

# Antineoplastic activities of MT81 and its structural analogue in ehrlich ascites carcinoma-bearing swiss albino mice

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**Key words:** antitumor activity and antioxidant status, MT81 and its acetic acid derivative, ehrlich ascites carcinoma, mean survival time, hematological parameters

Many fungal toxins exhibit *in vitro* and *in vivo* antineoplastic effects on various cancer cell types. Luteoskyrin, a hydroxyanthraquinone has been proved to be a potent inhibitor against Ehrlich ascites tumor cells. The comparative antitumor activity and antioxidant status of MT81 and its structural analogue [Acetic acid-MT81 (Aa-MT81)] having polyhydroxyanthraquinone structure were assessed against Ehrlich ascites carcinoma (EAC) tumor in mice. The *in vitro* cytotoxicity was measured by the viability of EAC cells after direct treatment of the said compounds. In *in vivo* study, MT81 and its structural analogue were administered (*i.p.*) at the two different doses (5, 7 mg MT81; 8.93, 11.48 mg Aa-MT81/kg body weight) for 7 days after 24 hrs. of tumor inoculation. The activities were assessed using mean survival time (MST), increased life span (ILS), tumor volume, viable tumor cell count, peritoneal cell count, protein percentage and hematological parameters. Antioxidant status was determined by malondialdehyde (MDA) and reduced glutathione (GSH) content, and by the activity of superoxide dismutase (SOD) and catalase (CAT). MT81 and its structural analogues increased the mean survival time, normal peritoneal cell count. They decreased the tumor volume, viable tumor cell count, hemoglobin percentage and packed cell volume. Differential counts of WBC, total counts of RBC & WBC that altered by EAC inoculation, were restored in a dose-dependent manner. Increased MDA and decreased GSH content and reduced activity of SOD, and catalase in EAC bearing mice were returned towards normal after the treatment of MT81 and its structural analogue. Being less toxic than parent toxin MT81, the structural analogue showed more prominent antineoplastic activities against EAC cells compared to MT81. At the same time, both compounds exhibit to some extent antioxidant potential for the EAC-bearing mice.

## Introduction

Cancer is one of the most dreaded diseases of the 20<sup>th</sup> century. It is the single most common cause of deaths and spreading further with continuance in 21<sup>st</sup> century in both developed and developing countries.<sup>1,2</sup> Statistics show that men are largely plagued by lung, colon, rectal and prostate cancer, while women increasing suffer from breast, stomach, colon and rectal cancer. From literature it is revealed that many natural products are available as chemo-preventive agents against commonly occurring cancer types. However, there is continuing need for identification, characterization and development of new chemo-preventive agents from enormous pool of synthetic, biological and natural products.

About 60% of currently used anticancer agents are obtained from natural sources, including plants, marine organisms and microorganisms. Fungal toxins (mycotoxins) though known to be toxic to the animal and human systems still find their use in therapeutic application. Mycophenolic acid,<sup>3</sup> penicillic acid,<sup>4</sup>

5-methoxy-sterigmatocystin,<sup>5</sup> a series of analogues of anguidine,<sup>6,7</sup> including triacetoxyscirpenol, three diacetoxyscirpenols, three monoacetoxyscirpenol and scirpenol, T-2 toxin and related tricothecenes,<sup>8</sup> cytochalasin B,<sup>9</sup> patulin,<sup>10</sup> aflastatin A,<sup>11</sup> 14-Hydroxytoxin B and 16-Hydroxyroridin E,<sup>12</sup> tenuazonic acid,<sup>13</sup> 4-beta-acetoxyscirpentiol,<sup>14</sup> gliotoxin,<sup>15</sup> fluorinated psurotin A, synerazol,<sup>16</sup> rubratoxin B,<sup>17</sup> beauvericin<sup>18</sup> showed anti-tumor activities in different types of cancer cell line and *in vivo*. Harri et al. reported that the tricothecenes verrucarins A and B and roridin A inhibited the growth of Ehrlich ascites tumor in mice and Walker carcinoma in rats. Myrocin C, a new diterpene from soil fungus *Myrothecium verrucaria* increases the life span of EAC-bearing mice.<sup>19</sup> Luteoskyrin, a hydroxyanthraquinone is proved to inhibit mRNA synthesis in Ehrlich ascites tumor cells.<sup>20,21</sup> Oxidative stress may be involved in both initiation and promotion of multi-step carcinogenesis. Some synthetic,<sup>22</sup> biogenic chemicals,<sup>23</sup> nanoparticles,<sup>24</sup> phytochemicals<sup>25,26</sup> can prevent oxidative stress<sup>22,23</sup> and thus can modulate the expression of genes related to tumor promotion.

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**Table 1.** Mean survival time and increased life span of EAC-bearing mice after the treatment of MT81 and acetic acid-MT81

Group	EAC challenge cell no./mouse	Mean survival time (days) Mean $\pm$ SEM	Increase in life span (%)	30 days survivors/ total no.
Saline control (0.9% NaCl w/v)	-	-	-	6/6
EAC control	$2 \times 10^6$	$15.66 \pm 1.67$	0.0	0/6
Vehicle control (0.01 ml propylene glycol/20 gm of body weight)	$2 \times 10^6$	$16.0 \pm 1.75$	2.17 <sup>a</sup>	0/6
MT81 low dose (5 mg/kg body weight)	$2 \times 10^6$	$24.0 \pm 1.67^*$	50 <sup>b</sup>	1/6
MT81 high dose (7 mg/kg body weight)	$2 \times 10^6$	$28.5 \pm 1.14^*$	78.12 <sup>b</sup>	1/6
Aa-MT81 low dose (8.93 mg/kg body weight)	$2 \times 10^6$	$23.0 \pm 1.65^*$	43.75 <sup>b</sup>	1/6
Aa-MT81 high dose (11.48 mg/kg body weight)	$2 \times 10^6$	$25.90 \pm 1.38^*$	61.88 <sup>b</sup>	1/6
5-Fluorouracil (20 mg/kg body weight)	$2 \times 10^6$	$40.2 \pm 1.08^*$	151.25 <sup>b</sup>	5/6

Data given are Mean  $\pm$  SEM; n = 6. <sup>a</sup>significant different at  $p < 0.05$ , <sup>\*\*</sup>significant different at  $p < 0.01$ , <sup>\*\*\*</sup>significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.

Mycotoxin MT81 was isolated, purified and identified in our laboratory from a locally isolated fungal strain of *Penicillium nigricans* (patent no. 156916 dated 15.2.82, Govt. of India). MT81 is a dextrorotatory polyhydroxyanthraquinone compound having molecular formula of  $C_{22}H_{18}O_7$  and molecular wt. of 394.<sup>27</sup> Its LD<sub>50</sub> value is 35.1 mg/Kg body wt. in mice. MT81 is a good hyperglycemic,<sup>28</sup> antimicrobial<sup>29</sup> and antileishmanial<sup>30</sup> agents. It produces massive bone marrow depression,<sup>31</sup> liver,<sup>32</sup> brain<sup>33</sup> and kidney<sup>34</sup> dysfunction. MT81 exhibits central nervous system depressant action.<sup>35</sup> MT81 has shown in vitro and in vivo antitumor activity against Ehrlich ascites tumor cells.<sup>36</sup>

To generate more potent and less toxic toxin, a structural analogue, acetic acid (Aa-MT81) MT81 was synthesized in our laboratory having LD<sub>50</sub> value 80.4 mg/kg body weight in mice. This analogue was reported to possess antimicrobial<sup>29</sup> and antileishmanial<sup>30</sup> effects. The Ehrlich ascites tumor cell (EAC) is a spontaneous murine mammary adenocarcinoma<sup>1</sup> and carried out in inbred mice by serial intraperitoneal (i.p.) administration. As (-)-luteoskyrin and (+)-rugulosin being hydroxyanthraquinone possess antitumor properties, present study was carried out to evaluate the antitumor and antioxidant activities of MT81 and its structural analogue (which are also polyhydroxyanthraquinone) against Ehrlich ascites carcinoma (EAC) in mice.

## Results

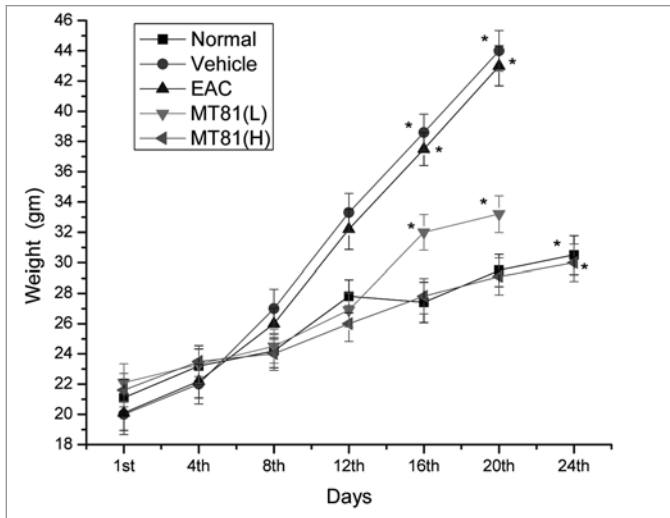
**Short-term (in vitro) cytotoxicity.** The in vitro cytotoxicity of MT81 and its structural analogue towards EAC cells showed that the IC<sub>50</sub> of MT81, Aa-MT81 were 17  $\mu$ g, 22  $\mu$ g/ml respectively. The degree of in vitro lethality is slightly more in case of MT81 due to its toxicity.

**Mean survival time (MST).** In the EAC control group, the mean survival time was  $16.0 \pm 1.75$  days, while it increased to  $24.0 \pm 1.67$  (3.88 mg/kg),  $28.5$  (5 mg/kg) days in the MT81-treated groups,  $23.0 \pm 1.62$  (8.93 mg/kg),  $25.9 \pm 1.38$  (11.48 mg/kg) days in the Aa-MT81-treated groups respectively. These results are almost comparable to that for 5-fluorouracil (20 mg/kg), the standard drug, for which the MST was  $40.2 \pm 1.08$  days.

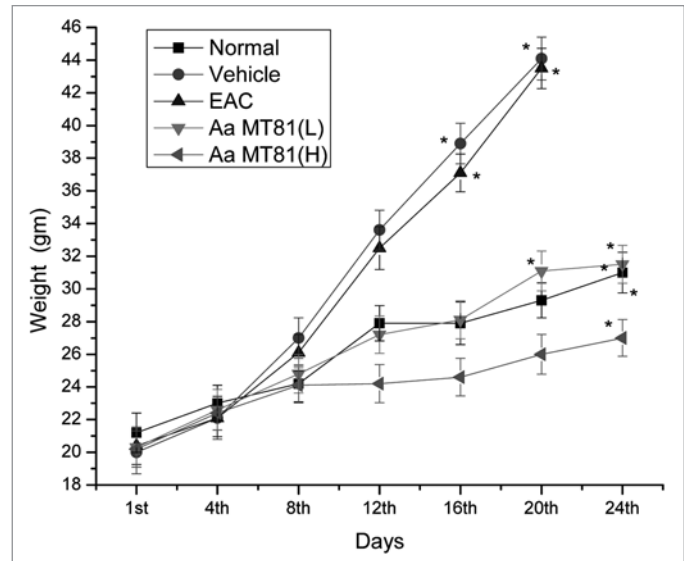
**In vivo treatment of MT81 and AaMT81 inhibit growth of EAC cells.** The result in Figures 1 and 2 indicates that control EAC-bearing mice (EAC and vehicle control groups) had a gradual increase in body weight of about 22 to 24 gm in 21 days from the day zero. When compared to the body weight of control EAC-bearing mice on day 21, the body weight of the treated mice decreased significantly by about 50%, indicating the effect of MT81 and Aa-MT81 in preventing the growth of Ehrlich ascites tumor cells. Inhibition of tumor growth in vivo expressed by the mean survival time and 30 days survivor has been summarized in Table 1. In case of EAC control, mean survival time is  $15.66 \pm 1.667$  (all died by 20 days) whereas with high dose of (7 mg/kg of body weight) of MT81, mean survival time is  $28.5 \pm 1.138$  days indicating 89.1% increase in longevity (Table 1) of the treated group with respect to EAC control. Aa-MT81 have demonstrated enhanced effect on the mean life span by 59.3%. The decrease in body weight in toxin-treated mice has been shown in Figures 1 and 2. The effects of MT81 and its structural analogue at different doses on tumor volume and viable tumor cell count are shown in Figures 4 and 5. MT81 and Aa-MT81 reduced the tumor volume, viable tumor cell count, packed cell volume (Fig. 11), and protein percent (Fig. 12).

**Effect on normal peritoneal cells.** The average number of peritoneal exudates cells in normal mice was found to be  $5.3 \pm 0.248 \times 10^6$ . Treatment of MT81 and its two derivatives increase the number of peritoneal cells significantly compared to the vehicle control group (Fig. 6).

**Hematological parameters.** Hematological parameters of EAC-bearing mice on day 14 were found to be significantly changed from normal (saline control) group (Table 2, Figs. 7–10). Hemoglobin content and RBC count in the EAC and vehicle control group were significantly ( $p \leq 0.01$ ) decreased in comparison to the normal group. MT81 increased the hemoglobin content and RBC counts to a less extent whereas Aa-MT81 increased them significantly ( $p < 0.05$ ). The total WBC counts and protein were found to be increased significantly in the vehicle control group ( $p < 0.01$ ). Administration of MT81 and its structural analogue at the above said doses reduces the WBC counts and protein as compared to the vehicle control.



**Figure 1.** Inhibitory role of MT81 on the body weight change in EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



**Figure 2.** Inhibitory role of AaMT81 on the body weight change of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.

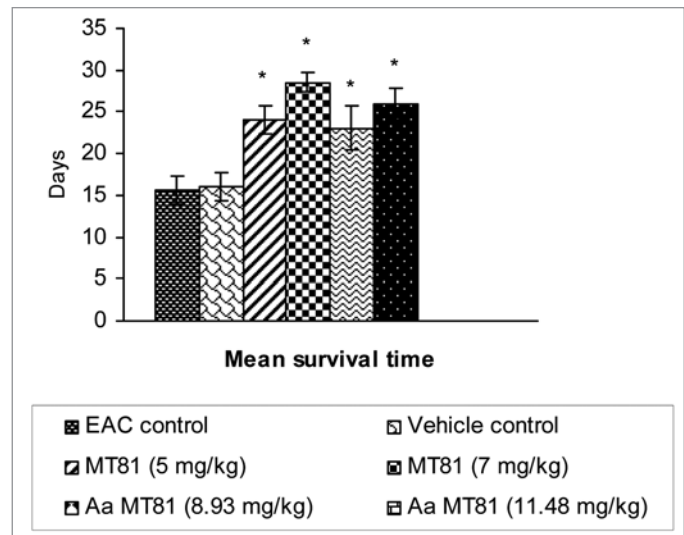
There was a decrease in lymphocytes in malignancy, accompanied by an increase in neutrophils. The treatment changed those altered parameters significantly ( $p < 0.01$ ), to near normal in a dose-dependent manner.

**Lipid peroxidation and glutathione content.** As shown in Figure 14, the levels of lipid peroxidation in liver tissue were significantly increased in EAC and vehicle control group as compared to the normal group ( $p < 0.01$ ). After administration of MT81 and its Acetic acid analogue to EAC bearing mice the levels of lipid peroxidation were reduced respectively as compared to EAC and vehicle control. Inoculation with EAC drastically decreased the GSH content in vehicle control group in comparison to normal group. The administration of Aa-MT81 increased GSH level in a dose dependent manner compared to MT81 but not significantly.

**Effect on antioxidant enzymes.** The activities of superoxide dismutase and catalase in the livers of EAC bearing mice decreased in EAC and vehicle control groups ( $p < 0.05$ ). Treatment with MT81 and its structural analogue increased these enzyme activities to some extent but not in a significant manner (Figs. 16 and 17).

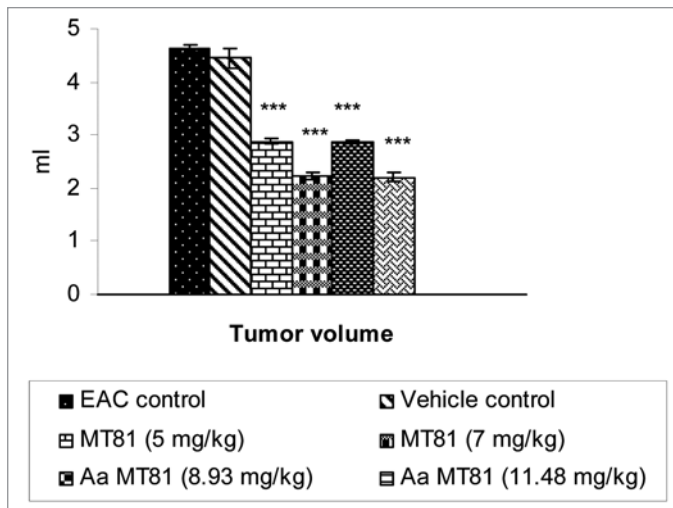
## Discussion

The present study was carried out to evaluate the antitumor effect and antioxidant status of mycotoxin MT81 and its structural analogue in EAC-bearing mice. The treated animals significantly inhibited the tumor volume, packed cell volume, viable tumor cell count, increased the mean survival time, peritoneal cell count. They also restored the hematological parameters to more or less normal levels. They decreased the hepatic lipid peroxidation and increased the antioxidant enzyme SOD and CAT as well as the GSH level.

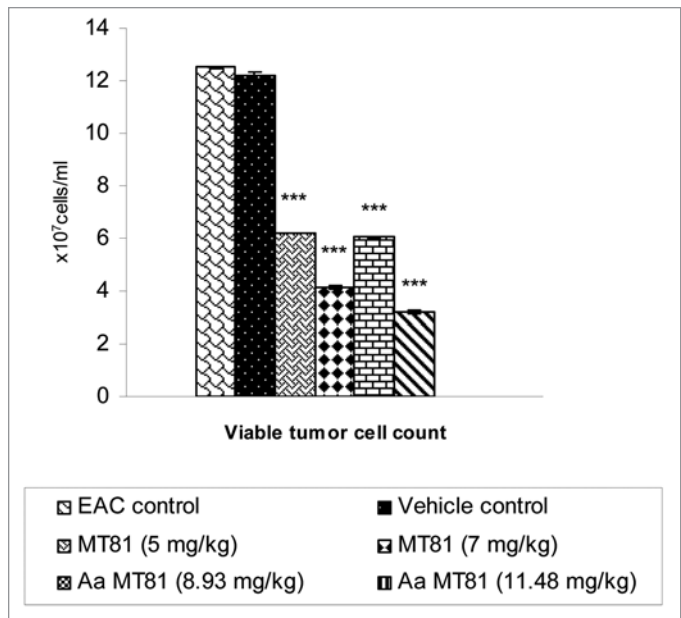


**Figure 3.** Modulatory role of MT81 and AaMT81 on Mean Survival Time of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.

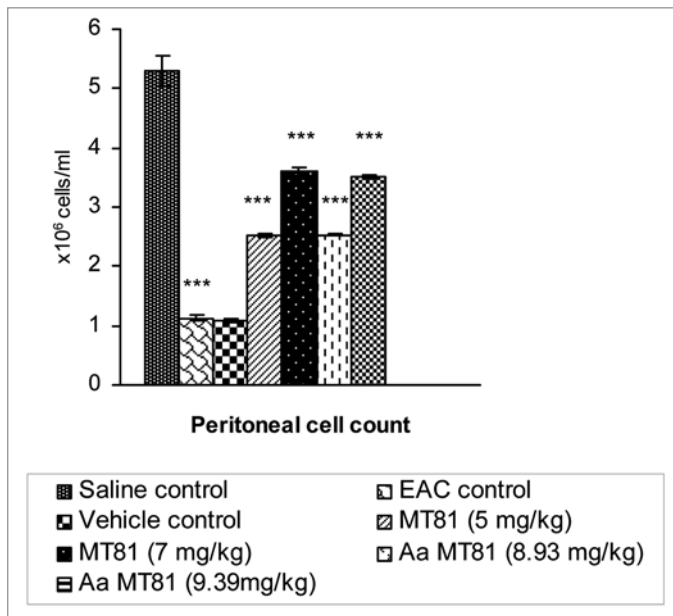
In EAC-bearing mice, a regular rapid increase in ascites tumor volume is seen. Ascites fluid is the direct nutritional source for tumor cell and so a rapid increase of this fluid is very necessary factor for tumor growth and nutrition. An anticancer drug is considered reliable if it can prolong the life span of animals.<sup>37</sup> MT81 and its Acetic acid analogue decrease the ascites fluid volume, viable EAC cell count and increase the percentage of life span.



**Figure 4.** Inhibition of Tumor Volume of EAC-bearing mice by MT81 and AaMT81. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; oryEAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



**Figure 5.** Inhibition of Viable Tumor Cell Count of EAC-bearing mice by MT81 and AaMT81 on Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



**Figure 6.** Increase of Peritoneal Cell Count of EAC-bearing mice by MT81 and AaMT81 on. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.

By decreasing the ascites fluid volume and arresting the tumor growth these toxins increase the life span of EAC bearing mice.

Myelosuppression and anemia are the major outcome after cancer chemotherapy.<sup>38</sup> In tumor-bearing mice, anemia occurs due to the diminution in RBC or hemoglobin percentage, and these may happens either due to iron deficiency or due to hemolytic

or myelopathic conditions.<sup>39</sup> Administration of Acetic acid analogue by restoring hemoglobin content, RBC and WBC count towards normal exhibit its protective role on hemopoietic system compared to its parent toxin, MT81. The hematological parameter showed that hemoglobin content and total RBC count were found to be lower in case of EAC control group in comparison with saline control. Most of the cases of low doses of the toxins, the values are found to be less significant. But in case of high dose of Aa-MT81, significant increase in hemoglobin content and RBC count were observed but which were not seen in case of high dose of MT81, most probably due to its higher toxicity than Acetic acid analogue. The toxin treated animals showed comparatively lower total WBC count than EAC and vehicle control. It may be due to the tumoricidal activity of the toxins.

Cancer is considered as a multifactor disease, where oxidative stress may be involved in both initiation and promotion of multi-step carcinogenesis. Reactive oxygen species (ROS) can accelerate DNA damage, stimulate pro-carcinogenesis, initiate lipid peroxidation, inactivate antioxidant enzyme systems and thus can modulate the expression of genes related to tumor promotion.<sup>40,41</sup>

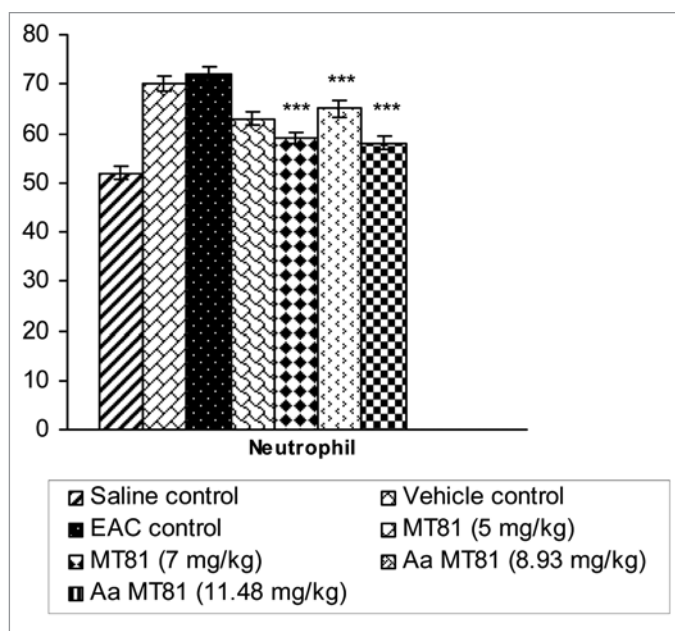
Excessive production of free radicals cause macromolecular damage and can induce lipid peroxidation in vivo.<sup>42</sup> Malondialdehyde (MDA) the end product of lipid peroxidation, are seen to be higher in cancer tissues than in non-diseased organ.<sup>43</sup> GSH, an important non-protein thiol, plays a significant role in protecting cells by scavenging ROS<sup>44</sup> and potent inhibitor of the neoplastic process. Aa-MT81 significantly decreased the lipid peroxidation compared to MT81 by reducing the MDA



**Table 2.** Modulatory role of MT81 and acetic acid-MT81 on the haematological parameters of EAC-bearing mice

Group	Haemoglobin conc. (g%)	Total RBC ( $\times 10^6/\text{mm}^3$ )	Total WBC ( $\times 10^3/\text{mm}^3$ )
Saline control (0.9% NaCl w/v)	8.9 $\pm$ 0.19	2.9 $\pm$ 0.12	7.6 $\pm$ 0.15
EAC control	6.8 $\pm$ 0.20	1.9 $\pm$ 0.07	8.6 $\pm$ 0.17
Vehicle control (0.01 ml propylene glycol/20 gm of body weight)	7.0 $\pm$ 0.12	2.0 $\pm$ 0.15	8.5 $\pm$ 0.15
MT81 low dose (5 mg/kg body weight)	7.3 $\pm$ 0.24	2.2 $\pm$ 0.12	8.2 $\pm$ 0.19
MT81 high dose (7 mg/kg body weight)	7.4 $\pm$ 0.11	2.3 $\pm$ 0.12	8.09 $\pm$ 0.11*
Aa-MT81 low dose (8.93 mg/kg body weight)	7.4 $\pm$ 0.31	2.4 $\pm$ 0.07	8.4 $\pm$ 0.10
Aa-MT81 high dose (11.48 mg/kg body weight)	7.75 $\pm$ 0.21*	2.5 $\pm$ 0.04*	8.19 $\pm$ 0.17**

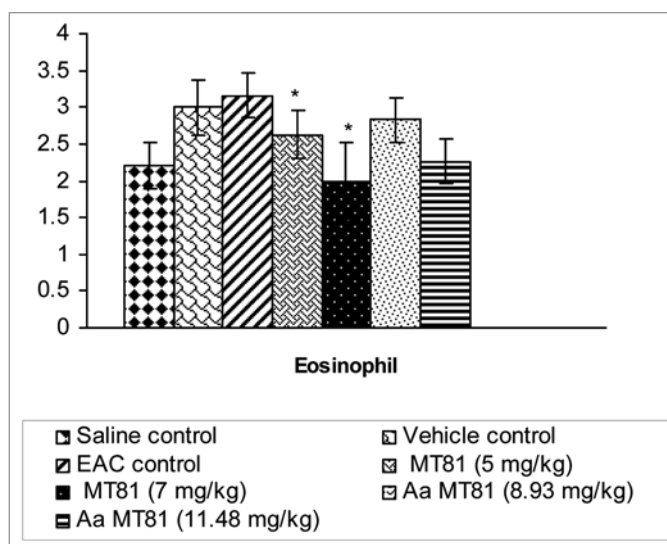
Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



**Figure 7.** Modulatory role of MT81, AaMT81 on Neutrophil Count of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.

level but the glutathione content is not increased significantly in a dose-dependent manner most probably due to their toxicity.

SOD and CAT, the antioxidant enzymes prevent  $\text{H}_2\text{O}_2$ -mediated intracellular damage, which is thought to be prerequisite for carcinogenesis.<sup>45</sup> SOD dismutates superoxide anions ( $\text{O}_2^-$ ) to  $\text{H}_2\text{O}_2$  and protects the cells against ( $\text{O}_2^-$ )-mediated lipid peroxidation. CAT acts on  $\text{H}_2\text{O}_2$  by decomposing it, thereby neutralizing its toxicity. Gupta et al. demonstrated that reduction in several antioxidant defense mechanisms correlates with the emergence of the malignant phenotype.<sup>46</sup> Consistent with this, a diminution in SOD activity in EAC-bearing mice may be due to the loss of  $\text{Mn}^{2+}$ -containing SOD activity in EAC cells and loss of mitochondria, leading to a decrease in total SOD activity in the liver.<sup>47</sup> A small amount of catalase in tumor cells was reported.<sup>48</sup>

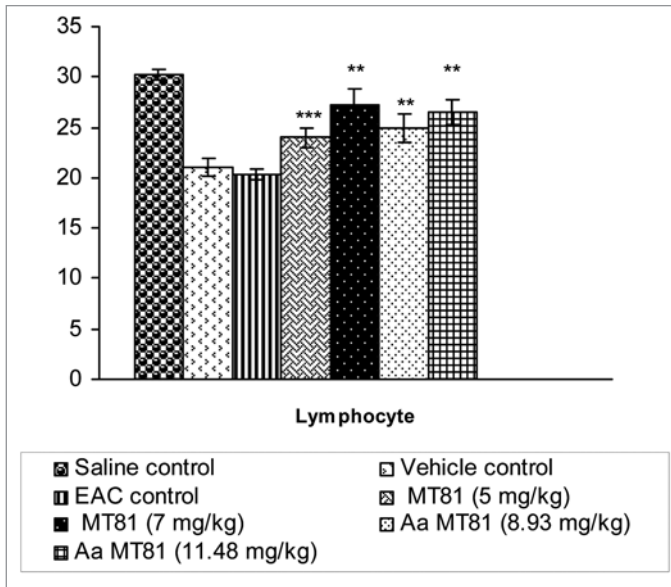


**Figure 8.** Modulatory role of MT81, AaMT81 on Eosinophil Count of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.

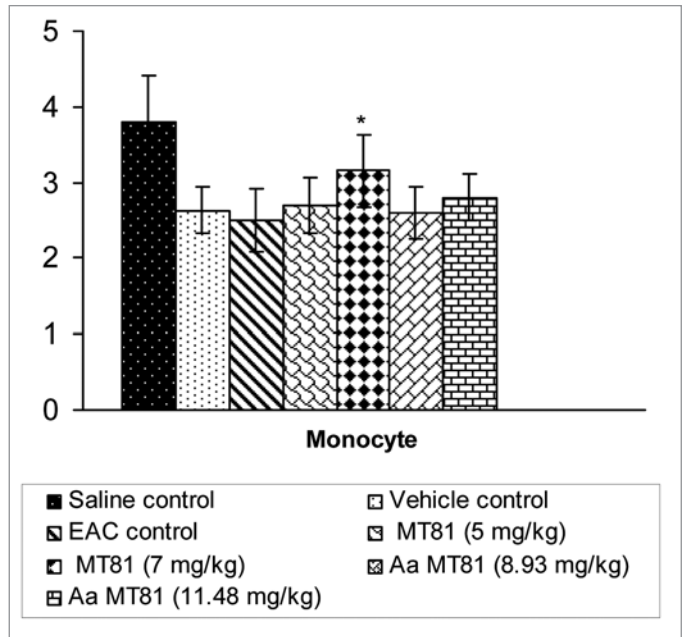
The inhibitions of SOD and CAT to some extent activities as a result of tumor growth were reported.<sup>49</sup> Similar findings were observed in the present study with EAC-bearing mice. The treatment of MT81 and Aa-MT81 at different doses increased the SOD and CAT levels to some extent in a dose-dependent manner like the chemopreventive efficacy of perillyl alcohol probably due to the inhibition of oxidative stress responses<sup>50</sup> and the structural analogue showed more potentiality.

The in vitro cytotoxicity and the significant inhibition of in vivo growth of EAC cells, increase in life span and of the tumor-bearing mice confirm the antitumorogenic effect of MT81 and its acetic acid derivative against EAC cells (Fig. 17).

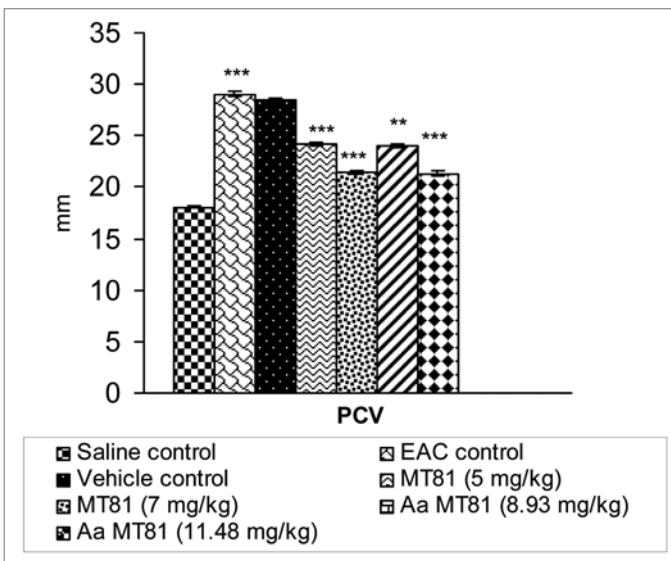
Being less toxic than parent toxin MT81, the Acetic acid analogue showed more prominent antineoplastic activities against EAC cells compared to MT81. At the same time both exhibit mild antioxidant potential for the EAC bearing mice in spite of their different toxic side effects.



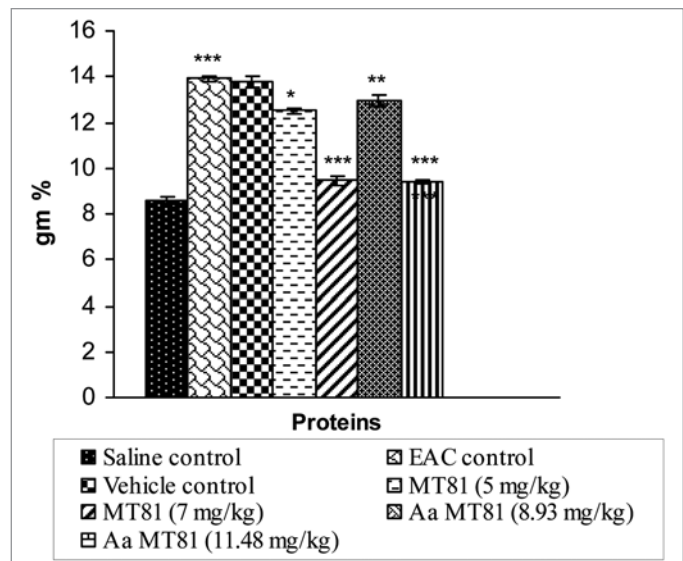
**Figure 9.** Modulatory role of MT81, AaMT81 on Lymphocyte Count of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



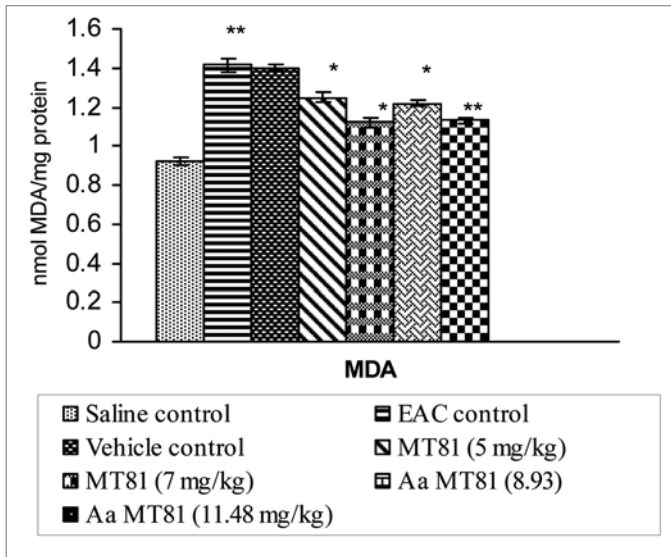
**Figure 10.** Modulatory role of MT81, AaMT81 on Monocyte Count of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



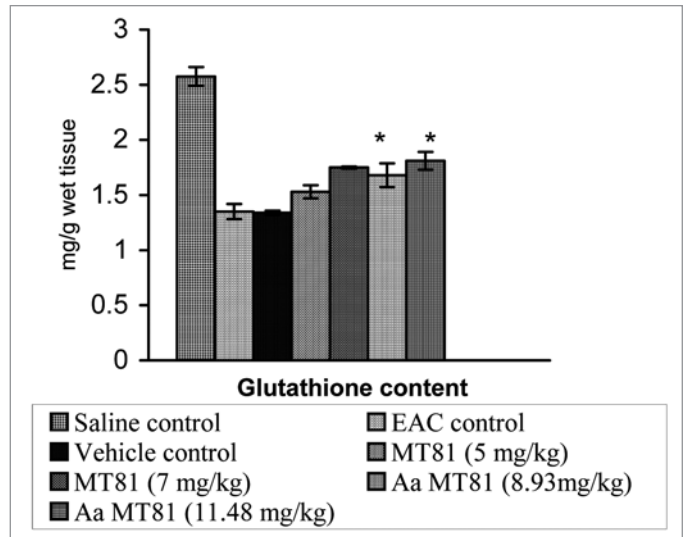
**Figure 11.** Role of MT81, AaMT81 on Packed Cell Volume (PCV) of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



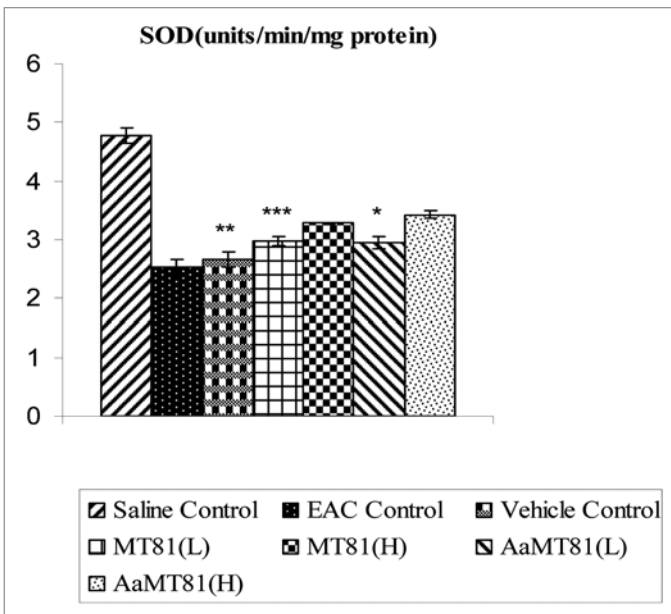
**Figure 12.** Role of MT81, AaMT81 on Proteins% of EAC-bearing mice. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



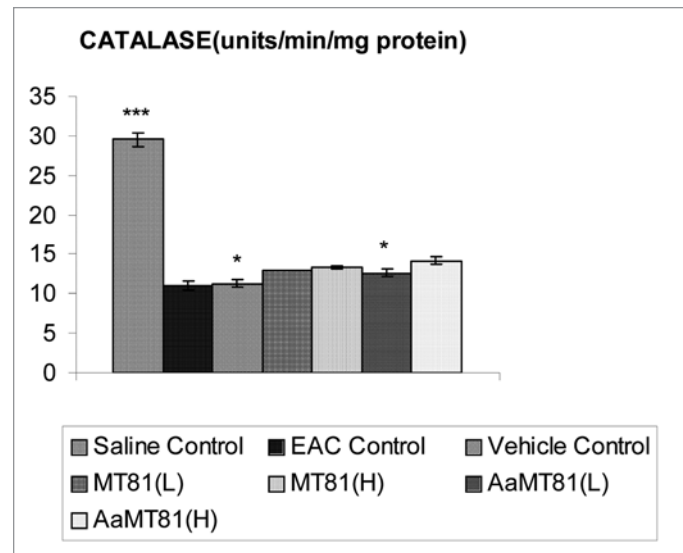
**Figure 13.** MDA content of EAC-bearing mice after the treatment of MT81 and AaMT81. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



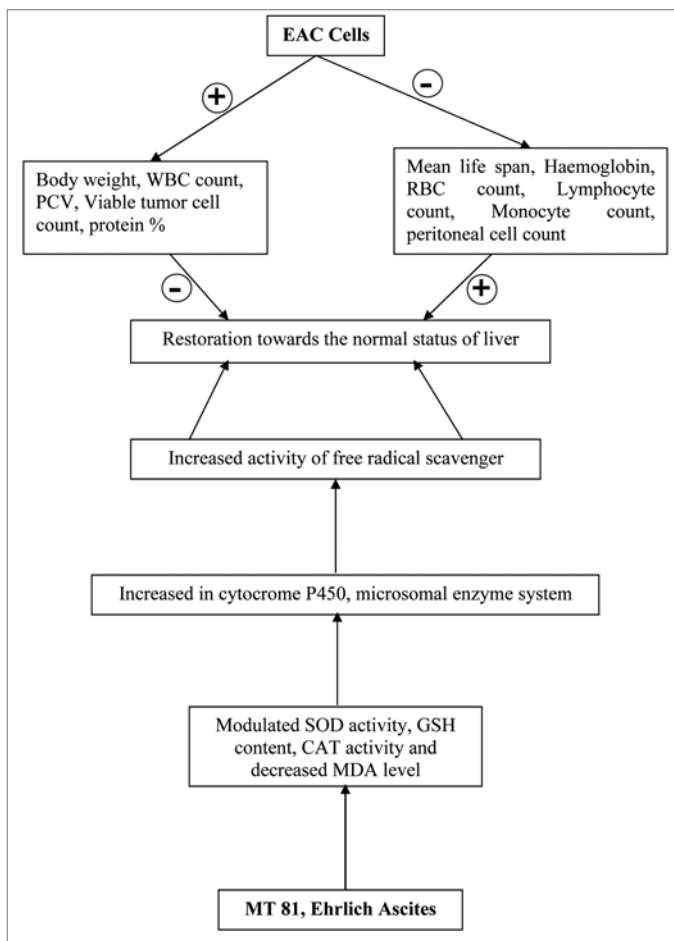
**Figure 14.** Glutathione content of EAC-bearing mice after the treatment of MT81 and AaMT81. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



**Figure 15.** SOD activity of EAC-bearing mice after the treatment of MT81 and AaMT81. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



**Figure 16.** Catalase activity of EAC-bearing mice after the treatment of MT81 and AaMT81. Data given are Mean  $\pm$  SEM; n = 6. \*significant different at  $p < 0.05$ , \*\*significant different at  $p < 0.01$ , \*\*\*significant different at  $p < 0.001$ ; EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control by one-way ANOVA followed by Student's t-test.



**Figure 17.** Possible potential anti-neoplastic cellular pathways of MT81 and AaMT81 on EAC-bearing mice. Where '+' denoted to stimulatory and '-' denoted to inhibitory effects to the respective parameters.

## Materials and Methods

**Chemicals and reagents.** All fine chemicals were obtained from Sigma Chemical, USA. Other chemicals used were analytical grade and obtained locally.

**Animals.** Male albino (Swiss) mice weighing between 18–25 g were used throughout the study. The mice were obtained from the animal house of Jadavpur University, Kolkata and grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than twelve animals per cage. They were maintained in a constant room temperature of 28–30°C and 55–65% humidity and a controlled day length, 14 hours light and 10 hours dark cycle. Standard pellet diet containing 66% starch, 20% casein, 8% fat, 2% standard vitamins and 4% salt was collected from Hindustan Lever Co., Ltd., (India) and given to the animals. Water was given ad libitum. The mice were acclimatized to laboratory conditions for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University animals Ethical Committee.

**Ehrlich's ascites carcinoma (EAC) cells and its transplantation.** EAC cells were obtained by the courtesy of Indian Institute

of Chemical Biology and were maintained by weekly intraperitoneal transplantation in the abovesaid mice at the concentration of  $2 \times 10^6$  cells/mouse. The EAC cells were harvested after 7–10 days. The washed cells free of contaminating RBC were taken in 0.14 M NaCl solutions. Cells were found to be 99% viable by the trypan blue exclusion assay.

**Study of in vitro cytotoxicity.** Washed and viable EAC cells of 7–10 days old tumor were suspended into isotonic solution (Phosphate buffer saline) and were adjusted to  $1 \times 10^6$  cells/ml. In a series of test tubes 1 ml of this suspension was taken and 0.01 ml of varying concentrations (5 to 25  $\mu\text{g}$ ) of MT81 and Aa-MT81 were added. One tube was kept as EAC control and in another tube marked as vehicle control, 0.01 ml of propylene glycol was added. The tubes were mildly shaken to mix the contents and incubated at 37°C for 3 hr under a  $\text{CO}_2$  atmosphere. After 3 hours, percentage of viability of EAC cells was determined by trypan blue exclusion method.<sup>51</sup>

**Assessment of in vivo antitumor activity.** Male albino mice were divided into nine groups each group containing twelve. Washed and viable EAC cells were resuspended in normal saline and inoculated (0.2 ml of  $2 \times 10^6$  cells/mouse) to animals of all groups intraperitoneally except the normal group. After 24 hrs, 5 ml/kg/day of normal saline were administered in Group 1 (Normal) and Group 2 (EAC control) and propylene glycol was administered in Group 3 (vehicle control group). MT81 (5 and 7.00 mg/kg/day), Aa-MT81 (8.93 and 11.48 mg/kg day) and the standard drug 5-fluorouracil<sup>52</sup> (20 mg/kg) were administered intraperitoneally in Groups 4, 5, 6, 7, 8, respectively for subsequent 7 days. After the last dose and 18 hr fasting, six mice from each group were sacrificed for the study of antitumor and antioxidant activities and hematological parameters. The rest of the animals of all groups were kept for study of the tumor growth response and host survival.

**Determination of tumor growth response and host survival.** Tumor growth was monitored by daily weight change and the survival time of host mouse by recording the mortality daily for 6 weeks and % ILS was calculated, Using the following equations<sup>53,54</sup>

$$\text{MST} = (\text{Day of first death} + \text{Day of last death})/2$$

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

The antitumor activity of MT81 and its structural analogues were determined by change in ascites tumor volume, viable and nonviable tumor cell count, mean survival time (MST), and percentage-increased life span (% ILS).

**Study on normal peritoneal cells.**<sup>55</sup> Peritoneal exudate cells were collected after the abovementioned treatment schedule by repeated intraperitoneal wash with normal saline and counted in each of the treated groups and compared with the saline and vehicle control group.

**Study of hematological parameters.** Total red blood cell (RBC), white blood cell (WBC) counts and haemoglobin content were measured from freely flowing tail vein



blood.<sup>56,57</sup> Differential leucocyte count of WBC was done from Leishman-stained blood smears<sup>58</sup> of normal, EAC control, MT81, Aa-MT81-treated groups, respectively.

**Antioxidant parameters.** After the collection of blood samples, the mice were sacrificed. The liver of the mice were then excised, rinsed in ice-cold normal saline followed by cold 0.15 M Tris-HCl (pH 7.4), blotted dry, and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-HCl buffer; a portion was utilized for the estimation of malondialdehyde<sup>59</sup> and a second portion, after precipitating proteins with trichloroacetic acid, was used for the estimation of glutathione (GSH).<sup>60</sup> The rest of the homogenate was centrifused at 1,500 rpm for

15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase, catalase and protein.<sup>61-63</sup>

**Statistical analyses.** The experimental results were expressed as the mean ± SEM. The data were statistically analyzed by one-way ANOVA followed by Student's t-test when EAC Control and Vehicle Control are compared to Saline control; Treated groups are compared to Vehicle Control. p < 0.05, p < 0.01, p < 0.001 was considered significant.

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