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CYPERUS ROTUNDUS EXTRACT EXERTS ANTICANCER ACTIVITY ON EHRLICH ASCITES CARCINOMA

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ABSTRACT

Introduction: Cancer is one of the most serious health concerns in both developing as well as the developed countries. Despite the advancements in the treatment modalities, mortality rates due to cancer are alarming. Adverse effects of chemotherapy and radiation therapy demands the search for alternative therapies, among which phytotherapy looks promising. ***Aims and objectives:*** This study was designed to evaluate the anticancer efficacy of *Cyperus rotundus* extracts on experimentally induced Ehrlich ascites carcinoma. ***Materials and Methods:*** *Cyperus rotundus* rhizomes were extracted in ethanol and water by maceration method. Ehrlich ascites carcinoma was induced in swiss albino mice by intraperitoneal injection of $2.5x10^6$ cells /mice viable tumor cells. Animals were treated with *Cyperus rotundus* extract per orally with 250 mg/kg and 500mg/kg body weight dosages. Mean survival time of the animals was recorded and compared with that of tumor control. A subpopulation from all the treatment groups was sacrificed and analyzed for hematological and biochemical parameters as per standard protocols. ***Results and Conclusion:*** *Cyperus rotundus* extract could increase the life span by 36% and 20% in groups treated with 250 mg/kg wt. and 500 mg/kg body wt. respectively. Hematological parameters were restored to normal levels in extract administered groups. Antioxidant activity assay indicated that the extract treatment could increase the levels of antioxidant enzymes. In conclusion, *Cyperus rotundus* ethanolic extract possessed potent anticancer activity against Ehrlich ascites carcinoma in *Mus musculus* system.

KEYWORDS: Anticancer, *Cyperus rotundus*, Mean survival time, Ehrlich ascites carcinoma.

INTRODUCTION

Cancer is the second most leading cause of death while heart disease being the first in the world.[1] Nearly 30% of deaths from cancer are due to high body mass index, low intake of fruits and vegetables, lack of physical activity, use of tobacco/alcohol and environmental effects. In developing countries, mortality rates are higher due to lack of healthcare facility and greater exposure to carcinogens. By 2020, world's total population is expected to have 15 million new cancer cases with about 12 million deaths due to cancer.[2] It is believed that the number of cancer patients dying will rise in future in both the developing as well as developed countries up to 70%.[3]

Despite of several advancements in the treatment modalities for cancer, mortality rates are still alarming. Cytotoxic drugs, chemicals, radioactivity, toxins and other substances which are used in cancer treatment impart innumerous physical damage as well as long-term side effects in the body. Thus, there is a need to establish safer alternative therapeutic strategies.

Ehrlich's ascites carcinoma (EAC) is a form of spontaneous breast cancer in female mouse[4] and is being used as a subcutaneously transplantable tumor in the mouse system. EAC is referred to the undifferentiated carcinoma, which is hyperdiploid in nature. It has the characteristics of rapid proliferation, high transplantation, short life-span, 100% malignancy with no tumor specific transplantation antigen.[5]

Plant products, due to their antioxidant property can modulate the physiology as well as metabolism effectively to significantly reduce the amount of free radicals and reactive oxygen species generated at tumor site. Due to this activity, they are even shown to regulate gene expression, differentiation of oncogenes as well as of genes involved in cell cycle, apoptosis, immune regulation and hormone metabolism. Due to the medicinal property of the phytochemicals, they are used during or after cancer therapy to neutralize and damage harmful consequences imposed by cancer therapeutics. Use of phytochemicals is known to possess long-term health benefits. Nature gives a great deal of effective anti-cancer agents such as dactinomycin and doxorubicin

derived from microorganisms and vinblastine, irinotecan, topotecan, vincristine and taxanes derived from plants which are used frequently in recent years. The herbs like *Brassica oleracea*, *Camptotheca*, *Catharanthu*s and *Podophyllum* are useful in the cancer treatment for many decades.[6]

Cyperus rotundus is an herb belonging to the family *Cyperaceae.* The oil extracts of *C.rotundus* were more widely used in ancient medicine for various kinds of health problems like stomach problems, constipation, fever, tooth problems and digestive disorders, as antispasmodic and control of menstrual irregularities.[7] Later, the wide use of *C. rotundus* phytochemical extract for mosquito repelleing, insecticidal, antibacterial, antimalarial, antimutagenic, anti‑diarrheal activity, anti‑spasmodic activity, antioxidant activity, antiepileptic effect, therapeutic uses for cardiovascular diseases, anti-cholesterol and wound healing activity was shown in various studies.[7,8,9,10,11,12,13,14] In further studies anti-carcinogenic activity, chemo-preventive activity, anti-proliferative activity against K562 erythroleukemia cells and apoptotic activity was shown from flavonoid extracts of *C. rotundus*.[15,16,17,18] This investigation was designed to assess the anticancer activity of *C. rotundus* ethanolic and aqueous extracts in Swiss albino mice induced with EAC.

MATERIALS AND METHODS

Plant material and extraction

Dried rhizomes of *C.rotundus* were collected from a local Ayurvedic pharmacy in Mangalore, Karnataka, India. The plant material was authenticated by Dr. Sunil Kumar, Senior Research officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi and voucher specimen (No.11110101) was deposited in the plant repository of SDM Research Center. The shade dried rhizomes of the *C.rotundus* were coarsely powdered and preserved at 4^{o} C for further studies.

Ethanolic and aqueous extracts of *C. rotundus* rhizome powder was prepared by maceration with the respective solvents for 48 h. The extracts were filtered and concentrated.

Animals

Eight to ten week old Swiss albino female mice, weighing 25 ± 5 g, selected from an inbred colony maintained in the Central Animal Research Facility of Manipal University were used for the experiments. Mice were housed in polypropylene cages (4 per cage) in an air conditioned room maintained at a comfortable temperature (23 ± 2°C) with a 12 h light-dark cycle. They were fed with standard feed pellets and tap water *ad libitum.* The study protocols were approved by the Institutional Animal Ethical Committee (IAEC) and were conducted according to the guidelines of CPCSEA (No: YU-IAEC/4/25/8/2011).

Cells and maintenance

EAC cells were obtained from Amala Cancer Research Center, Amala Nagar, Thrissur, Kerala, India. They were maintained and propagated by serial intraperitoneal inoculation (2 x10^{6}cells /mouse) in an aseptic environment. Cells propagated for 12-14 days were used for the experiments.

Acute toxicity studies

In vivo acute oral toxicity studies of the extracts were carried out as per OECD guidelines - 425[19] to explore the acute dose lethal to 50% of the animals thereby establishing the therapeutic index. The animals were fasted overnight and then were administered orally with a starting dose of 2000 mg/kg body weight of *C. rotundus* extract. After dosing, the animals were observed for 3 h and monitored upto 14 days for any mortality, behavioural changes, autonomic nervous system and central nervous system changes. The observations were recorded according to the specifications of Irwin's table.

Antitumor activity in EAC model

Swiss albino mice were divided into 7 groups (n = 6). A known number of viable EAC cells (2.5 x 10^{6}cells/mice) were injected intraperitoneally into all the groups in an aseptic condition except for the normal control group. The day of tumor inoculation was considered as day zero.[20]

Group 1 served as normal control where animals were fed with 5 ml/kg body weight of normal saline.

Group 2 animals received only tumor cells and served as tumor control.

Group 3 animals were injected with tumor cells and injected with standard drug Cisplatin (single dose of 3.5 mg/kg, *i.p.)* on day 1 which served as positive control.

Group 4 tumor-bearing mice received ethanol extract of *C.rotundus* 250 mg/kg body weight. Group 5 tumor-bearing mice received ethanol extract of *C.rotundus* 500 mg/kg body weight. Group 6 tumor-bearing mice received aqueous extract of *C.rotundus* 250 mg/kg body weight. Group 7 tumor-bearing mice received an aqueous extract of *C.rotundus* 500 mg/kg body weight. The extracts were dissolved in CMC 0.25% (carboxymethyl cellulose) daily just prior to the dosage and administered orally on days 1, 3, 5, 7, 9, 11 and 13 of tumor inoculation.

All the experimental animals were observed for the development of ascitic tumor. On the 15th day, 3 animals from all the groups were sacrificed by administering euthanizing agent thiopental sodium (300mg/kg). Remaining animals were observed up to 45 days with food and water *ad libitum* to check the mean survival time (MST) and percentage increase in mean life span (% IMLS). After sacrificing the animals, blood was withdrawn by cardiac puncture to evaluate the hematological parameters. Liver was collected from all the animals for estimation of *in vivo* antioxidants.

The antitumor activity of the extracts was measured in EAC animals with respect to following parameters like Mean survival time (MST), the percentage increase in mean life span (% IMLS).

Hematological parameters

On the 15th day of the tumor inoculation, blood was withdrawn by cardiac puncture and hematological parameters like total WBC count, total RBC count and hemoglobin were assessed following the standard procedures.[21]

Antioxidant assays

Preparation of tissue homogenate

Animals were sacrificed by cervical dislocation and were perfused transcardially with ice-cold saline. Liver was perfused *in situ* with ice-cold saline, dissected out, blotted dry and immediately weighed. 10% liver homogenate was prepared in ice-cold KCl (150mM) using Teflon – glass homogenizer (Yamato LSG LH-21, Japan). The homogenate was centrifuged at 10,000 rpm for 10 min and the pellet was discarded. The supernatant was again centrifuged at 20,000 rpm for 1 h at 4°C. The supernatant obtained was used for determining lipid Peroxidation[22], reduced glutathione[23] and catalase[24] activity.

Lipid Peroxidation activity

Tissue homogenate was treated with 10% TCA and the mixture was kept at room temperature and then centrifuged at 3000 g for 10 min to separate proteins. Supernatant was added with thiobarbituric acid followed by heating at 95°C for 60 minutes to generate malondialdehyde. Absorbance of the samples was measured at 532 nm using Beckman DU 64 spectrophotometer. The levels of lipid peroxides were expressed as nM of MDA/mg wet tissue.

Reduced Glutathione (GSH)

Tissue homogenates were treated with 0.02 M EDTA solution and kept on ice bath for 10 min. 2 ml of distilled water and 0.5 ml of 50% TCA were added. This mixture was kept on ice for 10-15 min and then centrifuged at 3000 g for 15 min. To the supernatant, 2.0 ml of Tris buffer was added followed by the addition of Ellman's reagent. OD was recorded at 412 nm.

Catalase (CAT)

Catalase activity was measured based on the ability of the enzyme to break down hydrogen peroxide. 10 µl samples were taken in tubes containing 3.0 ml of H_2O_2 in phosphate buffer. The time required for 0.05 optical density changes was observed at 240 nm against a blank containing the enzyme source in H_2O_2 free phosphate buffer. Absorbance was recorded at 240 nm and after the addition of enzyme; Δt was noted till OD was 0.45. Reading was taken at every 3 second interval. CAT activity was expressed as moles of H_2O_2 consumed/min.

Statistical analysis

Data were expressed as Mean ± SEM. Statistical analysis was carried out using one way ANOVA (Graph Pad Version 5.02, Instat Software) followed by Dennett's post hoc test. The values ***p<0.05 was considered as statistically significant, ****p<0.01 as highly significant and *****p<0.001 as extremely significant.

RESULTS

Effect of C. rotundus on mean survival time (MST) in EAC inoculated mice

In EAC inoculated control mice, MST was found to be 15.17 days, while median survival time was 15 days. In Cisplatin treated mice, a significant increase in MST (35.33) and % IMLS (133) was observed. Among the treatments, ethanol extract at 250 mg/kg showed a maximum increase in percentage life span (36%). Median survival time for Cisplatin, ethanol and aqueous extracts at 250 and 500 mg/kg was found to be 31, 23, 18, 17 and 16 days, respectively (Fig.1 & Fig.2). Fig. 3 depicts the effect of *C. rotundus* rhizome extracts on the Kaplan Meier's estimate of survival of EAC-bearing mice. After EAC inoculation (2.5 x 10^6 cells/mouse, i.p.), mice were treated with aqueous and ethanol extracts at two different concentrations and a single dose of Cisplatin (3.5 mg/kg, *i.p.*).

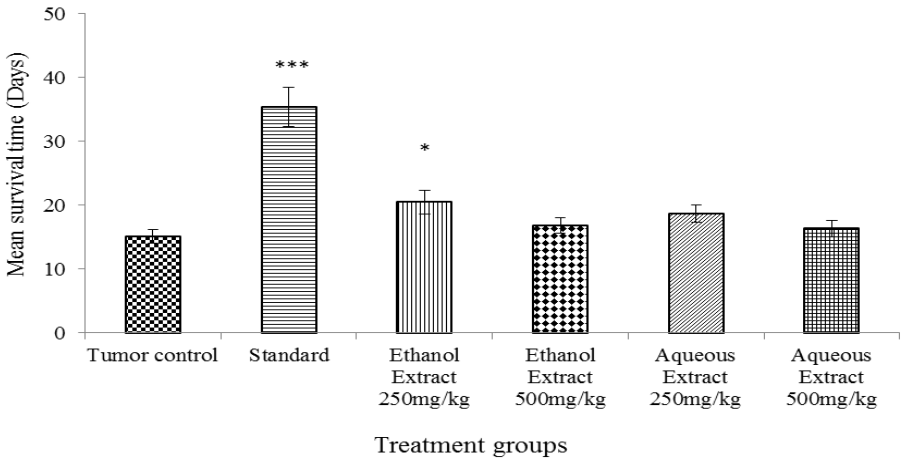

Fig. 1: Effect of *C. rotundus* rhizome extracts on MST of EAC induced mice

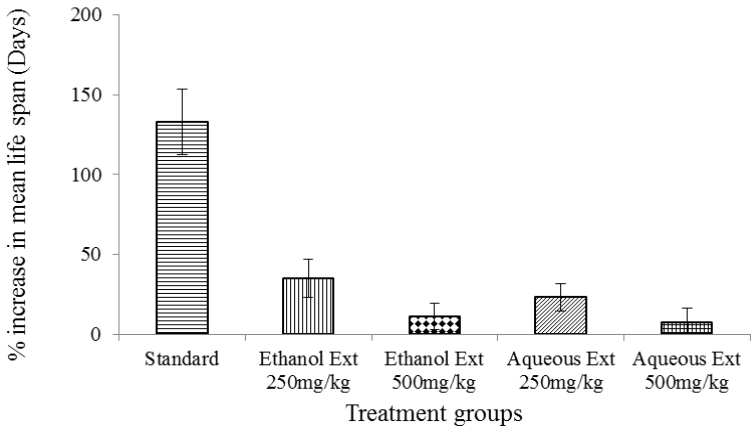

Fig. 2: Effect of *C. rotundus* rhizome extracts on the percentage increase in mean life span (% IMLS) of EAC inoculated mice

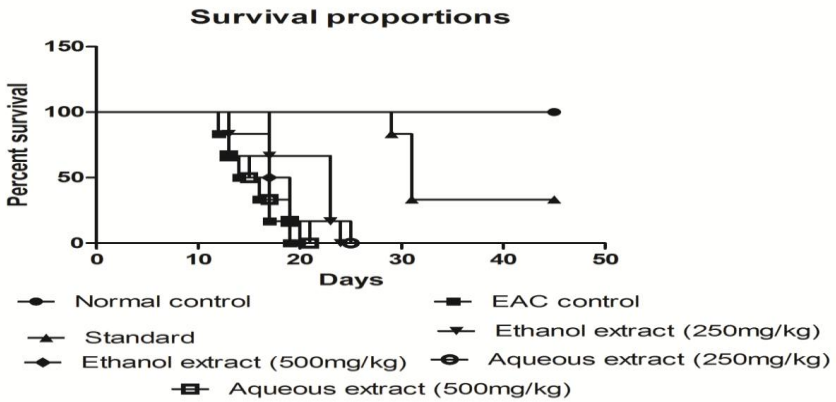

Fig. 3: Effect of *C. rotundus* rhizome extracts on the Kaplan Meier's estimate of survival of EAC bearing mice

Effect on tumor volume

Cisplatin treated group showed a significant reduction in the tumor volume. Tumor volume was significantly decreased in all the treatment groups except aqueous extract at 500 mg/kg dose (Fig. 4).

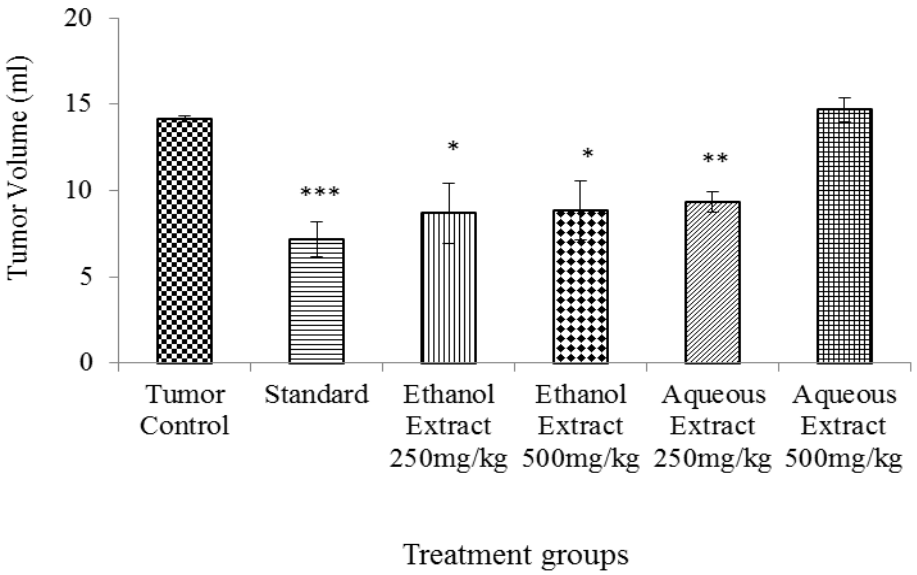

Fig. 4: Effect of *C. rotundus* rhizome extracts on the tumor volume in EAC inoculated mice

Effect of C. rotundus extracts on hematological parameters in EAC inoculated mice

RBC count was significantly increased in the standard (Cisplatin) group compared to EAC control group. Ethanol extract at both dosage levels (250 mg/kg and 500 mg/kg) showed a statistically significant increase (**p < 0.01) in the RBC count when compared to tumor control, whereas in aqueous extract treated group, there was no statistically significant elevation in RBC count (Fig. 5A).Hemoglobin level was reduced in EAC control group significantly (**p <0.01). In Cisplatin treated group, there was significant (***p < 0.001) reduction in hemoglobin level when compared to tumor control. Ethanol extract at 250 mg/kg showed a significant (*p < 0.05) elevation. Aqueous extract at both dosage levels showed a significant increase in Hb% when compared to tumor control (Fig.5B).

WBC count increased more than twofold in control EAC inoculated mice, which was significantly (**p < 0.05) reduced by Cisplatin treatment. Ethanol extract at 500 mg/kg prevented the rise in WBC count significantly (**p < 0.01). Aqueous extract at two dosage levels induced an increase in WBC count which is not statistically significant when compared to EAC control animals (Fig.5C).

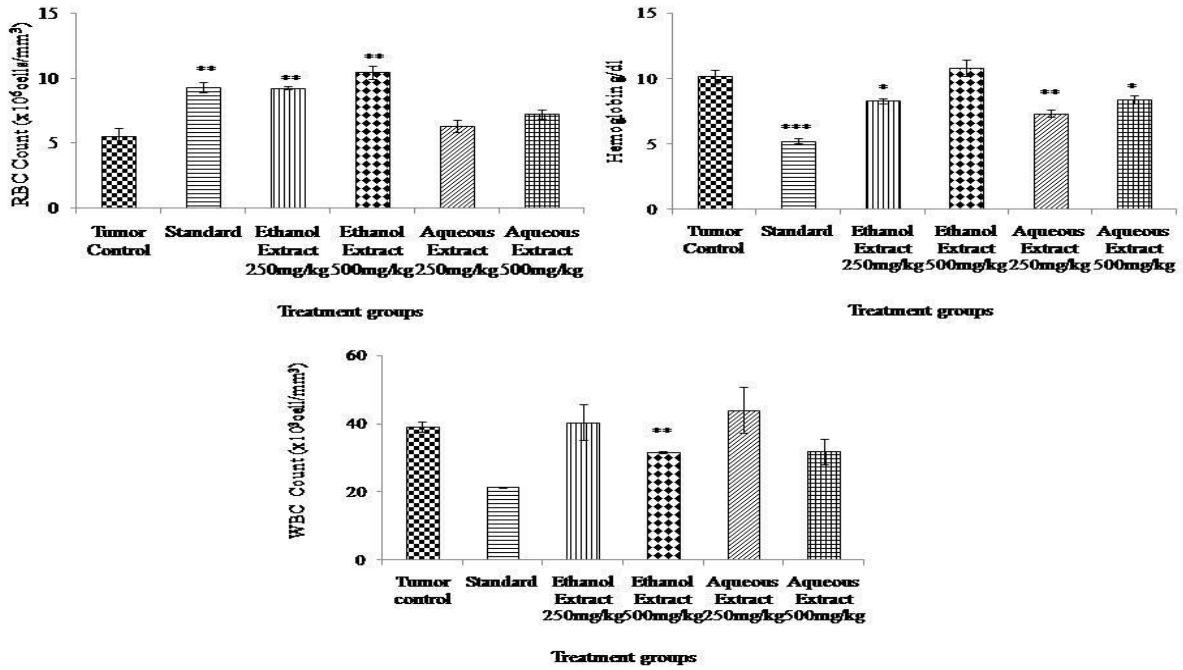

Fig. 5: Effect of *C. rotundus* extracts on haematological parameters of tumor inoculated mice

Effect of C. rotundus extracts on antioxidant parameters of EAC inoculated mice

Fig.6A contains data related to the effect of *C. rotundus* rhizome extracts and reference standard Cisplatin on lipid peroxidation in liver homogenate from different groups. In Cisplatin treated group, extremely significant (***p < 0.001) decrease in the lipid peroxidation was observed in comparison to EAC control group. Ethanol extract administered at 250 & 500 mg/kg dosages and aqueous extract at 500 mg/kg dose showed highly significant (**p < 0.01) reduction in lipid peroxidation when compared to EAC control group.

Modulations in the glutathione peroxidase activity by *C. rotundus* extracts is represented in fig. 6B. In Cisplatin treated group, a remarkable increase was observed in comparison to EAC control. However, the observed result was statistically non-significant. Ethanol and aqueous extracts administered at 250 and 500 mg/kg doses increased the glutathione peroxidase activity in comparison to EAC control group.

Fig. 6C depicts the changes in catalase activity in different treatment groups of tumor inoculated mice. In Cisplatin treated group, a remarkable increase was observed in comparison to EAC control (***p < 0.001) group. Ethanol extract at both dosage levels and aqueous extract at 500 mg/kg dose were also found to increase catalase activity significantly (***p < 0.001) in comparison to EAC control group.

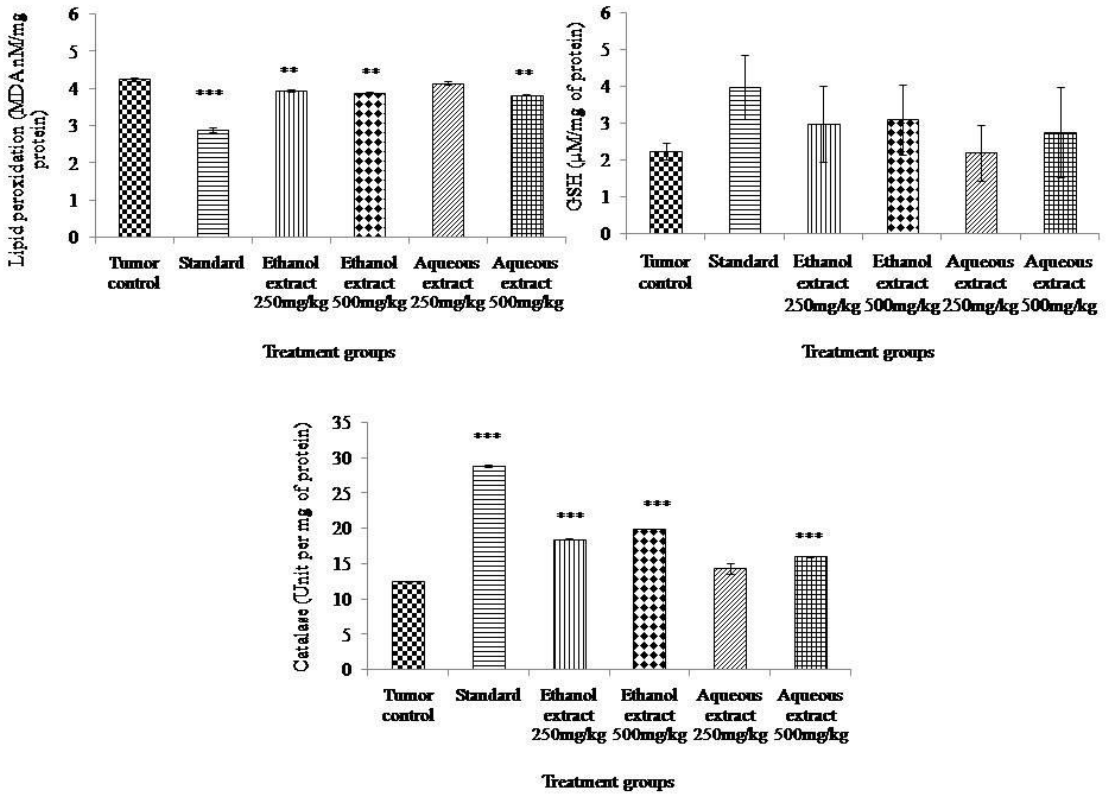

Fig. 6: Effect of *C. rotundus* extracts on antioxidant status of tumor inoculated mice

DISCUSSION

The confirmatory explanation for the anti-neoplastic property of ethanol extract of *C.rotundus* was provided by the percentage increase of the mean life span (% IMLS) in comparison with the standard drug cisplatin. Use of cisplatin in phase-II trials of cancer studies is reported previously.[25] The study of percentage survival, which was done using Kaplan Meier's estimate in EAC-bearing mice treated with ethanol extract of *C.rotundus* in comparison with cisplatin also demonstrated the maximum survival in the EAC bearing mice. Kaplan Meier's method is supposed to be the best method for analysis of survival.[26]

A decrease in tumor volume was also documented after 45 days of *C.rotundus* extract treatment in EAC-bearing mice. Along with all the improvements in physical features, it was interesting to note the improvement in hematological parameters after the treatment with ethanol extract of *C.rotundus.* Effect of phytochemicals on EAC cell lines manifested at the level of hematological and histopathological parameters has been documented in different studies.[27,28] Ethanol extract of *C.rotundus* could cause significant increase in the RBC count and hemoglobin levels in the EAC-bearing mice equivalent to normal levels with only exception of the aqueous extracts of *C.rotundus*, which could not make any increase in the RBC counts.

Antioxidants provide resistance against oxidative stress by modulation of many biochemical processes among which lipid peroxidation, glutathione peroxidase (GSH) activity and catalase activity are the major ones.[29,30] Inhibition of lipid peroxidation by *C.rotundus* extracts has been shown in rat liver homogenate[29] and splenocyte functions in mice[31] earlier. Similarly, in the present study both the ethanol and aqueous extracts of *C.rotundus* (except the aqueous extract at 250 mg/kg) exhibited a significant reduction in lipid peroxidation and increase in catalase activity in the EAC bearing mice. With regard to GSH activity, treatment with *C.rotundus* extracts resulted in increased GSH activity in EAC bearing mice, however, the increase was nonsignificant statistically. The reason of this nonsignificant increase needs to be reproved before consideration or may require more data points for broader analysis.

CONCLUSION

The present study demonstrates the efficient anti-neoplastic properties of ethanol and aqueous extracts of *C. rotundus* at doses of 250 mg/kg or 500 mg/kg. The results of this study showed that the ethanol extract of *C.rotundus* has more therapeutic effects over the aqueous extract. The results of the study bear its high relevance due to the fact that this herb is available locally and may represent a convenient and cost- effective cancer therapeutic after appropriate validation in clinical system.

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REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics.CA Cancer J Clin. 2007; 57: 43-66.
2. Brayand F, Moller B. Predicting the future burden of cancer. Nat Rev Cancer. 2006; 6: 63–74.
3. Imran A, Waseem A, Wani, Kishwar S. Cancer scenario in India with future perspectives. Cancer Therapy. 2011; 8: 56-70.
4. Aktaş E. Ehrlich Asit Sıvısının L-Hücrelerinin Çoğalma Hızına Etkisi. Yüksek Lisans Tezi. İstanbul Üniversitesi Fen Bilimleri Enstitüsü. İstanbul, 1996.
5. Kaleoğlu Ö, İşli N. Ehrlich-Lettre Asit Tümörü. Tıp Fakültesi Mecmuası 1997; 40: 978–84.
6. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol. 2005; 100(1–2): 72–79. doi:10.1016/j.jep.2005.05.011. PMID 16009521.
7. Puratchikody A, Nithya C, Nagalakshmi G. Wound healing activity of *Cyperus rotundus* Linn. Indian J Pharm Sci. 2006; 68: 97-101.
8. Chandratre RS, Chandarana S, Mengi SA. Lipid lowering activity of alcoholic extract of *Cyperus rotundus.* Int. J. Res. Pharm.Chem. 2011; 1(4): 1042-045.
9. Eun JS, Dong L, Jong HK, Sun ML, Yeong SK, Yi J. Antiplatelet effects of *Cyperus rotundus* and its component (+)-nootkaton. J Ethnopharmacol. 2011; 135: 48–54.
10. Majid NZA, Jabier MSJ, Wagi RI, Kareem HHAA. Extraction, identification and antibacterial activity of *Cyperus* oil from Iraqi *C. rotundus* Eng. & Technol. 2008; 26: 1.
11. Mohsen K, Zahra K, Mehrdad R, Azizi Y. Anticonvulsant and antioxidant effect of hydroalcoholic extract of *Cyperus rotundus* rhizome on pentylentetrazole induced kindling model in male mice. Med Plants Res. 2011; 5: 1140-146.
12. Shamkuwar PB, Hoshamani AH, Gonjari ID. Antispasmodic effect of *Cyperus rotundus* L. (Cyperaceae) in diarrhea. Scholars Res Library. 2012; 4: 522-24.
13. Imam H, Zarnigar, Sofi G, Seikh A, Lone A. The incredible benefits of Nagarmotha (*Cyperus rotundus*). Int J Nutr Pharmacol Neurol Dis. 2014; 4: 23-27.
14. Meena AK, Yadav AK, Niranjan US, Singh B, Nagariya AK , Verma M. Review on *Cyperus rotundus* - a potential herb. Int J Pharmaceut Clin Re. 2010; 2(1): 20-22.
15. Kilani S J, Ghedira Z, Nasr N, Krifa M, Ghedira K, Franca DMG, Ghedira CL. Evaluation of *in vitro* antioxidant and apoptotic activities of *Cyperus rotundus.* Asian Pac J Trop Med. 2014; 105-112.
16. Kilani S, Ledauphin J, Bouhlel I, Ben SM, Boubaker J, Skandrani I, Mosrati R, Ghedira K, Barillier D, Chekir-Ghedira L. Comparative study of *Cyperus rotundus* essential oil by a modified GC/MS analysis method, evaluation of its antioxidant, cytotoxic, and apoptotic effects. Chem Biodivers. 2008; 5(5): 729-42.
17. Sivapalan R. Medicinal uses and pharmacological activities of *Cyperus rotundus* Linn. - A review. Int. J. Sci. Res. Pub. 2013; 3(5): 1-8.
18. Mazzio EA, Soliman KF A. *In vitro* screening for the tumoricidal properties of international medicinal herbs. Phytother Res. 2009; 23(3): 385-98.
19. OECD, Guidelines for testing of chemicals, acute oral toxicity, environmental health and safety Monograph Series on Testing and Adjustment, 2001; 425: 1.
20. Jagetia GC, Baliga MS. Modulation of antineoplastic activity of cyclophosphamide by *Alstonia scholaris* in the Ehrlich ascites carcinoma-bearing mice. J Exp Ther Oncol. 2003; 3: 272–82.
21. Brandao R, Borges LP, de Oliveira R, Rocha JB, Noqueira CW. Diphenyldiselenide protects against haematological and immunological alterations induced by mercury in mice. J Biochem Mol Toxicol. 2008; 22: 311–19.
22. Okhawa H, Oshishi N, Yagi K. Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-58.
23. Charles C, Brian G, Michael BT. A direct enzymatic method for the determination of reduced glutathione in blood and other tissues. Biochem. Med. 1975; 13: 283- 97.
24. Sinha AK. Colorimetric assay of catalase. Anal. Biochem. 1972; 47(2): 389-94.
25. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C, Paoletti P. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol. 2003; 21(14): 2636-644.
26. Goel MK, Khanna P, Kishore J. Understanding survival analysis: Kaplan-Meier estimate. Int J Ayurveda Res. 2010; 1(4): 274-78. doi:10.4103/0974-7788.76794.
27. Navale AM, Shah GB, Pithadia AB. Tumor inhibitory activity of methanolic and ethyl acetate soluble extracts of *Thuja occidentalis* L. on mice bearing Ehrlich ascites carcinoma. Int J of Phyto Pharm. 2014; 4(3): 86-88.
28. Dhanya S, Isloor AM, Shetty P, Nayak PG, Pai KSR. *In vivo* anticancer and histopathology studies of Schiff bases on Ehrlich ascitic carcinoma cells: 1st Cancer Update. Arabian J Chem. 2013; 6(1): 25-33.
29. Yazdanparast R, Ardestani A. *In vitro* antioxidant and free radical scavenging activity of *Cyperus rotundus*. J Med Food. 2007; 10(4): 667-74.

30. Youcef N, Ahlem B. Aqueous extract of *Cyperus rotundus* rhizome protects against mercury (Ii) induced oxidative and renal stress in rats. Int J Pharm Pharm Sci. 2014; 6(8): 610-14.
31. Kilani-Jaziri S, Neffati A, Limem I, Boubaker J, Skandrani I, Sghair MB, Bouhlel I, Bhouri W, Mariotte AM, Ghedira K, Dijoux Franca MG, Chekir-Ghedira L. Relationship correlation of antioxidant and antiproliferative capacity of *Cyperus rotundus* products towards K562 erythroleukemia cells. Chem Biol Interact. 2009; 181(1): 85–94.