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EUPATORIUM AYAPANA LEAF EXTRACTS ENHANCE ANTIOXIDANT POTENTIAL IN EHRLICH'S ASCITES CARCINOMA - BEARING SWISS ALBINO MICE

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ABSTRACT

Medicinal plants have gained significant importance in the potential management of cancer. *Eupatorium ayapana*, belonging to the Asteraceae, is therapeutically used as an antiseptic, antidysenteric, antibacterial and haemostatic agent. The present study was aimed to evaluate the antioxidant potential of ethanolic (EEEAL) and water extracts (WEEAL) of *Eupatorium ayapana* leaf in Ehrlich's ascites carcinoma-bearing Swiss albino mice. EEEAL, WEEAL were administered intraperitoneally at the dose level of 150 mg/kg b ody weight/day for consecutive 14 days after 24 hours of EAC cell inoculation (1×10^6 cell) to mice using 5-fluorouracil as standard drug. Treatment with the extracts decreased the levels of hepatic and renal malon-di-aldehyde (MDA), oxidized glutathione (GSSG) and increased reduced glutathione (GSH) level, catalase (CAT) and super oxide dismutase (SOD) activity in EAC-bearing mice. The study reveals that the extracts of *Eupatorium ayapana* leaf exhibit antioxidant activities in Ehrlich's ascites carcinoma-bearing Swiss albino mice.

Keywords: *Eupatorium ayapana;* Ehrlich's ascites carcinoma; Antioxidant potential; Glutathione.

1. INTRODUCTION

From ancient time Indians using plants as source of medicines. Despite Ayurveda other systems and medicines like Unani, Siddha also used plants as source of medicine. Nowadays active principles are being isolated and their activities are being studied scientifically throughout India and world. Eupatorium ayapana Vent (Family: Asteraceae) is one of the most important plants used in herbal medicine. It is an aromatic shrub, native of South America and has long been naturalized in India in other tropical countries as well (Gupta M et al. 2004). Chemical constitutions like 7-ethoxy coumarin (ayapanin), 6, 7-dimethoxy coumarin (ayapin); carotene, vitamin-C and stigmasterol have been isolated from its leaves (Bose PK and Roy AC, 1936). Five additional coumarins, viz. hydrangetin, daphnetin, daphnetin-7-methyl ether dimethyl ether, and umbelliferone have also been isolated (Chaturvedi R and Mulchandani NB, 1989). The leaves are helpful to protect the liver, inflammation of the urinary tract, tetanus, sore throat, cough and dyspepsia. The leaf juice is highly used against snakebite in the Brazilian Amazon and for ulcers. The juice of fresh leaves is also used for digestion (Bose PK and Sarkar BB, 1937). In Europe, 'Ayapana tea' which is prepaired from the dried leaves of the plant used as a tonic. It is a potent haemostatic. Traces of finely divided ayapanin or ayapin reduced blood coagulation time in rabbit (Bose PK and Sen PB, 1941). Eupatorium ayapana showed antimicrobial activity (Gupta M et al. 2002).The plant is hepato-protective and it has

antioxidant effect against carbon tetrachloride induced hepatotoxicity in rats (Bose P et al.2007). Several research work explored that reactive oxygen species (ROS), e.g., superoxide radicals, hydroxyl radicals, and hydrogen peroxide, have been considered as significant causative agents in some radical-mediated conditions including aging (Finkel T and Holbrook NJ, 2000), cardiovascular disease (Shireiqi I. 2000) and cancer (Cerutti PA. 1994; Frei B. 1995). Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection against infection and degenerative diseases. The use of antioxidant treatments is emphasized now by many medical practitioners and researchers as a key strategy for reversing or inhibiting the process of carcinogenesis (Shireiqi I. 2000). At present, scientists are interested in elucidating the role of several therapeutic modalities, currently considered as elements of complementary and alternative medicine, on the control of certain diseases especially cancer. In recent years plant derived natural products such as terpenoids, coumarins and sterols etc. have received considerable attention due to diverse pharmacological and health beneficial activity (De Feudis FV et al. 2003; Takeoka GR and Dao LT, 2003). World-wide level research is going on to search potent natural antioxidants, especially from plant sources, as nutritional supplements, health food, and/or phytomedicine. The present study was carried out to evaluate the antioxidant activities of ethanol and water extract of Eupatorium ayapana in Ehrlich ascites carcinoma (EAC) bearing mice. It may help to correlate why the plant is used in herbal medicine as an antitumor remedy for its inhibiting and scavenging role to free radicals.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Thiobarbutaric acid(TBA), Trichloro acetic acid(TCA), Sodium chloride(NaCl), Hydrochloric acid(HCl), Sulfosalicyclic acid(SSA), 2,4,4-dithionitrobenzoic acid(DTNB), Pyrogallol, Hydrogen peroxide(H_2O_2), Tris HCl, Potassium dihydrogen phosphate(KH_2PO_4) were purchased from Merck Ltd., SRL Pvt. Ltd., Mumbai, India. All other chemicals used were analytical grade and obtained from Merck Ltd., SRL Pvt. Ltd., Mumbai, India.

2.2. Plant materials collection and Extraction

Leaves of the *Eupatorium ayapana* collected from the district of Purba and Paschim Midnapore, West Bengal and authenticated (A voucher specimen No. SMC-3, Collection date 15.03.2011) by the Department of Botany, Vidyasagar University, West Bengal, India. The leaves were shade dried at room temperature. Then it was allowed to crush in an electric grinder and powdered. In 450 ml of solvent (ethanol or water) 250 gm of powder was suspended for 48 hours in room temperature. The extracts were then filtered through filter paper separately. The filtrates were concentrated with a rotary evaporator at 40^0C under low pressure. The concentrated filtrates were then poured in petridishes and were incubated at 37^0C for drying to afford crude water and ethanolic extracts of *Eupatorium ayapana* leaves.

2.3. Animals

Male Swiss albino mice (20-25 g) were collected. The mice were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C; humidity 55 ± 5%) with 12 hrs dark/light cycle. They were acclimatized to laboratory conditions for 10 days before the beginning of the experiment. The study was approved by the Institution's animal ethical committee.

2.4. Tumor cells

Ehrlich ascites carcinoma (EAC) cells were collected from Dept. of Biotechnology, Indian Institute of Technology (IIT), Kharagpur. The cells were maintained by intraperitoneal inoculation of $1x10^6$ cells/mice and developed a milky white fluid containing rounded tumor cells. Ehrlich ascites carcinoma (EAC) cells were harvested after 21days. The washed and viable cells free of containing RBC were taken in 0.9gm% NaCl solution for transplantation.

2.5. Antioxidant studies

Thirty male albino mice were divided into five groups each group containing six animals. Washed and viable EAC cells were resuspended in normal saline and inoculated (0.2 ml of 1×10^6 cells/mouse) to animals of all groups intraperitoneally except the saline control group. After 24 hrs (from the second day), 5ml/kg/day of normal saline were administered in group 1(Saline- control) and group 2 (EAC-control) respectively. Standard drug 5-

fluorouracil (5 mg/kg), ethanolic and water extract of *Eupatorium ayapana* leaf (150mg mg/kg/day) were administered intraperitoneally in groups 3, 4, 5, respectively for subsequent 14 days. After the last dose and 18 hr fasting, all the mice were sacrificed for the estimation of malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG), Catalase (CAT), super oxide dismutase (SOD) level of kidney and liver (MaitiChoudhury S et al. 2010).The liver and kidney 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 used for the estimation of malondialdehyde (Ohkawa H et al. 1979), and a second portion, after precipitating proteins with trichloroacetic acid, was used for the estimation of oxidized and reduced glutathione(Grifth Mindr P. 1998). The rest of the homogenate was centrifuged at 1,500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (Marklund S and Marklund G, 1974), catalase (Aebi H. 1983) and protein (Lowry OH et al. 1951).

2.6. Statistical analysis

The experimental results were expressed as the Mean ± Standard error of mean (SEM). Statistical analysis of the collected data were done by Analysis of variance (ANOVA) followed by Student's t-test. Difference was considered significant when $p<0.05$.

3. RESULTS

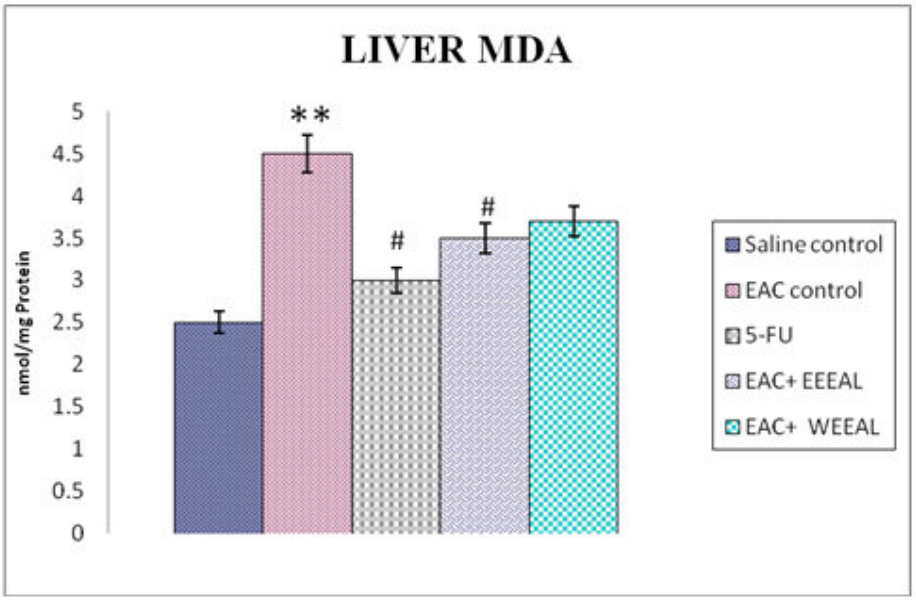

Figure 1

shows the effect of EEEAL and WEEAL on Liver MDA in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control and probability values are given in * and # respectively. # indicates significant difference at p<0.05; ** indicates significant differenence at p < 0.01.

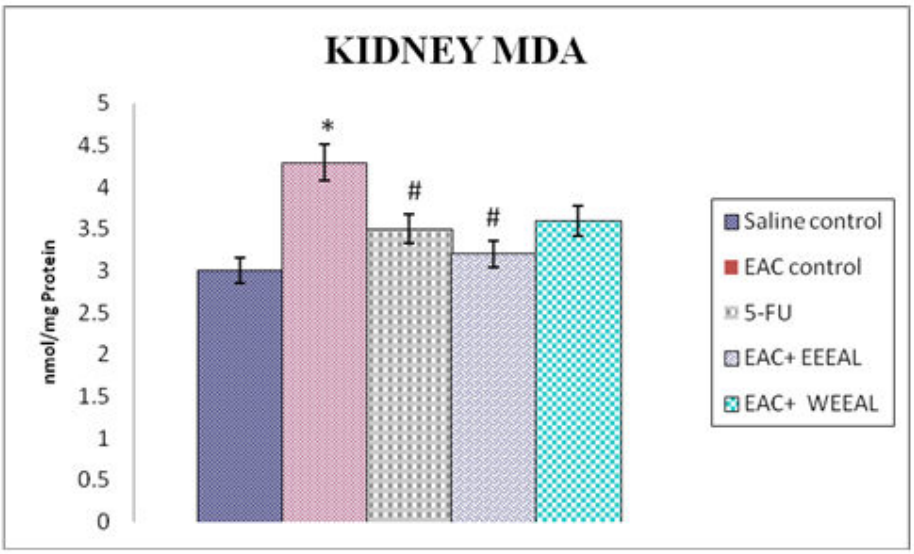

Figure 2

shows the effect of EEEAL and WEEAL on Kidney MDA in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control and probability values are given in * and # respectively. *, # indicates significant difference at p< 0.05.

Figure 1 and 2 shows the effect of the ethanolic (EEEAL) and water (WEEAL) extracts of *Eupatorium ayapana* leaf on the hepatic and renal malon-di-aldehyde (MDA) content in EAC-bearing mice respectively. Hepatic and renal MDA levels were decreased in EEEAL and WEEAL-treated groups. Hepatic MDA was decreased more significantly than renal MDA in the extracts-treated mice.

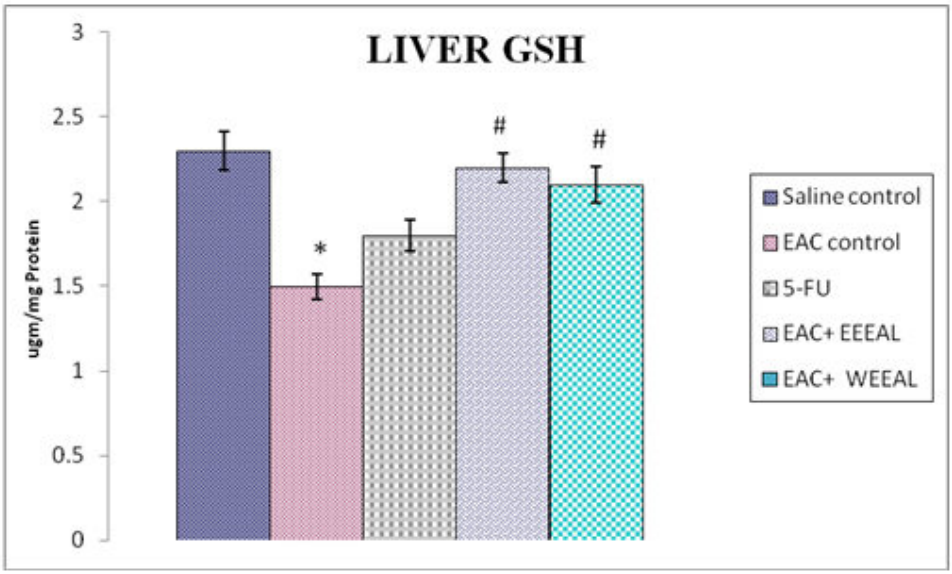

Figure 3

shows the effect of EEEAL and WEEAL on Liver GSH in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control and probability values are given in * and # respectively. *, # indicates significant difference at p < 0.05.

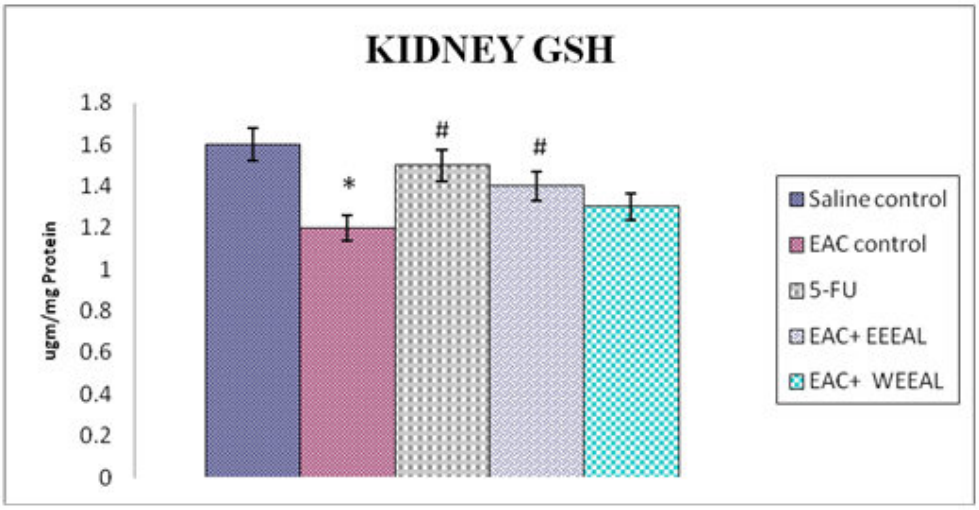

Figure 4

shows the effect of EEEAL and WEEAL on Kidney GSH in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control and probability values are given in * and # respectively. *, # indicates significant difference at p < 0.05.

The Hepatic and renal glutathione (GSH) level (Figure 3, 4) were reduced significantly in EAC-control group. From the figure 3 and 4, it is seen that renal GSH level has been restored more significantly than hepatic GSH level.

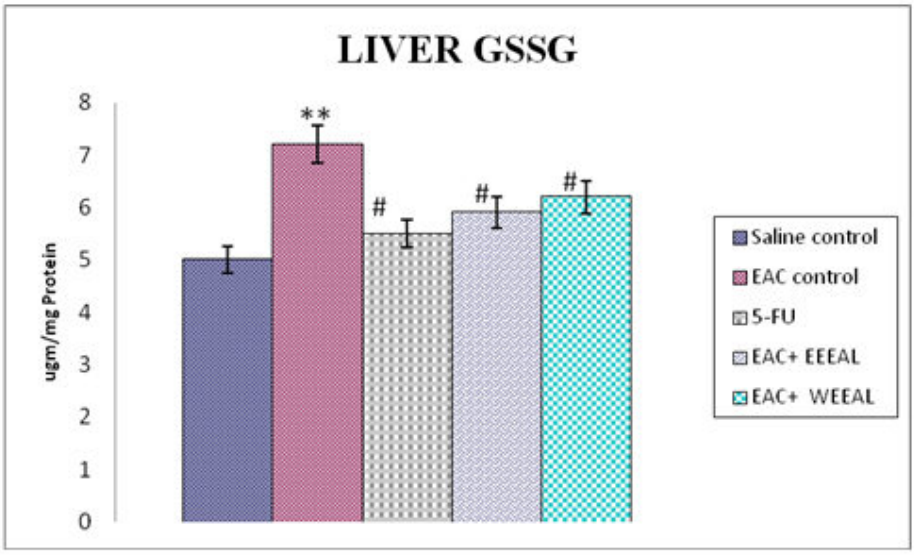

Figure 5

shows the effect of EEEAL and WEEAL on Liver GSSG in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control and probability values are given in * and # respectively. # indicates significant difference at p < 0.05; ** indicates significant differenence at p < 0.01.

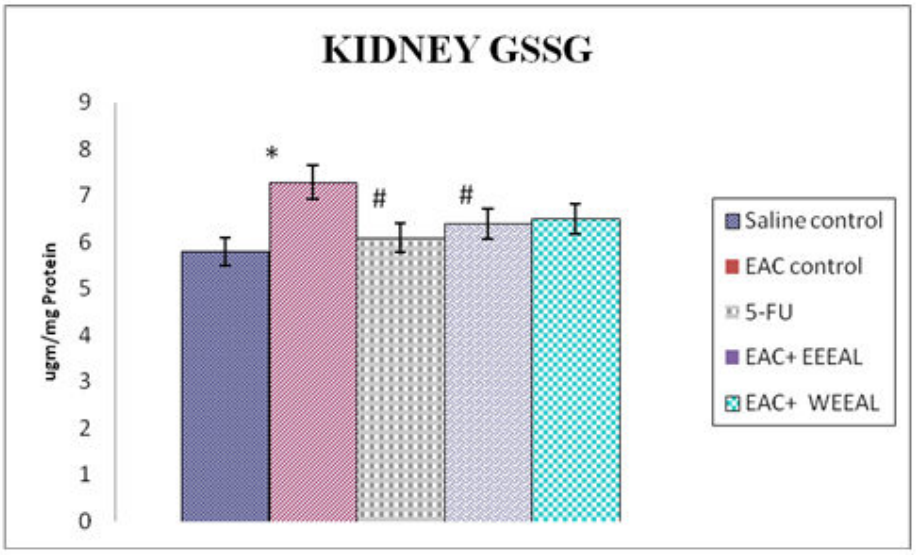

Figure 6

shows the effect of EEEAL and WEEAL on Kidney GSSG in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control and probability values are given in * and # respectively. *, # indicates significant difference at p < 0.05.

Figure 5 and 6 shows the effect of EEEAL and WEEAL on the hepatic and renal GSSG levels respectively in EAC-bearing mice. Hepatic and renal GSSG levels were increased in EAC-control group whereas those were reduced in the extracts-treated groups (Figure 5 and 6).

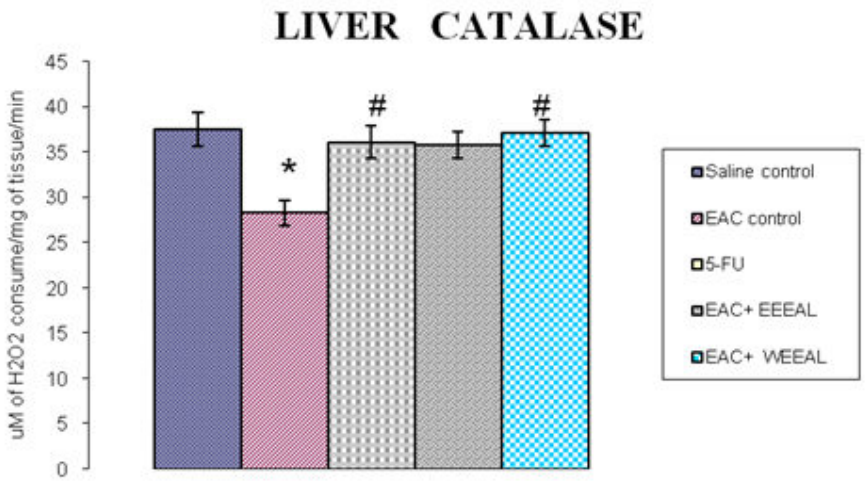

Figure 7

shows the effect of EEEAL and WEEAL on Liver Catalase in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control. Probability values are given in *and #. *, # indicates significant difference at p < 0.05.

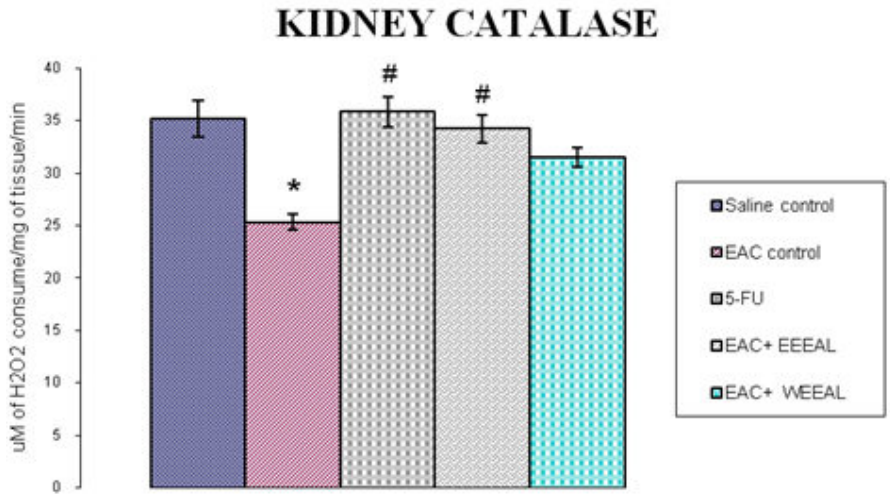

Figure 8

shows the effect of EEEAL and WEEAL on Kidney Catalase in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control. Probability values are given in *and #. *, # indicates significant difference at p < 0.05.

Figure 7 and 8 shows the effect of the ethanolic and water extracts of *Eupatorium ayapana* leaf on the activity of hepatic and renal catalase (CAT) in EAC-bearing mice respectively. The activity of hepatic and renal catalase (CAT) was increased in the extracts-treated groups and hepatic catalase (CAT) activity was restored more significantly than renal catalase (CAT) activity. In our study, the activity of hepatic and renal super oxide dismutase (SOD) was decreased in EAC-control group but it was elevated in EEEAL and WEEAL-treated groups (Figure 9, 10).

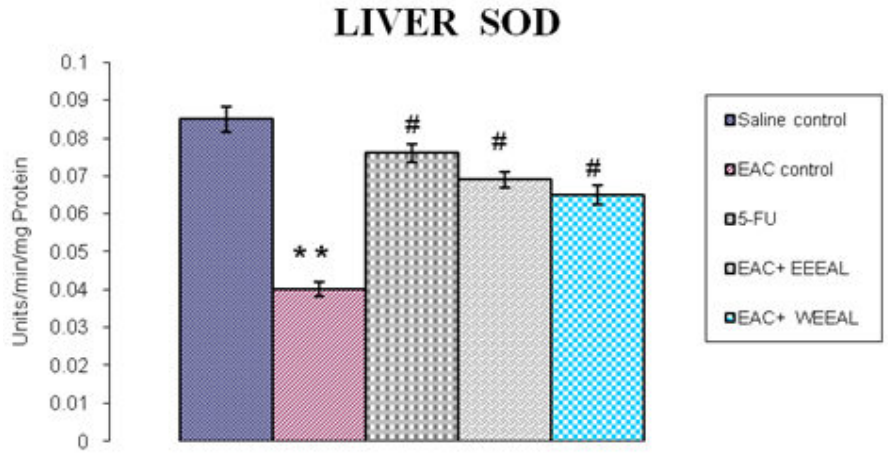

Figure 9

shows the effect of EEEAL and WEEAL on Liver SOD in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control. Probability values are given in *and #. # indicates significant difference at p < 0.05; ** indicates significant differenence at p < 0.01.

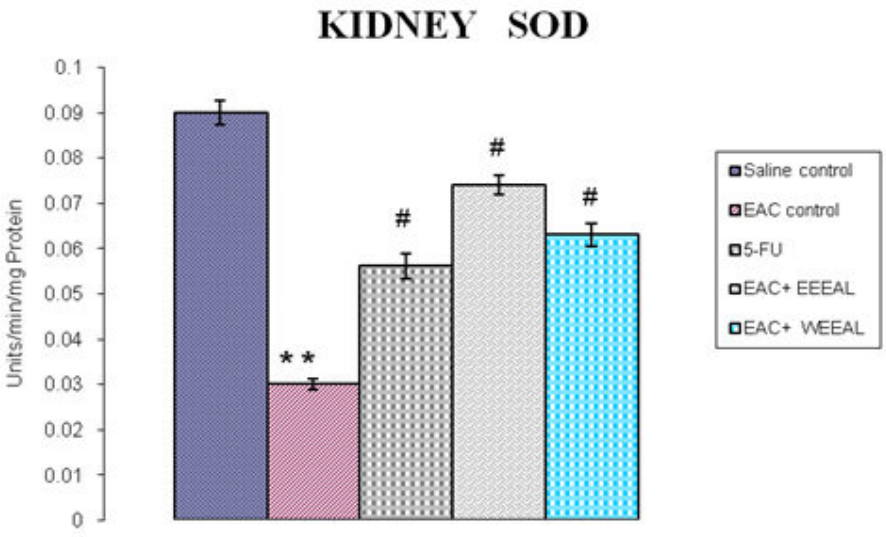

Figure 10

shows the effect of EEEAL and WEEAL on Kidney SOD in EAC-bearing mice. Results are expressed as Mean ± SEM. EAC-control is compared to Saline-control; Treated groups are compared to EAC-control. Probability values are given in *and #. # indicates significant difference at p < 0.05; ** indicates significant differenence at p < 0.01.

4. DISCUSSION

The present study was carried out to evaluate the antioxidant role of ethanolic (EEEAL) and water (WEEAL) extracts of *Eupatorium ayapana* leaf in Ehrlich ascites carcinoma (EAC) bearing mice. The EEEAL and WEEAL prepared from *Eupatorium ayapana* decreased the liver and kidney MDA and GSSG level in EAC-bearing mice. Simultaneously EEEAL and WEEAL increased hepatic and renal GSH, catalase and SOD activity. The tumor-bearing condition is also said to be under oxidative stress. This oxidative stress is related with active oxygen production by tumor cells and also due to abnormal oxidation-reduction control. Oxidative stress has been involved in many diseases, more as a consequence of the pathology than as the causative factor (Halliwell and Barry, 2005). In oxidative stress, lipid peroxidation is occurred due to excessive free radicals production and is considered a primary mechanism of cell membrane destruction and cell damage. Malondialdehyde (MDA) is the end product of lipid peroxidation. It is present at higher concentration in the carcinomatous tissue than normal tissue (Yagi K et al. 1991). In this study MDA level is increased in tumor control group. But ethanolic (EEEAL) and water (WEEAL) extracts of *Eupatorium ayapana* leaf decreased the levels of lipid peroxidation (MDA) in EAC-bearing mice. GSH plays a major role in protecting the cell against oxidative damage by reacting with ROS. In healthy human cells normally ~98% of the total GSH exists in the reduced form while a much smaller fraction (~1%) exists in the oxidized forms – GSSG. (Griffith OW. 1981; Dringen R and Hirrlinger J, 2003; Kennett E et al. 2005). When GSH is oxidized to glutathione disulphide (GSSG), GSH reductase, an enzyme (GR; EC 1.6.4.2) rapidly reduces it back to GSH, thus ensuring that the cycling of ROS does not alter the GSH to GSSG concentration ratio of ≥ 100 (Griffith OW. 1999; Griffith OW and Mulcahy RT, 1999).When the oxidative stress in cell reaches the high level in different diseases, a shift in redox buffer can occur, which results in oxidative damage to cellular constituents especially the vulnerable plasma membrane. (Kennett EC and Kuchel PW, 2006). In cancerous cells, GSH is readily oxidized into the GSSG and so, there is high level of GSSG present in the cancer cells. In this study, it is noted that treatment with EEEAL and WEEAL significantly decreased the levels of GSSG but increased the level of GSH in EAC-bearing mice compared to the EAC control group mice. Superoxide dismutase

(SOD) is involved in the clearance of superoxide and catalase (CAT) catalyzes the disproportion of hydrogen peroxide (H_2O_2). The inhibition of SOD and CAT activities is occured as a result of tumor growth (Sun Y et al. 1989). In the present investigation, decreased levels of SOD, catalase levels were observed in EAC-bearing mice. After the treatment of ethanolic (EEEAL) and water (WEEAL) extracts of *Eupatorium ayapana* leaf, the activities of these enzymes were significantly increased. The implication of free radicals in tumors is well known (Feng Q et al. 2001). The free radical hypothesis supports the fact that the antioxidants effectively inhibit the tumor. The present study demonstrated that EEEAL and WEEAL decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in EAC tumor bearing mice and the observed properties may be attributed to the antioxidant principles present in the plant extract. *Eupatorium ayapana* leaves exhibited the antioxidant and free radical scavenging property, which is one of the most essential properties of any anticancer chemotherapy drug.

5. CONCLUSION

From the above discussion it can be concluded that *Eupatorium ayapana* leaf is a potent antioxidant natural remedy against Ehrlich ascites carcinoma (EAC)-bearing Swiss albino mice and viewing the overall result it can be concluded that among the two extracts ethanolic extract is more effective.

DECLARATION OF INTEREST

Authors declare that there are no conflicts of interests.

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