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Antitumor and structure antioxidant activity relationship of colchicine on Ehrlich ascites carcinoma (EAC) in female mice

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Abstract

Colchicine has been reported to play important roles in hepatoprotection, anti-inflammation *in vitro* anti cancer activity. The present study was initiated to evaluate antioxidant and anti-cancer effects of colchicine (10µg/mice, i.p.) in mice after subcutaneous implantation of ehrlich ascites carcinoma (EAC) for 21 days. On the 22th day, the mice were sacrificed for the estimation of tumor growth, and biochemical parameters (glucose, insulin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), lipid peroxides (TBARS), protein thiols (Pr-SHs), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, 17β-estradiol and progesterone). The results of this study showed that administration of colchicine and 5-Flourouracil individually for 21 days to the carcinoma induced mice demonstrated a significant (P<0.01) decrease in tumor weight and a significant (P<0.01) improvement in biochemical parameters and life span compared to the EAC control mice. In addition, the results clearly suggest that colchicine induced antioxidant activity on experimental EAC control mice.

Keywords: Colchicine, 5-Flourouracil, breast cancer, Ehrlich ascites cells and antioxidants.

Introduction

Cancer is one of the most prevalent groups of disorders in the population in many countries worldwide (1). Cancer is a term describing conditions characterized by uncontrolled cellular proliferation and differentiation (2). Oxidative stress is involved in the process of development of cancer and tumors; due to reactive oxygen species (ROS) that can damage the macromolecules as lipids , react with metals (as free iron and copper) , produce aldehydes and synthesize malondialdehyde inducing mutations (3) or cause breaks in the double chain, produce modifications in guanine and thymine bases, and sister chromatid exchanges (4). The alkaloids represent the largest single class of plant secondary metabolites. They have a remarkable range of often dramatic pharmacological activity, and are also often toxic to man (5). Many alkaloids are used in therapeutics and as pharmacological tools. A wide range of biological effects has been reported for alkaloids, including antitumor and anti-inflammatory activities (6). Colchicine is an alkaloid drug, chemically known as N-[(7S)-1, 2, 3, 10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a] heptalen-7-yl] acetamide, and widely used for the treatment of gout disease (7).

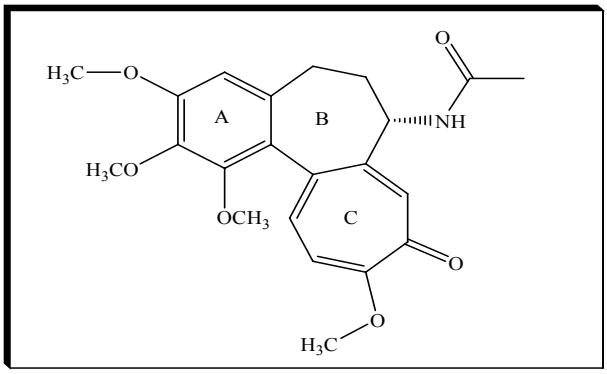


Figure. 1: Structure of colchicines

Colchicine has a high market value and consistent demand in the field of medicine (8). The alkaloid, colchicine is the drug of choice to relieve acute attack of gout, familial Mediterranean fever (9) and a cure for cancer related diseases (10, 11). Also, colchicine, a recognized liver protector which prevents the assembly of cytoplasmatic microtubules, inhibits the transcellular movement of collagen (12, 13), stimulates the production of collagenase in cultures of synovial tissue (14) and exerts a stabilizing effect on the plasma membranes of the hepatocyte (15). It prevents infiltration reverses $CC1_4$-induced liver cirrhosis in rats (16, 17).

Not surprisingly, alkaloid such as colchicine contains 4 methoxy groups are excellent scavengers of ROS and represent promising anti-tumor effects. *In vivo* tests have been conducted with colchicine to determine for example, its hepatoprotective (16), anti-inflammatory (7) and *in vitro* anticancer activity (11). To our knowledge, there are no reports about *in vivo* antitumor activity of colchicine. The present study aimed to evaluate the antitumor activity of colchicine as well as compared to 5-Flourouracil in female albino mice.

Materials and Methods

Mice

This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of October 6 University. Adult mice weighing around 25 ± 2gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were individually housed in cages in an air-conditioned room with a temperature of 22 ± 2ºC, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet *ad-libitum*.

Chemicals

5-fluorouracil and colchicine were obtained from Merck Ltd., Germany. All the other reagents used were of analytical grade and were obtained commercially.

Experimental design

EAC cells were obtained from the National Cancer Institute, Cairo University. The cells maintained *in vivo* in Swiss albino mice by subcutaneous transplantation ($2x10^6$ cells per mouse) to the animals of all groups except the first group (18).

The animals enrolled in the present study were divided into 4 groups, each group consists of 8 animals:

Group (1): Control negative non tumor bearing mice (TB), received 2ml saline.

Group (2): EAC control (tumor bearing mice (TB)) received 2 ml saline.

Group (3): EAC (tumor bearing mice (TB)) + colchicines (10µg/mice) day after day for 3 weeks after subcutaneous implantation of EAC (19).

Group (4): EAC (tumor bearing mice (TB)) + 5-fluorouracil (20mg/kg) was given by intraperitoneal injection on alternate days for 3 weeks after subcutaneous implantation of EAC (20).

The 8 mice from each group were dissected and the ascites fluid was collected from peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. The tumor weight was measured by taking the weight of mice before and after collection of ascites fluid from peritoneal cavity (21, 22).

At the end of the study, all mice were sacrificed, blood was collected, centrifuged, and plasma was used freshly for estimation of plasma glucose (23). The plasma insulin, progesterone and 17β-estradiol concentration were measured using the insulin ELISA kit (Shibayagi Co. Japan) (24-26, respectively), as well as transaminases (L-alanine and L-aspartate) (27), alkaline phosphatase (ALP) (28). Also, lactate dehydrogenase (LDH) (29), TBARS, Pr-SHs and GSH levels in plasma were done by the methods described by Buhl and Jackson (30), Uchiyama and Mihara (31), Koster, et al., (32) and Chanarin (33), respectively. Plasma Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were carried out Paglia and Valentine (34), Marklund and Marklund (35), respectively. Plasma triglyceride, total cholesterol and HDL- cholesterol were determined using commercially available kits (Asan and Youngdong Pharmaceutical Co., Korea) (36-38). Plasma LDL-cholesterol level was calculated from Friewald et al (39) formula (LDL-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol).

Statistical analysis

All analyses utilized SPSS 15.0 statistical package for Windows (SPSS Inc., Chicago, IL) (40). A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value < 0.05 was accepted as statistically significant with LSD test as the post –hoc test. All the results were expressed as mean ± SD for eight separate determinations.

Results

Injection of colchicine and 5-fluorouracil to mice transplanted with carcinoma resulted in a significant decrease in tumour weight compared to the group that received subcutaneous implantation of EAC (Table 1). The decrease in tumour weight in group of mice which injected with 5-fluorouracil (Group 4) was more pronounced than colchicine injected mice (Group 3) ($p< 0.01$).

Table 1: Effect of Colchicine and 5-fluorouracil on tumor volume and weight

No.	Groups	Tumor weight (gm)
(I)	Normal (Non-tumor bearing mice (NTB)) 2ml saline , 0.9%	0.0 ± 0.00
(II)	EAC control (tumor bearing mice (TB)) 2 ml saline, 0.9%	6.2 ±0.30*
(III)	Colchicine (10µg/kg. b.w. i.p)	2.7 ± 0.40*
(IV)	5-Flourouracil (20mg/kg b.w. i.p)	1.60 ± 0.20*

5-Flourouracil was given i.p. day after day at a 20mg/kg b.w. The test colchicine was given i.p. daily for 3 weeks. Values are given as mean ± SD for groups of eight animals each.

* Significantly different from normal group at $p< 0.01$.

Subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant decrease in plasma glucose and insulin compared to the normal control group (table 2). Intraperitoneal administration of colchicine to mice resulted in a

significant increase in plasma glucose and insulin when compared to the group that received subcutaneous implantation of EAC (p< 0.01). However, i.p. injection of 5-fluorouracil resulted in significant increase of glucose (p< 0.05) and non-significant increase of insulin compared to the group that received subcutaneous implantation of EAC.

Table 2: Level of plasma glucose and insulin in normal and experimental groups of mice

No.	Groups	Glucose (mg/dl)	Insulin (uIU/ml)
(I)	Normal (Non-tumor bearing mice (NTB)) 2ml saline, 0.9%	110.73 ± 6.48	13.44 ± 2.05
(II)	EAC control (tumor bearing mice (TB)) 2ml saline, 0.9%	67.83 ± 8.17*	5.61 ±1.27*
(III)	Colchicine (10µg/kg. b.w. i.p)	101.08 ± 7.41@	10.19 ± 1.56*
(IV)	5-Flourouracil (20mg/kg b.w. i.p)	77.64 ± 5.40@	5.98 ± 1.77

5-Flourouracil was given i.p. day after day at a 20mg/kg b.w. The test colchicine was given i.p. daily for 3 weeks. Values are given as mean ± SD for groups of eight animals each.
* Significantly different from normal group at *p< 0.01*. @ Significantly different from control group at *p< 0.05*.

Tables 3,4 and 5 showed that subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant increase in plasma AST, ALT, ALP, LDH and TBARs as well as decrease in plasma Pr-SHs, blood GSH, SOD and GPx compared to the normal control group (p< 0.01). Intraperitoneal administration of colchicine to mice resulted in a significant decrease in plasma AST, ALT, ALP, LDH and TBARs as well as increase in plasma Pr-SHs (p< 0.01), blood GSH, SOD and GPx (p<.0.05) compared to the group that received subcutaneous implantation of EAC (p< 0.05). However, injection of 5-fluorouracil to mice resulted in a significant decrease in plasma AST, ALT, ALP, LDH and TBARs as well as increase in plasma Pr-SHs (p< 0.05) non significant change in blood GSH, SOD and GPx compared to the group that received subcutaneous implantation of EAC (p< 0.05).

Table 3: Level of plasma alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) in serum of normal and experimental groups of mice

No.	Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
(I)	Normal (Non-tumor bearing mice (NTB)) 2ml saline, 0.9%	27.95± 4.70	32.88 ± 5.22	134.61± 10.07
(II)	EAC control (tumor bearing mice (TB)) 2ml saline, 0.9%	50.16 ± 6.38*	67.08 ±8.32*	274.36 ± 15.91*
(III)	Colchicine (10µg/kg. b.w. i.p)	26.72 ± 5.30*	29.66± 4.24*	152.48± 10.95*
(IV)	5-Flourouracil (20mg/kg b.w. i.p)	35.74± 6.85@	38.00 ± 5.00@	182.22± 13.94@

5-Flourouracil was given i.p. day after day at a 20mg/kg b.w. The test colchicine was given i.p. daily for 3 weeks. Values are given as mean ± SD for groups of eight animals each.
* Significantly different from normal group at *p< 0.01*. @ Significantly different from control group at *p< 0.05*.

Table 4: Levels of plasma lactate dehydrogenase (LDH), lipid peroxides (TBARS) and protein thiols (Pr-SHs) of normal and experimental groups of mice

No.	Groups	LDH (U/L)	TBARS (nmol/ml)	Pr-SHs (µmol/l)
(I)	Normal (Non-tumor bearing mice (NTB))2ml saline, 0.9%	110.37 ± 9.18	40.52± 3.29	152.16± 9.42
(II)	EAC control (tumor bearing mice (TB)) 2ml saline, 0.9%	246.05± 14.23*	92.44 ±7.03*	68.90 ± 7.64*
(III)	Colchicine (10µg/kg. b.w. i.p)	113.05± 10.51*	36.11± 4.60*	129.40± 11.25*
(IV)	5-Flourouracil (20mg/kg b.w. i.p)	135.60 ± 18.19@	52.18± 6.45@	85.40± 9.32@

5-Flourouracil was given i.p. day after day at a 20mg/kg b.w. The test colchicine was given i.p. daily for 3 weeks. Values are given as mean ± SD for groups of eight animals each.
* Significantly different from normal group at *p< 0.01*. @ Significantly different from control group at *p< 0.05*.

Table 5: Level of reduced glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood of normal and experimental groups of mice

No.	Groups	GSH (mg%)	SOD (U/g Hb)	GPx (U/g Hb)
(I)	Normal (Non-tumor bearing mice (NTB))2ml saline, 0.9%	25.46 ± 3.22	62.82± 3.29	95.74± 9.42
(II)	EAC control (tumor bearing mice (TB)) 2ml saline, 0.9%	14.08± 2.97*	42.63 ±3.95*	67.90 ± 4.38*
(III)	Colchicine (10µg/kg. b.w. i.p)	22.13± 4.77@	56.70± 8.05@	92.05± 5.14@
(IV)	5-Flourouracil (20mg/kg b.w. i.p)	12.41 ± 4.10	43.60± 5.21	61.05± 7.33

5-Flourouracil was given i.p. day after day at a 20mg/kg b.w. The test colchicine was given i.p. daily for 3 weeks. Values are given as mean ± SD for groups of eight animals each. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the haemoglobin (Hb) concentration.Values are given as mean ± SD for groups of eight animals each.

* Significantly different from normal group at $p< 0.01$. @ Significantly different from control group at $p< 0.05$.

Tables 6 showed that subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant decrease in plasma cholesterol (TC), triglycerides (TG), HDL-C and LDL-C compared to the normal control group (p< 0.01). Intraperitoneal administration of colchicine to mice resulted in a significant increase in plasma TC, TG, HDL-C and LDL-C compared to the group that received subcutaneous implantation of EAC (p< 0.01). In addition, injection of 5-fluorouracil to mice resulted in a significant increase in plasma TC, TG, HDL-C and LDL-C compared to the group that received subcutaneous implantation of EAC (p< 0.05).

Table 6: Level of plasma total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C of normal and experimental groups of mice

No.	Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
(I)	Normal (Non-tumor bearing mice (NTB)) 2ml saline, 0.9%	124.43 ± 9.61	109.16± 6.39	34.29± 5.05	68.31± 4.32
(II)	EAC control (tumor bearing mice (TB)) 2ml saline, 0.9%	73.76± 10.81*	67.24±5.90*	20.64 ± 3.95*	39.67± 6.22*
(III)	Colchicine (10µg/kg. b.w. i.p)	101.42± 10.23*	92.35± 8.67*	31.22± 5.39 *	51.73± 3.11*
(IV)	5-Flourouracil (20mg/kg b.w. i.p)	81.46 ± 6.85@	59.57± 6.18@	25.68± 4.39@	43.87@± 5.33

5-Flourouracil was given i.p. day after day at a 20mg/kg b.w. The test colchicine was given i.p. daily for 3 weeks. Values are given as mean ± SD for groups of eight animals each. Values are given as mean ± SD for groups of eight animals each. LDL-C (mg/dl) = TC-HDL-[TG / 5],

* Significantly different from normal group at $p< 0.01$. @ Significantly different from control group at $p< 0.05$.

Tables 7 showed that subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant decrease in plasma estrogen and progesterone compared to the normal control group (p< 0.01). Intraperitoneal administration of colchicine and 5-fluorouracil to mice resulted in a significant increase in plasma 17β-estradiol (p< 0.01) and progesterone (p<0.05) compared to the group that received subcutaneous implantation of EAC.

Table 7: Level of plasma 17β-estradiol and progesterone of normal and experimental groups of mice

No.	Groups	17β-estradiol (pg/ml)	Progesterone (ng/ml)
(I)	Normal (Non-tumor bearing mice (NTB)) 2ml saline, 0.9%	12.70 ± 1.35	20.63± 2.54
(II)	EAC control (tumor bearing mice (TB)) 2ml saline, 0.9%	2.54± 0.39*	12.58±2.03*
(III)	Colchicine (10µg/kg. b.w. i.p)	9.21± 3.54*	18.05± 3.20@
(IV)	5-Flourouracil (20mg/kg b.w. i.p)	10.03 ± 1.76*	14.93± 3.71@

5-Flourouracil was given i.p. day after day at a 20mg/kg b.w. The test colchicine was given i.p. daily for 3 weeks. Values are given as mean ± SD for groups of eight animals each. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. @ Significantly different from control group at $p< 0.05$.

Discussion

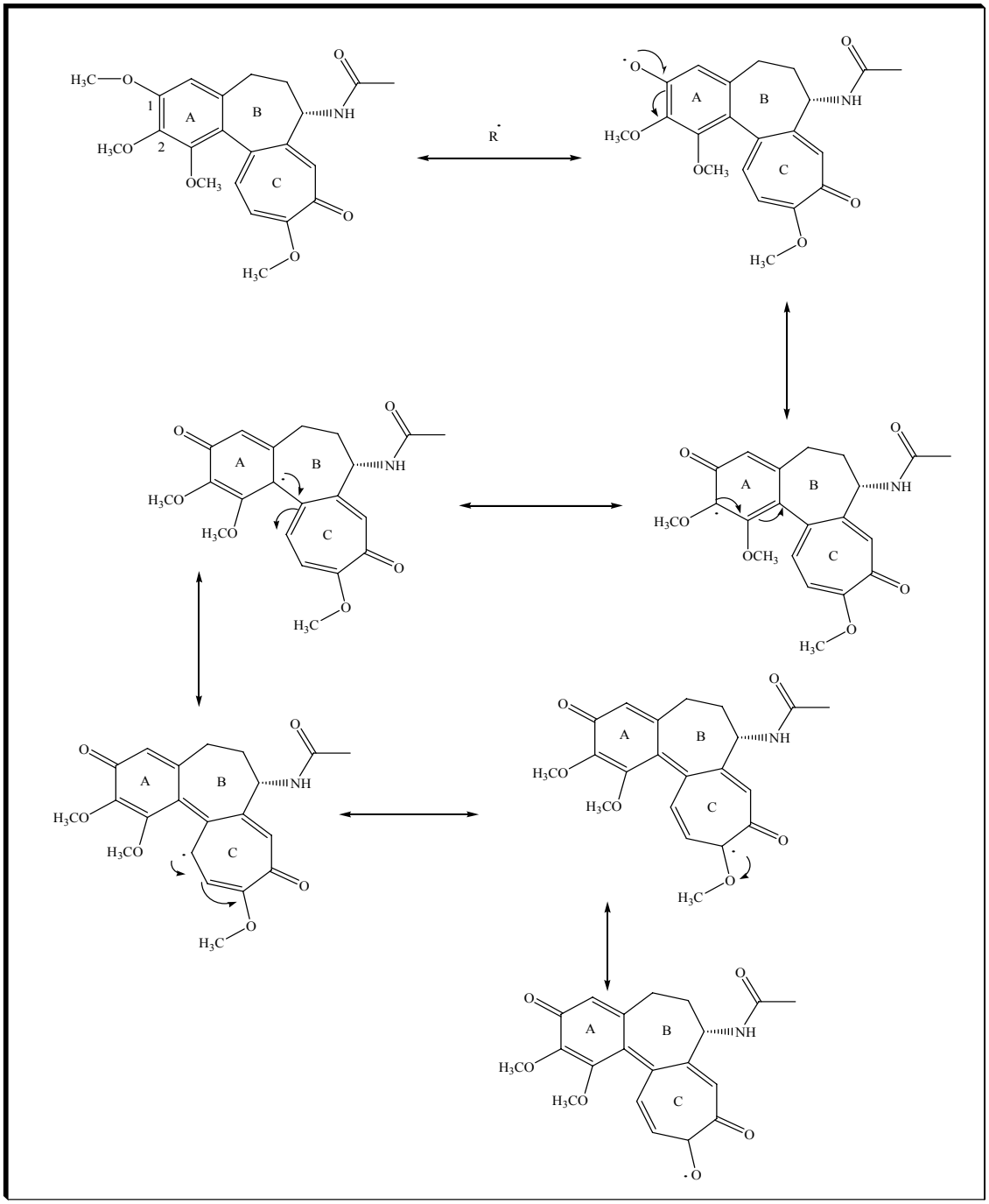
Colchicine, a heterocyclic alkaloid (41) has been used for centuries in acute gout arthritis (42). During the recent decades, it has been employed for an increasing number of disorders such as BD, FMF, liver cirrhosis, dermatologic disorders and scleroderma (43) and free radical generation was found in some of these diseases (44, 45).

The present article aimed to study the antitumor activity of colchicine, 10µg/mice, day after day for 3 weeks in EAC bearing mice compared to 5-Flourouracil 20mg/kg. b.w. a standard antitumor drug. Cancer is a pathological state involving uncontrolled proliferation of tumor cells. Reduced weight of tumor indicated a decrease in abnormal cell divisions, *i.e.* tumor proliferation (46, 47).

The present results showed that EAC implantation caused fall of blood glucose and insulin in EAC control mice. Hypoglycemia was proportional to the number of tumor cells inoculated into the host. One reason for hypoglycemia could be an augmented consumption of glucose by the cells of the tumor (48, 49). Indeed, hypoglycemia was most expressed in mice with large tumors, i.e., with the highest tumor volume and weight due to transport of glucose through the membrane of tumor (50). Facilitated transport of glucose is attributed to the changes of the membrane of tumor cells (51) and increase of insulin-like (glucose-lowering) substances level in the tumor cells, or produces an insulin-like (glucose-lowering) principle itself. Several authors have described higher concentration of insulin-like substances in the plasma of mice with some tumors (52, 53). However, we have found a decrease of insulin activity in the plasma of EAC control group. Supplementation of colchicine and 5-Flourouracil resulted in increase glucose and insulin levels compared to EAC control group. The antioxidant effect of colchicine was investigated by Das et al. (16) which may decrease the rate of glucose and insulin transport to the tumor cells.

Liver is considered to be the main organ of drug detoxifying organ, some liver marker enzyme levels were measured from serum. AST, ALT, ALP, LDH and TBARs levels were increased in EAC controlled mice, whereas Pr-SHs, GSH, SOD and GPx levels were decreased. In the present study, subcutaneous implantation of EAC into the mice resulted in a significant decrease in blood GSH, SOD and GPx as well as plasma TC, TG, HDL-C and LDL-C with a significant increase in plasma TBARs compared to the normal control group. These results were in agreement with Raju and Arockiasamy (54) who reported that the consumption of free amino acid for building the proteins of rapidly dividing tumor cells might result in the disturbance of the enzyme activity in the liver (55). Treatment with colchicine altered liver enzymes level and restored them to that of the normal group.

Alterations of cholesterol metabolism, including increased cholesterol synthesis and accumulation of cholesterol esters in tumor tissues associated with a decrease of high density lipoprotein cholesterol in serum, were previously observed in different models of neoplastic cell proliferation including haematological malignancies. A number of studies had indicated that reactive oxygen species (ROS) are involved in a variety of different cellular processes ranging from apoptosis and necrosis to cell proliferation and carcinogenesis. In this study; there was a significant decrease in levels of GSH, SOD, GPx & Pr-SHs and elevation in liver enzymes and MDA levels of EAC control group. According to our results, it can be stated that the antioxidant activity of colchicine (44, 45) due its structure property, this important property may be responsible for its antitumor activity against EAC *in vivo*. The structural requirement considered essential for effective radical scavenging by colchicine is the presence of *P*-dimethoxy groups at carbon number 1 and 2 in A ring and conjugated double bond. The presence of double bond in A ring makes the electrons more delocalized to form quinone structure which possesses electron donating properties and is a radical target (56) (scheme 1).



Scheme 1: Proposal mechanism of colchicine antioxidant activity

The present work showed that EAC implantation caused fall of plasma sex hormones; 17β-estradiol and progesterone as compared with normal control mice. EAC bearing mice associated with increase receptor population (57, 58) and altered 17β-estradiol and progesterone levels were brought back to normal by colchicine and 5-Flourouracil treatment.

Therefore, from the present study it can be concluded that colchicine showed promising antitumor potential in Ehrlich ascites carcinoma bearing albino mice which can be attributed to its structure requirements.

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