Original Article

The Toxicological and Histopathological Effects of Aqueous and Ethanolic Extracts of *Cyperus rotundus* Rhizomes in Ehrlich Ascites Carcinoma Induced in Swiss Albino Mice

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Abstract

Introduction: *Cyperus rotundus*, commonly known as mustha, is a perennial weed and possesses the ability to adopt to various soil types, temperatures, and moisture levels. It has several pharmacological and medicinal applications such as astringent, diuretic, antispasmodic, carminative, vermifuge, and antimicrobial properties. This study was designed to assess the toxicological and histopathological effects of aqueous and ethanolic extracts of *C. rotundus* rhizomes in Ehrlich ascites carcinoma (EAC)-induced Swiss albino mice. **Material and Methods:** Toxicity evaluation was carried out according to the OECD guidelines and histopathological assessment of the liver and kidney tissues was made using hematoxylin and eosin staining. **Results:** Results indicated that both ethanolic and aqueous extracts did not induce any toxicity up to 2000 mg/kg body weight doses. Examination of ascitic fluid revealed that ethanol extract at 250 mg/kg dosage induced degenerative changes, whereas aqueous extract at both dosage levels showed mild signs of apoptosis. Gross pathology of the liver and kidney indicated that the extracts did not alter the normal cytoarchitecture of these tissues. **Discussion and Conclusion:** Findings from this study interpret that *C. rotundus* rhizome extracts can be used as a complementary therapeutic in the EAC.

Keywords: *Cyperus rotundus, Ehrlich's ascites carcinoma, histopathology, toxicology*

Introduction

Due to the diverse medicinal properties of the phytochemicals, they are used during or after cancer therapy to neutralize and damage harmful consequences imposed by cancer therapeutics. Use of phytochemicals has long-term health benefits; however, they are used in parallel to cancer therapy because cancer therapy can cause significant damage to tumor cells within a short period of time.[1] There is an increasing interest in exploring the indigenous plants to identify their possible therapeutic aspects most probably due to their presumed safety, cost-effectiveness, and easy availability.[2]

Cyperus rotundus is a 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 such as stomach problems, constipation, fever, tooth problems, and digestive disorders, as antispasmodic and control of menstrual irregularities.[3] Later, the wide use of *C. rotundus* phytochemical extract for mosquito repelling, insecticidal, antibacterial, antimalarial, antimutagenic, antidiarrheal activity, antispasmodic activity, antioxidant activity, antiepileptic effect, therapeutic uses for cardiovascular diseases, anticholesterol, and wound healing activity was shown in various studies.[4-7] In further studies, anticarcinogenic activity, chemopreventive activity, antiproliferative activity against K562 erythroleukemia cells, and apoptotic activity were shown from flavonoid extracts of *C. rotundus*.[8,9] Flavonoids from *C. rotundus* have shown activity against metastasis and also showed antitumorigenic, antiproliferative activity in many studies.[10,11]

Ehrlich's ascites carcinoma (EAC) originally appeared as spontaneous breast cancer in female mouse[12] and is being used as a subcutaneously transplantable tumor in the mouse system. EAC is an undifferentiated carcinoma, which is hyperdiploid in nature. It has the characteristics of rapid proliferation, high transplantation, short life span, and 100% malignancy with no tumor-specific

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transplantation antigen.[13] The ascites liquid obtained from the peritoneum is a gray-white, or light bloody color viscous liquid which contains millions of neoplastic cells. After inoculation, the EAC cells grow in three phases in the peritoneal cavity of mice. These are a proliferating phase, where the cells increase in their number exponentially followed by a plateau phase and then a resting phase, where the number of cells remains constant.[14] Due to the resemblance of EAC and human tumors which are more sensitive to chemotherapy, it can be used as an efficient model in *in vivo* anticancer studies.

This investigation was designed to study the toxicological and histopathological effects of aqueous and ethanolic extracts of *C. rotundus* rhizomes in EAC-induced Swiss albino mice.

Material 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 −20°C for further studies. Ethanol and aqueous extracts of *C. rotundus* were prepared as per the standard procedures.[15]

Animals

Eight-to-ten-week-old Swiss albino female mice, weighing 20–30 g, selected from an inbred colony maintained in the Central Animal Research Facility of Manipal University, were selected for the study. The mice were housed in polypropylene cages (four per cage) in an air-conditioned room maintained at a comfortable temperature (23°C ± 2°C) with a 12-h light/dark cycle. They were fed with standard feed pellets and tap water *ad libitum*. The experiments on mice were approved by the Institutional Animal Ethical Committee (IAEC) and were conducted according to the guidelines of Control and Supervision of Experiments on Animals (No: YU-IAEC/4/25/8/2011).

Acute toxicity studies

The *in vivo* acute oral toxicity studies of the extracts were carried out as per the OECD guidelines 425 to explore the acute dose lethal to 50% (LD_{50}) of the animals, and 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 in a single dose. After dosing, the animals were observed for 3 h and monitored for 14 days for any mortality, behavioral changes, autonomic nervous system, and central nervous system changes.

In vivo anticancer activity of *Cyperus rotundus* extracts in Ehrlich's ascites carcinoma cells

The EAC cells were obtained from Amala Cancer Research Center, Amala Nagar, Thrissur, Kerala, India. They were maintained and propagated by serial intraperitoneal inoculation of 2×10^6 cells/mouse in an aseptic environment. Cells propagated for 12–14 days were used in the experiments.

Antitumor activity in Ehrlich ascites carcinoma model

Swiss albino mice were divided into seven groups ($n = 6$). A known number of viable EAC cells (2.5×10^6 cells/mice) were injected intraperitoneally into all the groups in an aseptic condition except for the normal group. The day of tumor inoculation was considered as day 0. All the experiments on tumor-bearing mice were conducted 24 h after EAC transplantation and that day was considered as day 1.

Group 1 served as normal control where animals were fed with 5 ml/kg body weight of normal saline (0.9% NaCl w/v). 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 a standard. 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 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 other than the normal group, and on the 15th day, three animals from all the groups were sacrificed by administering euthanizing agent thiopental sodium (300 mg/kg).

Blood was withdrawn by cardiac puncture immediately after euthanizing the animals to evaluate the hematological parameters. Ascitic fluid was collected from the peritoneal cavity of each animal to observe the appearance of neoplastic cells. The liver and kidney were collected from all the animals for histopathological studies.

Histopathological studies

Pathology of the ascitic tumor

Gross pathology of ascitic tumor was carried out according to the standard protocol.[16] A small amount of the ascitic fluid was withdrawn aseptically from

the peritoneum of the mice on the 15th day of tumor inoculation. A drop of the fluid was placed on the slide and made into a smear. The slide was kept aside, and the smear was fixed with methanol for ½ h. Then, a few drops of Leishman's reagent were added to the smear. The slide was allowed to stain for 3 min and was washed with an excess of distilled water. Then, the slide was allowed to stand for ½ h. It was fixed with xylene and examined under the microscope at × 100 magnification. Photomicrographs were recorded focusing the appropriate regions in the smear.

Histopathology of the liver and kidney

At the end of the treatment, both control and experimental mice were sacrificed by administering euthanizing agent thiopental sodium (300 mg/kg). The liver and kidney tissues from all groups were collected, fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol, and mounted in molten paraffin at 58°C –62°C. About 5 µ sections were obtained, stained with Harris hematoxylin and eosin, and evaluated for any histopathological changes under a bright-field microscope.[17] Five slides were screened from each animal. Liver sections were evaluated for pathological changes such as cellular infiltration, nuclear abnormalities, and central vein dilatation. Kidney sections were evaluated for pathological changes such as fatty changes, necrosis, and hemorrhagic spots.

Results

Acute toxicity study

The aqueous and ethanol extracts of rhizomes of *C. rotundus* were found safe up to a dose of 2000 mg/kg body weight Therefore, 250 mg/kg body weight and 500 mg/kg body weight were selected as treatment doses for further evaluation.

The results of the present study indicated that the toxic dose of the extracts was not found up to the tested concentrations, and therefore, may be accepted as safe OECD 425. The test animals did not display any significant changes in behavioral pattern such as trembling, diarrhea, salivation, breathing, impairment in food intake, water consumption, postural abnormalities, hair loss, sleep, lethargy, and restlessness, or in physical appearances such as eye color, mucous membrane, salivation, skin/fur effects, body weight, and injury when compared to the control at the end of 14 days of general observation.

In general, safety studies on herbal medicines are carried out by performing acute and subacute toxicity tests in laboratory animals such as rodents and nonhuman primates. Acute effects are normally observed soon after a single exposure of test agent, the subchronic effects are usually monitored over an extended period during which there is repeated exposure of test agent.[18]

Histopathological assessment of ascitic, liver, and kidney tissues in different treatment groups

Morphological analysis of ascitic cells

Figure 1 represents the appearance of EAC cells under ×100 magnification. In cisplatin-treated group, there were degenerative changes such as membrane blebbing, vacuolization, binucleated cells, intercellular bridging, and a reduction in the staining intensity [Figure 1b]. Ethanol extract at 250 mg/kg dosage showed clear degenerative changes, whereas aqueous extract at both dosage levels showed mild signs of apoptosis such as nuclear fragmentation, blebbing, and vacuolization [Figure 1c-f].

Histopathology of the liver and kidney

Examination of liver sections obtained from normal mice revealed normal cytoarchitecture when observed under a light microscope at ×40 magnification [Figure 2a]. Figure 2b shows the structure of liver in tumor control mice. The histology studies of the liver of cisplatin-treated group showed cellular infiltration (inflammation), nuclear abnormality, congestion, and mild central vein dilatation as seen in Figure 2c. The liver sections obtained from ethanol extract- and aqueous extract-treated groups, at both doses showed normal cytoarchitecture [Figure 3a-d].

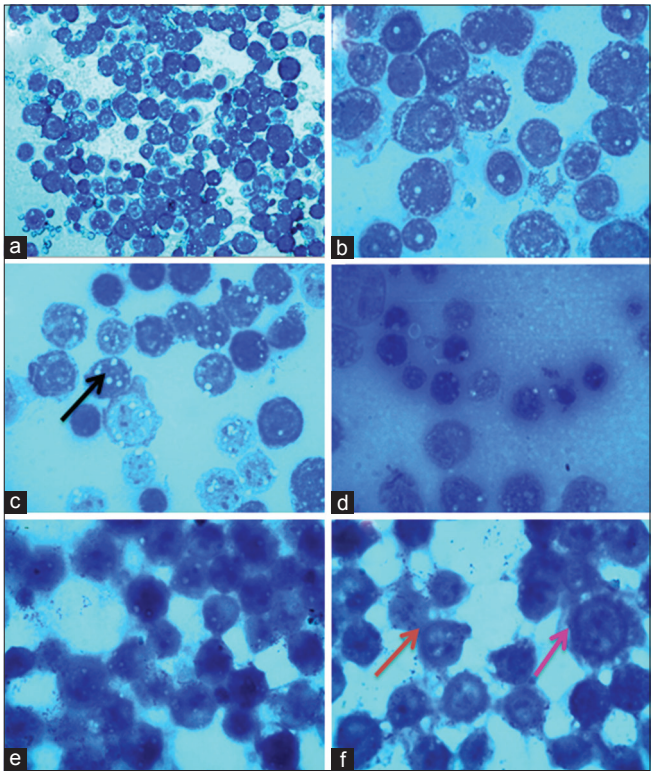

Figure 1: Morphology of Ehrlich ascites carcinoma cells at × 100 magnification (a) tumor control, (b) cells treated with cisplatin, (c) ethanol extract at 250 mg/kg, (d) ethanol extract at 500 mg/kg, (e) aqueous extract at 250 mg/kg, and (f) aqueous extract at 500 mg/kg of *Cyperus rotundus*. Black arrow: Vacuolization, Orange arrow: Bridging, Pink arrow: Blebbing

Examination of the kidney sections obtained from normal mice revealed normal histological appearance in both the cortex and medulla under a light microscope at ×40 magnification [Figures 4-7]. The cisplatin-treated group showed signs of nephrotoxicity such as fatty changes, necrosis, and hemorrhagic spots [Figures 4c and 6c]. The sections obtained from ethanol extract-treated groups and aqueous extract-treated groups, at both dosage levels did not show any signs of nephrotoxicity [Figures 5a-d and 7a-d].

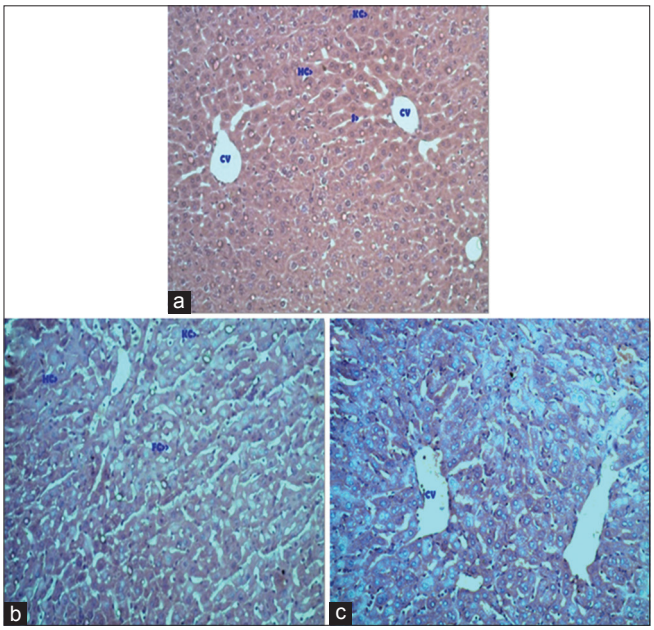

Figure 2: Histopathology of the liver at × 40 magnification (a) normal liver, (b) tumor control, and (c) treated with cisplatin. HC: Hepatocytes, KC: Kupffer cells, CV: Central vein, S: Sinusoid

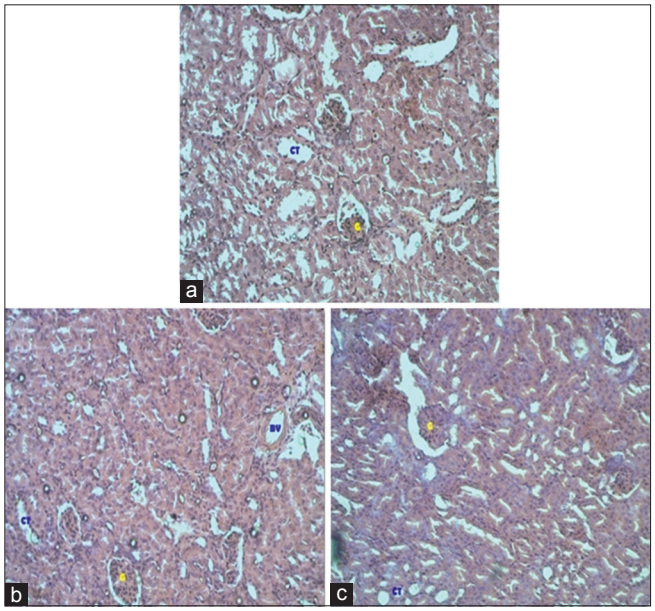

Figure 4: Histopathology of the cortex of kidney at × 40 magnification (a) normal kidney, (b) tumor control, and (c) treated with cisplatin. G: Glomerulus, CT: Collecting tubule

Discussion

According to the WHO study, the dependence on traditional medicines is considered to be 80% by the remote area population.[19] Utilization of medicinal plants as drugs is gaining popularity in developing countries because of their safety and efficacy. Phytochemicals isolated from such plants are regarded as nontoxic without causing any

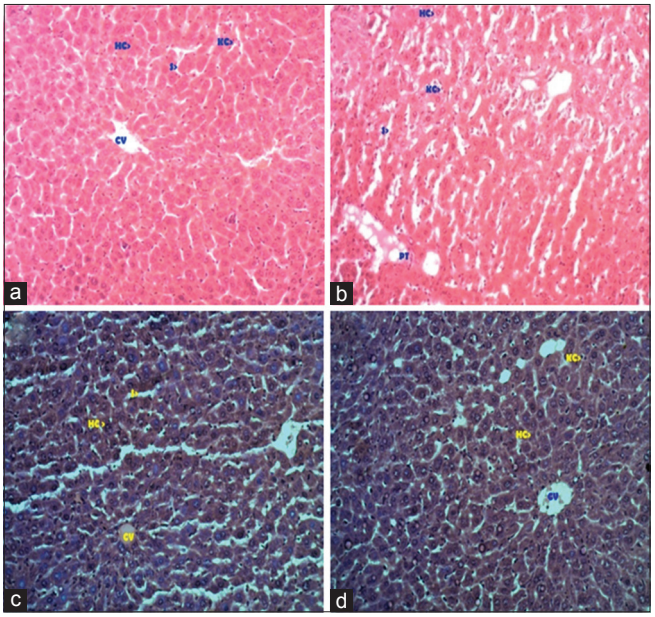

Figure 3: Histopathology of the liver at × 40 magnification (a) treated with ethanol extract at 250 mg/kg, (b) ethanol extract at 500 mg/kg, (c) aqueous extract at 250 mg/kg, and (d) aqueous extract at 500 mg/kg of *Cyperus rotundus* rhizome. HC: Hepatocytes, KC: Kupffer cells, CV: Central vein, S: Sinusoid

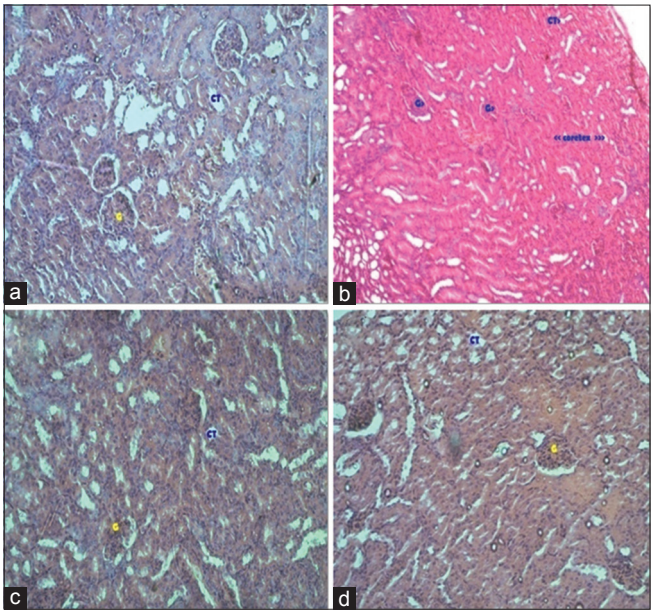

Figure 5: Histopathology of the cortex of kidney at × 40 magnification (a) treated with ethanol extract at 250 mg/kg, (b) ethanol extract at 500 mg/kg, (c) aqueous extract at 250 mg/kg, and (d) aqueous extract 500 mg/kg of *Cyperus rotundus* rhizome. G: Glomerulus, CT: Collecting tubule

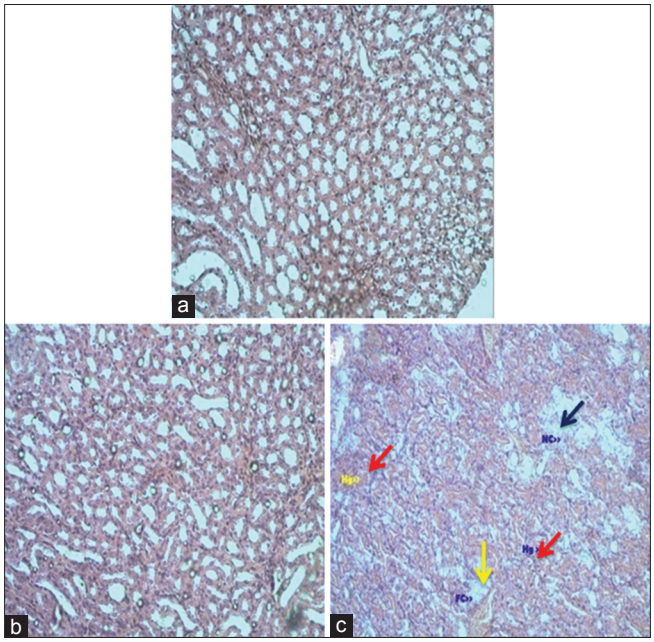

Figure 6: Histopathology of the medulla of the kidney at × 40 magnification (a) normal kidney, (b) tumor control, and (c) treated with Cisplatin. FC: Fatty changes (Yellow arrow), NC: Necrotic changes (Blue arrow), Hg: Hemorrhagic spot (Red arrow)

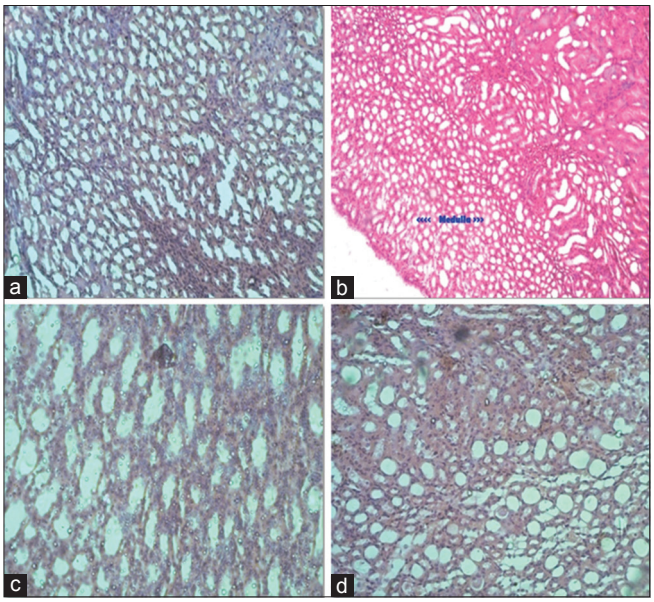

Figure 7: Histopathology of the medulla of kidney at × 40 magnification (a) ethanol extract at 250 mg/kg, (b) ethanol extract at 500 mg/kg, (c) aqueous extract at 250 mg/kg, and (d) aqueous extract at 500 mg/kg of *Cyperus rotundus* rhizome

undesirable health effects.[20] Drugs originated from plant sources are known to play a key role in the management of various chronic diseases including cancer and are considered to be potential alternatives for conventional allopathic treatment modalities.[21] This study was intended to evaluate the toxicity and histopathological effects of *C. rotundus* rhizome extracts on EAC-induced Swiss albino mice.

Safety of the plant extracts will be generally evaluated using oral acute toxicity studies. Results of acute toxicity study indicated that there were no major behavioral changes on the administration of *C. rotundus* extracts. Compounds with oral LD_{50} higher than 2000 mg/kg body weight are considered as safe for administration.[22] Histopathological assessments of liver and kidney tissues from the treated animals indicated that there were no significant differences in the morphology, thereby proving the safety of the extracts.

Conclusion

Finally, to conclude that since this herb showed moderate anticancer activity and less toxicity to major organs such as liver and kidney; it can be advised as a prophylactic agent after appropriate validation from clinical trials. The results of the study bear its high relevance because this herb is available locally and may represent a convenient and cost-effective means to cope with many types of cancer such as breast cancer and colorectal cancers, which are the two leading causes of mortality in India. Further studies are needed to assess the active ingredients of *C. rotundus* rhizome extracts involved in the antiproliferative or cytotoxic effects. A detailed study could be conducted to show the exact molecular mechanism by which the components in ethanol extract could attain the therapeutic efficacy. There is scope to explore the *in vivo* anticancer activity of active components identified from the extracts individually as cancer therapeutics.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Chu YF, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activities of common vegetables. J Agric Food Chem 2002;50:6910-6.
2. Sawangjaroen N, Sawangjaroen K. The effects of extracts from anti-diarrheic Thai medicinal plants on the *in vitro* growth of the intestinal protozoa parasite: *Blastocystis hominis*. J Ethnopharmacol 2005;98:67-72.
3. Puratchikody A, Nithya C, Nagalakshmi G. Wound healing activity of *Cyperus rotundus* Linn. Indian J Pharm Sci 2006;68:97-101.
4. Seo EJ, Lee DU, Kwak JH, Lee SM, Kim YS, Jung YS, *et al*. Antiplatelet effects of *Cyperus rotundus* and its component (+)-nootkatone. J Ethnopharmacol 2011;135:48-54.
5. Majid NZ, Jabier MS, Wagi RI, Kareem HH. Extraction,

identification and antibacterial activity of *Cyperus* oil from Iraqi *C. rotundus*. Eng Technol 2008;26:1.

6. Imam H, Zarnigar R, Sofi G, Seikh A, Lone A. The incredible benefits of Nagarmotha (*Cyperus rotundus*). Int J Nutr Pharmacol Neurol Dis 2014;4:23-7.
7. Meena AK, Yadav AK, Niranjan US, Singh B, Nagariya AK, Verma M. Review on *Cyperus rotundus* – A potential herb. Int J Pharmaceut Clin Res 2010;2:20-2.
8. Soumaya KJ, Zied G, Nouha N, Mounira K, Kamel G, Geneviève FD, *et al.* Evaluation of *in vitro* antioxidant and apoptotic activities of *Cyperus rotundus*. Asian Pac J Trop Med 2014;7:105-12.
9. Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, *et al.* Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. Int J Cancer 2000;87:595-600.
10. Kandaswami C, Lee LT, Lee PP, Hwang JJ, Ke FC, Huang YT, *et al.* The antitumor activities of flavonoids. *In Vivo* 2005;19:895-909.
11. Huang YT, Hwang JJ, Lee PP, Ke FC, Huang JH, Huang CJ, *et al.* Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. Br J Pharmacol 1999;128:999-1010.
12. Aktaş E. Ehrlich's Ascites tumor cells proliferation rate. İstanbul: İstanbul Üniversitesi Fen Bilimleri Enstitüsü; 1996.
13. Kaleoğlu O, İşli N. Ehrlich-Lettre Ascites Tumor. Tıp Fakültesi Mecm 1977;40:978-84.
14. Song Z, Varani J, Goldstein IJ. Differences in cell surface carbohydrates, and in laminin and fibronectin synthesis, between adherent and non-adherent Ehrlich ascites tumor cells. Int J Cancer 1993;55:1029-35.
15. Raaman N. Phytochemical Techniques. New Delhi: New India Publishing Agency; 2006. p. 9-18.
16. Ramalingam R, Sivakumar T. *In vitro* and *in vivo* anti-cancer activity of leaves of *Plumeria alba* Linn. J Pharm Res 2009;2:11-4.
17. El-Sayyad HI, Ismail MF, Shalaby FM, Abou-El-Magd RF, Gaur RL, Fernando A, *et al.* Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats. Int J Biol Sci 2009;5:466-73.
18. Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, *et al.* Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. J Ethnopharmacol 2004;94:205-17.
19. Kifayatullah M, Waheed I, Das SK, Sisugoswami M, Izarullah. Evaluation of hydroethanolic extract of *Opuntia monacantha* Haw. For analgesic activity. World J Pharm Pharm Sci 2014;3:1006-20.
20. Vaghasiya YK, Shukla VJ, Chanda SV. Acute oral toxicity study of *Pluchea arguta* boiss extract in mice. J Pharmacol Toxicol 2011;6:113-23.
21. Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation, on rats. J Health Sci 2007;53:351-8.
22. Adeneye AA, Olagunju JA. Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn in Wistar rats. Biol Med 2009;1:1-10.