect of MPO on body weight and Median Survival Time (MST)

Table 1 depicts that EAC bearing mice showed an increase in body weight (29.8 g) due to increase in ascites volume by actively proliferating tumor cells compared to normal group (19.71g). Further, ingestion of MPO or 5-Fluorouracil initiated a gradual reduction in body weight of EAC bearing mice. A reduction in body weight was found to be dose dependent as a dose level of 5 mg MPO per kg body weight showed an efficient decrease in body weight after 14 days of tumor implantation.

The reduction trend in body weight was reflected on MST and % ILS. The reduction in body weight and increase in MST could be due to the delaying impact of plant extract on cell division as observed by Gupta *et al* (2004) who tested the anticancer activity of *Indigofera aspalathoides* and *Wedelia calenduloceae*, and reported a decrease in volume of EAC (body weight) with increase in MST of mice. Further, the dietary phytochemical capsaicin content of MPO would have strengthen the chemoprevention activity as supported by Shylaja and Peter (2008) who identified such effect with the capsaicin. In addition Mori *et al* (2006) also stated that capsaicin an active component of the red pepper inhibits the clonal proliferation in a dose dependent manner.

Table 1 Effect of MPO on body weight, MST and %ILS of EAC bearing Swiss albino mice

Experimental groups	Body weight (g)	MST	% ILS
Group I (Normal)	19.71 ± 0.75	-	-
Group II (Cancer control)	29.80 ± 0. 83	14.33 ± 0.57	-
Group III (EAC + 0.5 mg MPO/kg body weight)	26.20 ± 0.83	23.16 ± 0.76	61.61
Group IV (EAC + 2.5 mg MPO/kg body weight)	25.80 ± 0.83	24.16 ± 0.29	68.59
Group V (EAC + 5 mg MPO/kg body weight)	23.83± 0.98	26.16 ± 0.76	82.55
Group VI (EAC + 5 FU 20mg /kg body weight)	22.66 ± 0.51	27.66 ± 0.76	93.02

Values are mean ± SD (n=6)

Effect of MPO on hematological indices

Table 2. Effect of MPO on hematological indices

Groups	Haemoglobin (g)	Total RBC (million/mm^3)	Total WBC (10^3 cells/mm^3)
Group I (Normal)	13.27 ± 0.29 ^{a}	6.92 ± 0.25 ^{a}	5.07 ± 0.17 ^{e}
Group II (Cancer control)	10.40 ± 0.57 ^{d}	4.00 ± 0.29 ^{f}	17.1 ± 0.98 ^{a}
Group III (EAC + 0.5 mg MPO/kg body weight)	11.45 ± 0.47 ^{c}	5.07 ± 0.15 ^{e}	12.42 ± 0.96 ^{b}
Group IV (EAC + 2.5 mg MPO/kg body weight)	11.85 ± 0.12 ^{c}	5.42 ± 0.15 ^{d}	8.92 ± 0.70 ^{c}
Group V (EAC + 5 mg MPO/kg body weight)	12.10 ± 0.18 ^{b}	5.85 ± 0.12 ^{c}	7.25 ± 0.40 ^{d}
Group VI (EAC + 5 FU 20 mg /kg body weight)	12.20 ± 0.21^b	6.17 ± 0.17 ^{b}	6.85 ± 0.50 ^{d}
CD (5%)	0.522	1.023	0.296

Values are mean ± SD (n=6), A column means followed by common superscripts are not significant at 5% level by DMRT

Results indicated that the mean haemoglobin level of normal rats was 13.27 ± 0.29 g against the EAC control with the mean value of 10.40 ± 0.57 g per cent which was significantly (P<0.05) lower compared to normal. Increase in haemoglobin level was noticed in the rats supplemented with the encapsulated oleoresin powder and 5-Fluorouracil. Further, the haemoglobin level of EAC bearing mice ingested with higher level of MPO as 5 mg (12.1 g) was closer and comparable to animals treated with 5 Fluorouracil (12.2 g), indicating the efficacy of MPO on improving the haemoglobin content. The total red blood cell count was decreased in EAC bearing mice (4.00 ± 0.29) compared to normal (6.92 ± 0.25). However, an increase in RBC count was noticed in EAC bearing mice treated with MPO at different doses demonstrated a significant (P<0.05) increase in the total RBC count compared to EAC control. An apparent increase in WBC count was noticed in EAC induced animals. A distinct reduction in the count as 12.42 ± 0.96, 8.92 ± 0.70 and 7.25 ± 0.40 x 10^3 cells/mm^3 was noticed when treated with MPO at 0.5, 2.5, 5 mg respectively. The results confirmed that encapsulated oleoresin facilitated to reverse the changes in haematological parameters caused by the tumor growth and could reduce the toxic effects. This corroborates with the results of Ito *et al* (2004) who stated that capsaicin induced *in vitro* growth inhibition of leukemic cells by causing G0-G1 cell cycle arrest and apoptosis. In addition, pathological analysis revealed no capsaicin-induced tissue changes in any of the organs which proved that capsaicin had no toxic effects on mice.

Effect of MPO on differential white blood cells

Table 3. Effect of MPO on differential count of WBC on EAC bearing mice

Groups	Neutrophils (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Group I (Normal)	16.25 ± 0.63 ^{e}	0.50 ± 0.06 ^{f}	77.32 ± 2.47 ^{a}	1.73 ± 0.09 ^{a}
Group II (Cancer control)	61.50 ± 2.67^a	1.86 ± 0.10 ^{a}	25.05 ± 3.72 ^{f}	0.82 ± 0.02 ^{c}
Group III (EAC + 0.5 mg MPO/kg body weight)	48.87 ± 3.58 ^{b}	1.39 ± 0.01 ^{b}	43.30 ± 4.21 ^{e}	0.93 ± 0.04 ^{c}
Group IV (EAC + 2.5 mg MPO/kg body weight)	36.97 ± 2.34 ^{c}	1.08 ± 0.11 ^{c}	54.62 ± 3.26 ^{d}	1.01 ± 0.06 ^{c}
Group V(EAC + 5 mg MPO/kg body weight)	31.05 ± 2.56 ^{d}	0.89 ± 0.03 ^{d}	60.27 ± 1.66 ^{c}	1.22 ± 0.17 ^{b}
Group VI (EAC + 5 FU 20mg /kg body weight)	31.80 ± 1.42 ^{d}	0.80 ± 0.06 ^{e}	69.55 ± 6.23^b	1.37 ± 0.26 ^{b}
CD (5%)	3.565	0.112	5.741	0.203

Values are mean ± SD (n=6), A column means followed by common superscripts are not significant at 5% level by DMRT

Table 3 indicates that supplementation with MPO at different concentrations and 5 FU led to a significant (P<0.01) decrease in the counts of neutrophils and eosinophil in the tumor bearing mice. Neutrophils and eosinophil count of animals treated with 5 mg MPO was on par with that of the counts identified in the 5-FU treated group, indicating the positive attributes of capsaicin. Subsequently, the reduction in lymphocytes was brought closer to normal level on ingestion of MPO and 5- FU. A similar observation was seen in monocyte count also. The mean counts of monocyte was found to be 0.82 ± 0.02 per cent in EAC control (group II) which was very low compared to the normal control (group I) as 1.73 ± 0.09 per cent. The counts were increased to 1.22 ± 0.17 and 1.37 ± 0.26 per cent on treatment with 5 mg of MPO (group V) and 5- FU (group VI) respectively. However no statistical difference was observed between lymphocyte and monocyte counts. The results revealed that the treatment with MPO brought back the WBC cell counts near to normal values

signifying the protective action of MPO on the heamotopoietic system.

Effect of MPO on tumor volume, viable and non-viable tumor cell count

Table 4. Effect of MPO on tumor volume, packed cell volume, viable and non-viable tumor cell count

Groups	Tumor Volume (ml)	Packed Cell Volume (ml)	Viable Tumor Cell Count (10^6 cells/ml)	Non-Viable Tumor Cell Count (10^6 cells/ml)
Group I (Normal)	--	--	--	--
Group II (Cancer control)	4.0 ± 0.26 [a]	1.95 ± 0.12 [a]	11.46 ± 0.15[a]	0.49 ± 0.04[d]
Group III (EAC + 0.5 mg MPO/kg body weight)	3.42 ± 0.17 [b]	1.7 ± 0.08 [b]	8.67 ± 0.06[b]	0.69 ± 0.05[c]
Group IV (EAC + 2.5 mg MPO/kg body weight)	2.97 ± 0.05 [c]	1.2 ± 0.14 [c]	6.15 ± 0.16[c]	0.70 ± 0.03[c]
Group V(EAC + 5 mg MPO/kg body weight)	2.8 ± 0.08 [c]	1.07 ± 0.15 [c]	5.95 ± 0.14[d]	0.72 ± 0.03[b]
Group VI (EAC + 5 FU 20mg /kg body weight)	1.82 ± 0.17 [d]	1.72 ± 0.17 [d]	4.95 ± 0.15[e]	1.05 ± 0.16[a]
CD (5%)	0.193	0.205	0.179	0.103

Values are mean ± SD (n=6), A column means followed by common superscripts are not significant at 5% level by DMRT

The results given in table 4 showed that there was a perceptible decrease in tumor volume, packed cell volume and viable cell count in experimental groups supplemented with MPO and 5 FU. Tumor volume of cancer control group was 4.0 ± 0.26 ml, and it was reduced to 3.42 ± 0.17, 2.97 ± 0.05 and 2.8 ± 0.08 ml on treatment with different concentrations of MPO. Similar results were observed in packed cell volume and viable tumor cell count with mean level ranged from 1.7 ± 0.08 to 1.07 ± 0.15 ml and 8.67 ± 0.33 to 5.92 ± 0.29 x 10^6 cells/ml respectively when fed with 0.5 to 5 mg of MPO. The PCV of 5-FU (groupVI) and 5mg of MPO ingested was 1.72 ± 0.17 and 1.07 ± 0.15 ml respectively and no significant difference was seen between these two groups indicating that the therapeutic action of 5mg MPO was nearer to 5-FU.

Viable tumor cell count found to be higher in cancer control compared to experimental groups of mice. Ingestion of different doses of MPO 0.5, 2.5 and 5 mg gradually reduced the viable tumor cell count to 8.67, 6.15 and 5.95 x 10^6 cells /ml respectively. Non-viable cell count increases satisfactorily on treatment with MPO. The findings coincide with the studies of Morre *et al* (1996) who stated that capsaicin abrogates tumor growth in animal models. Ito *et al* (2004) have reported that a component of hot pepper (*Capsicum)*-capsaicin, induced death signaling which is mediated through the mitochondrial-dependent pathway and had no toxic effects on NOD/SCID mice leukemia model. Capsaicin did not affect cellular proliferation of normal bone marrow cells of healthy volunteers which concluded that capsaicin might be used as a new potent anticancer agent for the management of hematological malignancies.

Effect of MPO on biochemical indices

Effect of MPO on catalase and superoxide dismutase in EAC bearing mice

Table 5 Effect of MPO on catalase and superoxide dismutase in EAC bearing mice

Groups	Catalase (U*/mg protein)	Superoxide dismutase (U^{Δ}/mg protein)
Group I (Normal)	2.80 ± 0.06[a]	4.33 ± 0.20 [a]
Group II (Cancer control)	1.47 ± 0.06[f]	1.52 ± 0.18 [e]
Group III (EAC + 0.5 mg MPO/kg body weight)	2.30 ± 0.05[e]	2.70 ± 0.29 [d]
Group IV (EAC + 2.5 mg MPO/kg body weight)	2.53 ± 0.05 [d]	3.55 ± 0.31 [c]
Group V(EAC + 5 mg MPO/kg body weight)	2.61 ± 0.06 [bc]	3.60 ± 0.22 [bc]
Group VI (EAC + 5 FU 20 mg/kg body weight)	2.70 ± 0.08[b]	3.73 ± 0.26 [b]
CD Value	0.094	0.36

Values are mean ± SD of six samples in each group, A column mean followed by common superscripts are not significant at 5% level by DMRT

* Decomposition of µmol of H2O2/min/mg protein

$^{\Delta}$ 50 % inhibition of NBT reduction/min/mg protein

Table 5 showed that there was a remarkable increase in antioxidant enzymes in MPO and drug treated animals. The mean value of catalase showed a significant difference between treated groups and control group indicating the potential effect of MPO even at a minimum dosage level of 0.5mg. Similarly, in case of radical scavenging enzyme SOD the enzyme level was less as 1.52 ± 0.18 units in cancer control when compared to the level in normal mice as 4.33± 0.20 units. The enzyme level increased to 2.70 ± 0.29, 3.55 ± 0.31 and 3.60 ± 0.22 units on increasing concentration of MPO supplementation as 0.5, 2.5 and 5mg/ kg body weight respectively. Further, there was no significant difference between mice treated with 5mg of MPO and 5-FU in catalase and SOD activity.

Effect of MPO on lipid peroxidation and reduced glutathione in EAC Bearing Mice

Table 6. Effect of MPO on lipid peroxidation and reduced glutathione in EAC Bearing Mice

Groups	LPO (nm*/mg protein)	Reduced Glutathione (mg/100gm)
Group I (Normal)	0.87 ± 0.06[c]	2.77 ± 0.10 [a]
Group II (Cancer control)	3.37 ± 0.17[a]	1.32 ± 0.15 [e]
Group III (EAC + 0.5 mg MPO/kg body weight)	2.25 ± 0.24 [b]	1.87 ± 0.09 [d]
Group IV (EAC + 2.5 mg MPO/kg body weight)	1.66 ± 0.08 [bc]	2.14 ± 0.19 [c]
Group V (EAC + 5 mg MPO/kg body weight)	1.42 ± 0.07 [bc]	2.20 ± 0.14 [c]
Group VI (EAC + 5 FU 20mg /kg body weight)	1.23 ± 0.03 [c]	2.44 ± 0.10 [b]
CD value	0.92	0.203

values are mean ± SD of six samples in each group, A column mean followed by common superscripts are not significant at 5% level by DMRT

*****n moles of malonaldehyde formed / mg protein

Critical difference of LPO (Table 6) revealed that the mean value of group II (cancer control) significantly differ from the mean value of all other (normal and treated) groups. No significant difference was seen in LPO of mice treated with maximum dose (5mg-group V) of MPO with 5-FU (group VI)

whereas, a significant difference was observed between the group III (treated with the least concentration of MPO (0.5mg) and VI (20mg of 5-Fluro Uracil) which means that the effect of MPO at minimum level was not up to the effect of the drug 5-FU. The results indicated that MPO at 5 mg level inhibit the deleterious consequences of oxidative stress. The findings are supported by the statement of Iqbal *et al* (2007) who mentioned that flavanoids can act as antioxidants by acting as hydrogen donors and may help to control the degree of lipid peroxidation. One way Analysis of variance result showed that a significant ($P<0.05$) increase in the mean levels of glutathione was observed between the EAC control and treated groups. The efficiency of MPO is revealed by altering the levels of these scavengers in a dose dependent manner. The results confirmed with studies of Ahuja and Ball (2006) who pointed out that a regular consumption of moderate amounts of chilli, in addition to providing flavour, may help in resisting lipoprotein oxidation.

Effect of MPO on hepatic marker enzymes in serum

Table 7. Effect of MPO on hepatic marker enzymes (AST and ALT) in EAC bearing mice

Groups	AST (U/L)	ALT(U/L)
Group I (Normal)	47.1±1.21[a]	63.44±0.388[a]
Group II (Cancer control)	21.63±3.70[e]	39.50±0.693[f]
Group III (EAC + 0.5 mg MPO/kg body weight)	29.89±0.41[d]	51.87±0.479[e]
Group IV (EAC + 2.5 mg MPO/kg body weight)	36.78±0.47[c]	55.34±0.804[d]
Group V (EAC + 5 mg MPO/kg body weight)	38.53±1.20[c]	57.53±0.897[c]
Group VI (EAC + 5 FU 20mg /kg body weight)	41.62±2.84[b]	60.31±0.576[b]
CD (5%)	3.015	0.986

Values are mean ± SD (n=6), A column means followed by common superscripts are not significant at 5% level by DMRT

The activities of hepatic markers viz., alanine transaminase (ALT) and aspartate amino transferase (AST) were significantly reduced in EAC control (group II) compared to normal mice. Statistical analysis proved that on treatment with different doses of MPO effectively restored the activities of the hepatic marker enzymes and thereby reduced the burden to liver caused by the peritoneal EAC cells. These findings are in line with the study of Karthigayan *et al* (2007) who reported that inoculation of EAC cells into the mice significantly reduced the activity of ALT and increased the activity of serum AST, on treatment with the octopus venom ameliorated and these changes to near normal levels, indicating the protective role on liver.

Conclusion

MPO treated animals at 5 mg/kg body weight inhibited the body weight, tumour volume, packed cell volume viable tumour cell count and also brought back the haematological parameters to more or less normal levels. The extracts also restored the activity of free radical scavenging enzyme, reduced glutathione and antioxidant enzymes such as catalase and superoxide dismutase which in turn reduced the level of lipid peroxidation in tumour bearing mice to normal level. It is proposed that the additive and synergistic antioxidant activity of phytochemicals such as alkaloid, flavanoid, carotenoid etc., present in MPO are responsible for its potent anticancer activity.

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