Original Article

Evaluation of Antitumor and Antioxidant Activity of Oxalis Corniculata Linn. against Ehrlich Ascites Carcinoma on Mice

Kathiriya A[1], Das K[2], Kumar EP[1] , Mathai K B[1]

Abstract

Background: Present investigations were carried out to evaluate an ethanolic extract of Oxalis corniculata Linn. For its anticancer and antioxidant activity in Ehrlich acsites carcinoma (EAC) - induced in swiss albino mice.

Methods: Cancer was induced in mice by aspiration of peritoneal fluid of EAC inoculated mice and diluted to get $1x10^6$ cells/ml in phosphate buffer. Cancerous animals were divided into four groups having six mice in each in which Group III and IV cancerous animals were treated with the ethanolic extract of Oxalis corniculata Linn. One hundred and 400 mg/kg b.w. respectively, whereas Group V cancerous animals received standard drug cyclophosphamide 25 mg/kg b.w. The test and standard drugs were administered orally 24 hrs after the inoculation of EAC for 9 days. The several changes of antitumor potential of EEOC were accessed. Biochemical parameters such as total protein, albumin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), lipid peroxidation and enzymatic antioxidants were determined.

Results: The present study revealed that EEOC showed significant antitumor and antioxidant activities in EAC bearing mice. The dose dependent reduction in body weight, tumor volume, packed cell volume, tumor cell counts and increase in median survival time (MST) and percentage increase in life span in EEOC treated animals were observed. There was a significant increase in RBC count; Hb content in EEOC treated animals and reduction in total WBC count. There was a significant increase in total protein and albumin content, but decrease in AST, ALT and ALP contents in EEOC treated animals. A significant decrease in liver MDA levels and increase in catalase and reduced glutathione levels were observed in EEOC treated animals.

Conclusion: Results conclude that the EEOC was effective in inhibiting the tumor growth in ascitic and solid tumor models. The biochemical and antioxidants studies were also supported its antitumor properties.

Keywords: Oxalis corniculata; Anticancer activity; Antioxidants activity; Ehrlich ascites carcinoma

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1. Dept of Pharmacology and Toxicology, St. John's Pharmacy College, Bangalore, Karnataka, India
2. Dept of Pharmacognosy and Phytochemistry, St. John's Pharmacy College, Bangalore, Karnataka, India

Corresponding Author:
Anil Kumar Kathiriya M.Pharm, Pharmacologist
Email: anilkumarkathiriya@gmail.com
Tel: (+91) 90 35 30 51 50

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Introduction

Cancer is a disease of misguided cells which have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is world's second killer after cardiovascular disease and it ever was killed 7.6 million people in 2005, out of them three quarters were from in low and middle income countries. That number is expected to increase to 9.0 million up to 2015 and rise further to 11.5 million in 2030[1]. New cancer patients in India are estimated between 7 to 9 lakhs [2]. Chemotherapy, radiotherapy and surgery are only three major existing modes of treatment in modern medicine. Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells, whereas the rest may damage healthy cells and tissue [3]. Role of free radicals are also to be considered an important factor for human body that damage against reactive oxygen species, viz.

hydroxyl, peroxyl and superoxide radicals which leds to implication to the cancer, diabetes and coronary artery disease [4, 5]. Hence the impact of herbals in cancer treatment should be considered to discover new drug molecule or its derived compounds for cancer research.

Oflate, Oxalis *corniculata* Linn. is a sub-tropical plant (Family: Oxalidaceae) being native of India, are commonly known as creeping woodsorrel [6]. It is a delicate-appearing, low growing, herbaceous plant and abundantly distributed in damp shady places, roadsides, plantations, lawns, nearly all regions throughout the warmer parts of India, especially in the Himalayas up to 8,000 ft-cosmopolitan [7,8]. Traditionally the plant is well known with its varsatile medicinal uses likely treatment for stomach ache, relieve the intoxication produced by Datura, as a refrigerant, decoction of roots is useful for worms, giddiness, diarrhea and dysentery [7]. The leaves are useful for cough, cold, fever and as antihelmintic [9]. But no such literatures are revealed for its activity against treatment for pandemic cancer and hence the present study was carried out to evaluate the antioxidant and antitumor activity of the ethanolic extract of Oxalis *corniculata* Linn. (EEOC) against Ehrlich ascites carcinoma (EAC) in mice.

Materials and Methods

Preparations of plant extract

Healthy, disease free plant material (Oxalis corniculata Lin.) was collected in the month of September 2009 from Tirupati, Andhra Pradesh, India and was authenticated by Prof. Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati (Andhra Pradesh), India. The oven dried plant material (500 gms) were powdered and further extracted with 80% ethanol (HPLC grade) in a soxhlet apparatus for 8 hours. The extract was then concentrated to dryness under reduced pressure, 500-600 C to yield semi-solid crude extract (yield 12-13% w/w), which was stored in glass bottle and preserved in a refrigerator at

0-6^{0}C. The crude extract was used for further investigations.

Phytochemical estimations

Qualitative phytochemical investigations of ethanol extract was performed according to the procedure of Indian Pharmacopoeia 1985. Results revealed the presence of several phytochemicals viz. phytosterol, glycosides, flavonoids and tannins.

Chemicals

Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were procured from Loba Chemicals (Mumbai, India). 5, 5'-Dithio bis-2-nitro benzoic acid and reduced glutathione were from Sisco Research Laboratory (Mumbai, India). Hydrogen peroxide was purchased from Nice Chemicals. All the other reagents were used of analytical grades.

Tumor cells

Ehrlich ascites carcinoma (EAC) was developed by Loewenthal and Jahn from one of the several lines of Ehrlich carcinoma that arise from spontaneous epithelial tumors, most probably of mammary gland origin [10]. EAC inoculated mice was supplied by Division of Radiobiology and Toxicology, Manipal Life science Centre, Manipal University, India for this present investigation. The EAC cells were maintained by weekly intraperitoneal (i.p.) inoculation of $2x10^6$ cells/mice [11].

Animals

Healthy Swiss albino mice (20-25 g) were procured from the Central Animal Facilities of the NIMHANS, Bangalore. Animals were housed at St. John's institute's animal house facilities until they gained significant weight (25 $\pm$ 0.5 g) suitable for the present investigation (approved by the Institute Animal Ethical Committee, ref no. IJAHSM/IAEC/2008/008). Animals were housed in polypropylene cages and maintained under standard conditions (12$\pm$1 h light/dark cycle, 22 $\pm$ 2^0 C and 55 $\pm$ 5% relative humidity). They were fed with standard rat pellet diet and water *ad libitum*. The animals were maintained in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) guidelines for the care and use of laboratory animals.

Acute toxicity study

Acute oral toxicity study of EEOC was carried out according to OECD guidelines (425, adopted March 23, 2006) for determination of LD_{50} [12].

Experimental protocol

The cancer animals' randomly divided into 5 groups of 12 mice each.

Group-I: Normal animals (negative control)

Group-II: EAC-cancer animals (positive control) + Normal Saline (5ml/kg)

Group-III: EAC-cancer animals + EOC (400 mg/kg)

Group-IV: EAC-cancer animals +EOC (100 mg/kg)

Group-VI: EAC-cancer animals + Cyclophosphamide (25 mg/kg)

The test and standard drugs (Cyclophosphamide) were suspended in water for injection solution as vehicle and administered orally 24 hours after the inoculation of EAC for 9 days. After administration of last dose, 6 mice from each groups were kept fasting for 18 hours and sacrified for the study of antitumor activity, heamatological parameters, biochemical estimations and liver antioxidant studies. The remaining animals in each of the group were kept aside to determine the median survival time (MST) and the body weight analysis of the tumor bearing mice [13].

Tumor growth response

The antitumor potential of EEOC was accessed by change in body weight analysis, survival time, total ascites fluid volume, packed cell volume and hematological parameters. Viable and non-viable cells were counted in a hemocytometer by using trypan blue dye exclusion (0.4%). Animal survival time was recorded and expressed as median survival time (MST) in days and percentage increase in lifespan (ILS) of treated mice was calculated as following formula:

Median survival time (MST) =
(Day of 1st death + Day of last death) / 2
Percentage increase in life span =
[(MST of treated group/ MST of control group) – 1] x 100.

An enhancement of lifespan upto 25% or more was considered as an effective response [14]. Consequently weekly body weight was also recorded during the study.

Hematological parameters

In order to detect influence of EEOC on hematological status of EAC bearing mice, comparison were made amongst four groups (group I-IV) of mice on the 14th day after inoculation. Blood was withdrawn from free flowing tail vein blood and hemoglobin content [15], red blood cell (RBC) [16], white blood cell (WBC) [17] and differential leukocyte count of WBC [18] were determined.

Normal peritoneal cells

Five groups of normal mice (n=6) were used for the study. Group one was untreated and used as a control. Second and third groups were treated respectively with 100 mg/kg and 400 mg/kg, p.o. of EEOC only once for a single day. Whereas fourth and fifth groups were received same treatment for two consecutive days. Peritoneal exudates cells were collected after 24 h treatment by repeated intraperitoneal wash with normal saline and counted in each of treated groups and compared with those of untreated groups.

Solid tumor

Two groups of mice (n=5) were divided for this study. The mice of group 1 were used as a tumor control. Group 2 were received EEOC (400 mg/kg) orally for 5 alternative days. Then tumor mass was measured from the 11th day of inoculation. The measurement was carried out every 5th day for a period of 30 days. The volume of tumor mass was calculated using the formula $V= 4/3\ \pi r^2$ where r is the mean of r^1 and r^2 which are two independent radii of tumor mass [19].

Biochemical parameters

After 18 hours of fasting and after 24 hours of the last dose, the blood sample was collected by puncturing retro-orbital plexus. Further the mice were sacrified and liver were collected for antioxidant study. Blood was used for the assay of biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), albumin, creatinine and total protein by using the Span Diagnostic Kits, Surat, India.

Antioxidant study

The liver was excised, rinced in ice-cold normal saline followed by cold 0.15M Tris HCl (pH 7.4), dried and weighed. A 10% w/v homogenate was prepared in 1.15% KCl. A homogenate was utilized for estimation of lipid peroxidation [20], glutathione [21] and catalase [22] activity.

Statistical analysis

The experimental result was expressed as Mean ± SEM. The data were accessed by the method of one way ANOVA followed by Dunnett's t-test. P-values of 0.05 were considered as statistically significant.

Results

The present study revealed that EEOC at different doses level showed significant antitumor and antioxidant activity against tumor bearing mice.

Effect on mean survival time

The effect of EEOC on the survival of tumor bearing mice is shown in table 1. The MST of control group was 19.83±0.79 days, whereas it was 28.83±0.70 days and 32.16±0.70 days for 100 and 400 mg/kg on EEOC treated groups,

Table 1. Effect of EEOC on survival time against EAC induced animals

Group	Median survival time	Percentage increase of life span
Normal	----------	----------
EAC control	19.83±0.79	----------
EAC + EEOC (100mg/kg)	28.83±0.70#	45.38
EAC + EEOC (400mg/kg)	32.16±0.70#	62.17
EAC + Cyclophosphamide (25mg/kg)	38.33±1.05#	93.29

Values are expressed as mean±SEM (n=6). # $p < 0.01$. **#** Value are significantly different from cancer control

Table 2. Effect of EEOC on tumor growth response against EAC induced animals

Group	Increase in body weight (gm)	Tumor volume (ML)	Packed cell volume (ML)	Viable cell count (%)	Non-viable cell count (%)
Normal	1.25±0.10	------	------	------	------
EAC control	7.51±0.53**	4.12±0.25	1.94±0.18	81.6±3.32	18.4±3.32
EAC + EEOC 100mg/kg)	5.26±0.17#	3.28±0.15#1	1.28±0.08#	57.6±2.18#	42.4±2.18#
EAC + EEOC 400mg/kg)	3.41±0.18#	1.96±0.16#	0.77±0.10#	33.8±2.95#	66.2±2.95#
EAC + cyclophosphamide (25mg/kg)	2.46±0.17#	1.38±0.07#	0.59±0.04#	28.8±3.13#	71.2±3.13#

Values are expressed as mean ±SEM (n=6). ** $p < 0.01$, #1 $p < 0.05$ and # $p < 0.01$. ** Values are significantly different from control, **#** Value are significantly different from cancer control.

Table 3. Effect of EEOC on hematological parameters against EAC induced animals

Group	Hb (gm %)	R.B.C. ($1x10^6$cells)	W.B.C. ($1x10^3$ cells)	Monocytes (%)	Lymphocyte (%)	Neutrophil (%)
Normal	12.88±0.27	5.13±0.09	7.041±0.18	1.83±0.16	76.2±0.58	21.6±0.81
EAC control	8.09±0.5**	3.65±0.14**	21.95±0.81**	1.8±0.2**	35.2±1.8**	60.8±1.85**
EAC + EEOC 100mg/kg)	9.76±0.18#	4.13±0.06#	16.29±0.94#	1.6±0.24#	57.1±1.41#	38.4±1.96#
EAC + EEOC 400mg/kg)	11.14±0.18#	4.78±0.04#	12.97±0.27#	1.4±0.24#	68.6±2.82#	26.2±1.74#

Values are expressed as mean ±SEM (n=6). ** $p < 0.01$, # $p < 0.01$. ** Values are significantly different from control, **#** Value are significantly different from cancer control.

respectively. The standard drug cyclophosphamide (25 mg/kg) treated groups showed 38.33±1.05 days. The increase in the life span of tumor bearing mice treated with EEOC doses (100 and 400 mg/kg) and cyclophosphamide was found to be 45.58%, 62.17% and 93.29% respectively as compared to the control groups. EEOC treated groups, at both dose levels, reduced body weight, compared to the EAC treated control groups.

Effect on tumor growth

EEOC treated groups at the doses of 100 and 400 mg/kg significantly ($p<0.05$ and $p<0.01$) reduced tumor volume and packed cell volume in a dose dependant manner as compared to that of the EAC control groups (Table2). Furthermore, percentage of non-viable tumor cell count at different doses of EEOC was effectively increased in a dose dependent manner.

Effect on haematological profile

Table 3 shows the effect of EEOC on hematological parameters against EAC induced animals estimated on 14th day of treatment Hb content and R.B.C. count in the EAC control group were significantly ($p < 0.01$) decreased as compared to normal group. Treatment with EEOC at the doses of 100 and 400 mg/kg significantly ($p < 0.01$) increased the R.B.C. count and Hb content as compared to EAC control groups. The total W.B.C. count was found significantly increased in the EAC control group when compared with the normal group ($p < 0.01$). Administration of EEOC at doses of 100 and 400 mg/kg in EAC bearing mice significantly (p

Table. 4 Effect of EEOC on peritoneal cells in normal mice

Treatments	No. of days	Peritoneal cells (1x10⁶)
Normal mice	----------	5.37±0.18
EEOC	1	7.62±0.23***
	2	8.89±0.12***

Values are mean of 6 animals *** $p < 0.001$. *** Values are significantly different from normal mice.

Table5. Effect of EEOC on solid tumor volume in EAC bearing mice

Treatment	Dose	Tumor volume (ml)	VDT (Days)	GD (Days)
G1	25 ml/kg	2.52±0.20	3.86±0.07	-----
G2	100 mg/kg	1.63±0.03**	4.83±0.06**	4.5±0.28
G3	400 mg/kg	1.34±0.10**	5.82±0.29**	6.8±0.37

Values are expressed as mean ± SEM (n=6). ** $p < 0.01$

Table 6. Effect of EEOC on biochemical parameters against EAC induced animals

GROUP	T. Protein (g/dl)	Albumin (g/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Normal	5.46±0.47	1.52±0.06	33.78±1.18	27.48±2.09	79.75±2.71
EAC control	2.78±0.17**	1.15±0.08*	63.18±3.09**	58.032±3.69**	120.02±4.33**
EAC + EEOC (100mg/kg)	3.56±0.05#	1.26±0.12N.S.	46.07±2.29#	43.84±1.87#	99.72±2.59#
EAC + EEOC (400mg/kg)	4.26±0.12#	1.32±0.07N.S.	38.03±2.13#	36.85±0.811#	86.75±4.26#
EAC + cyclophosphamide (25mg/kg)	4.88±o.30#	1.45±0.03#1	40.46±1.36#	33.25±1.10#	81.61±1.48#

Values are expressed as mean±SEM (n=6). ** $p < 0.01$, #1 $p < 0.05$ and # $p < 0.01$. N.S. = non significant. ** Values are significantly different from control, # Value are significantly different from cancer control.

< 0.01) reduced the W.B.C. count as compared with the EAC control. In a differential count of W.B.C, the presence of neutrophils increased, while the lymphocyte count decreased in the EAC control group. Treatment with EEOC at different doses changed these altered parameters approximately to the normal values significantly ($p < 0.01$).

Effect on peritoneal cells

The effect of EEOC on peritoneal cells in normal mice is shown in table 4. The average number of peritoneal exudates cell was found to be 5.37±0.18 x 10^6. Single treatment of EEOC (100mg/kg) enhanced peritoneal cells to 7.62±0.23 x 10^6 ($p < 0.001$), while two consecutive treatment enhanced the number to 8.89±0.12 x 10^6 ($p < 0.001$).

Effect of EEOC on solid tumor

Table 5 depicts the effect of EEOC on solid tumor in EAC bearing mice. All doses of EEOC at 100 and 400 mg/kg showed significant ($p < 0.01$) decrease in tumor volume when compared to EAC induced control animals. EEOC treated animals also shown significant increase in VDT when compared to EAC control animals. This finding suggested a marked effect on cell growth kinetics. There was a significant decrease in tumor growth rate in EEOC treated groups. The tumor growth delays about 5 days with 100mg/kg and 400mg/kg treatment resulting about 7 days.

Effect of EEOC on biochemical parameters

Table 6 shows the effect of EEOC on biochemical parameters against EAC induced animals. The total protein and albumin were found significantly decreased in the EAC control group when compared with the normal group ($p < 0.01$). Administration of EEOC at doses of 100 and 400 mg/kg and cyclophosphamide in EAC bearing mice significantly ($p < 0.01$) induced the total protein as compared with the EAC control. There were no significant changes in albumin levels when EEOC treated as compared with EAC control groups. Biochemical parameters like SGOT, SGPT and ALP in the EAC control group were significantly ($p < 0.01$) increased as compared to the normal group. Treatment with EEOC at doses of 100 and 400 mg/kg and cyclophosphamide 25 mg/kg significantly ($p < 0.01$)

Table 7. Shows effect of EEOC on liver enzymatic antioxidants of EAC bearing animals

GROUP	Catalase (units/mg protein/min)
Normal	4.10±0.27
EAC control	1.07±0.07**
EAC + EEOC (100mg/kg)	2.24±0.16#
EAC + EEOC (400mg/kg)	3.14±0.19#

Values are expressed as mean±SEM (n=6). ** p < 0.01 and # p < 0.01. ** Values are significantly different from control, **#** Value are significantly different from cancer control.

Table 8. Shows effect of EEOC on liver non-enzymatic antioxidants of EAC bearing animals

GROUP	Reduced glutathione (mMol/gm wet liver tissue)
Normal	28.12±1.59
EAC control	8.09±1.002**
EAC + EEOC (100mg/kg)	13.28±1.23#1
EAC + EEOC (400mg/kg)	22.545±0.71#

Values are expressed as mean±SEM (n=6). ** p < 0.01, #1 p < 0.05 and # p < 0.01. ** Values are significantly different from control, # Value are significantly different from cancer control.

Table 9. Shows the effect of EEOC on lipid peroxidation in liver tissue of EAC induced animals

GROUP	Lipid peroxidation (nmol/mg protein)
Normal	162.81±3.87
EAC control	439.28±6.34**
EAC + EEOC (100mg/kg)	324.58±5.24#
EAC + EEOC (400mg/kg)	234.68±5.84#

Values are expressed as mean±SEM (n=6). ** p < 0.01, #1 p < 0.05 and # p < 0.01. ** Values are significantly different from control, # Value are significantly different from cancer control.

decreased the SGOT, SGPT and ALP as compared to EAC control groups.

Effect of EEOC on antioxidant study

Table-7 depicts the effect of CAT enzymes in EEOC treated cancerous animals. EAC control animals have shown a significant decrease in CAT ($p < 0.01$) when compared with normal animals. Administration of EEOC at doses of 100 and 400 mg/kg significantly normalized the levels of CAT ($p < 0.05$ and $p < 0.01$ respectively) when compared with EAC control groups.

Table-8 depicts the effect of EEOC on liver non-enzymatic antioxidants of EAC bearing mice. EAC control animals have shown a significant decrease in GSH ($p < 0.01$) when compared with normal animals. After administration of EEOC at different doses of 100 and 400 mg/kg significantly increased the levels of GSH ($p < 0.05$ and $p < 0.01$ respectively) when compared with vehicle treated EAC groups of animals.

Table-9 summarizes the effect of EEOC on lipid peroxidation in liver tissue of EAC bearing mice. MDA levels in liver of EAC induced animals were measured as an index of lipid peroxidation. EAC control animals have shown a significant increase in MDA levels ($p < 0.01$) when compared with normal animals. All doses of EEOC 100 and 400 mg/kg showed significant ($p < 0.01$) protection against lipid peroxidation as compared to EAC control groups.

Discussion

Chemotherapeutic agent has been shown that synthetic cancer drugs cause nonspecific killing of cells, whereas natural products offer protective and therapeutic actions to all cells with low cytotoxicity and are beneficial in producing nutrient repletion to compromised people [23]. Therefore, there is a need for new prototypes, new templates, to use in the design of potential chemotherapeutic agents. Natural products are providing such templates. The natural products are economical and of low toxicity and this is perhaps the advantage over synthetic agents,

which exhibit normal tissue toxicity. With the desire to find new and different types of compounds with anticancer and radiosensitizing activity, and to open new areas of productive research, selecting *Oxalis corniculata* Linn. (Fam. Oxalidaceae) started a study, a plant with established medicinal properties, for testing its possible antitumor potential.

The *in vitro* cytotoxic activity of EEOC against EAC cell lines partially explains its significant antitumor activity against ascites and solid tumor. The EEOC treated animals at doses of 100 and 400 mg/kg inhibited the body weight, tumor volume, packed cell volume, tumor cell count and also reverted the hematological parameters to approximately normal levels. Processed extract may have direct tumorocidal effect and thereby it may maintain the normal hematological profile. In EAC bearing hosts, regular rapid increase in ascites tumor volume was observed. The ascites fluid is the direct nutritional source for tumor cells, and the faster increase in ascites fluid with tumor growth could possibly be a means to meet the nutritional requirements of tumor cells [24].

The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal [25] and disappearance of WBC from blood [26]. The EEOC decreased the ascites fluid volume and thereby increased the percentage of lifespan. Viable tumor cell count was significantly inhibited in all EEOC treated groups. The percentage of trypan blue positive dead tumor cells also increased in the treated groups as compared with EAC control hosts. It may be concluded that EEOC, by decreasing the nutritional fluid volume and arresting the tumor growth, thereby increased the lifespan of EAC bearing mice.

Furthermore in cancer chemotherapy, the major problems encountered are of myelosuppression and anemia [27, 28]. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin content and leucocytes, and may this occur either due to iron deficiency or due to hemolytic or myelopathic conditions [29, 30]. Whereas, our results says that EEOC have significantly enhanced the erythrocyte count and hemoglobin level when compared to that EAC bearing mice. The WBC level is reduced when compared to that of EAC bearing mice. These indicating parameters reveal that EEOC possesses protective action on the hemopoietic system.

Enzymes in serum have been studied for many years as possible early indicators of neoplasia and as aids in following the progression and regression of disease [31]. Hepatotoxicity may occur due to cytotoxic agent itself, or due to its toxic metabolites. In certain circumstances they can be carcinogenic (i.e. they may themselves cause cancer). If there is rapid cell destruction with extensive purine catabolism, urates may precipitate in the renal tubules and cause kidney damage [32, 33]. Results concluded that EAC inoculated increased levels of liver enzymes SGOT, SGPT, ALP and decrease the levels of total protein and albumin. These levels were markedly reversed by the administration of EEOC.

Increased lipid peroxidations would cause degeneration of tissues and lipid peroxide formed in the primary site would be transferred through circulation and provoke damage by propagating the process of lipid peroxidations [34]. MDA, the end product of lipid peroxidations was reported to higher in carcinomatous tissue than in non disease organs [35], and their levels were correlated with advanced clinical stages and the impairment is related to tumor progression [36]. Moreover, it has been claimed that MDA acts as a tumor promoter and co-carcinogenic agent because of its high cytotoxicity and inhibitory action on protective enzymes [37]. Glutathione, a potent inhibitor of neoplastic process, plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process [34]. EEOC reduced elevated levels of lipid peroxidation and increased the glutathione content in EAC bearing mice.

CAT is present in all oxygen metabolizing cells and their function is to provide a defense against the potentially damaging reactivities of hydrogen peroxide and superoxides [38]. Similar findings were observed in the present study like the administration of EEOC at different doses increased CAT levels in a dose-dependent manner, which might be indicating the antioxidant and free scavenging property of EEOC.

The antitumor activity of EEOC was accompanied with the increase of antioxidant status. The free radical hypothesis supported that the antioxidant effectively inhibited tumor and observed investigations might be attributed to the antitumor and antioxidant principles present in the extract. The extract also restored the hepatic lipid peroxidation and free radical scavenging GSH as well as antioxidant enzymes such as CAT in tumor-bearing mice to approximately normal levels.

To evaluate whether EEOC treatment indirectly inhibited tumor cell growth, the effect of EEOC treatment was examined on the peritoneal exudates cells of normal mice. Normally, each mouse contains

5 x 10^6 intra-peritoneal cells, 50% of which are macrophages. EEOC treatments were found to enhance peritoneal cell counts. These results demonstrated the indirect effect of EEOC on EAC cells, probably mediated through enhancement and activation of macrophages or through some cytokine product inside the peritoneal cavity [39] produced by EEOC treatment.

Ascites as well as in solid tumor reduction studies the prophylactic treatment of EEOC exhibited significant tumor reducing property.

Conclusion

Results conclude that the ethanolic extract of *Oxalis corniculata* Linn. was effective in inhibiting the tumor growth in ascitic and solid tumor models. Further the biochemical and antioxidants studies were also supported its antitumor properties.

Acknowledgement

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Conflict of Interest

Surveys have found that half or more of patients with cancer have tried complementary or alternative medicine. Complementary and alternative medicine (CAM) encompasses hundreds of diverse medical and healthcare systems and practices and thousands of products that are not considered part of conventional medicine. This includes practices such as acupuncture and yoga, herbal medicines, nutritional supplements, homeopathy, and others. CAM treatments are not necessarily proven to work, although some do have evidence backing them up. The list of practices that are considered CAM changes continually as practices and therapies that are proven safe and effective become accepted as mainstream healthcare practices. Hence the present research work is the Challenge of Herbal Therapies for Cancer disease.

Authors' Contribution

AK was the first author who has contributed his research work towards cancer study in his Master of Pharmacy study.DK has co-guided this research study and helped by carrying out the extraction procedure and other technical matters in the same.EPK has selected this present research protocol and served as principle guide for the research study.MKB has provided the space and supplied the animal for the present research work.

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