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Original Research

Antitumor potential of some selective medicinal plants on experimental tumor ascites

Mohamed Labib Salem[1], Abeer Abdel-Hamid Khamis[2], Abdel-Halim Abdel-Hady Mostafa[3], Ehab Mostafa Ali[2]

[1]Department of Immunology Zoology, Faculty of Science, Tanta University, Tanta, Egypt, [2]Department of Chemistry, Biochemistry Division, Faculty of Science, Tanta University, Tanta, Egypt, [3]Department of Biochemistry, Faculty of Science, Ain Shams University, Cairo, Egypt

Address for correspondence:
Dr. Mohamed Labib Salem, Department of Immunology Zoology, Faculty of Science, Tanta University, Tanta, Egypt.
Tel.: +20-01274272624.
Fax: +20-40-3350804.

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ABSTRACT

Background: Conventional anticancer chemotherapy is limited due to the associated with toxicity. Exploring new agents which can lower toxicity and enhance the efficacy of anticancer drug is of a paramount significance. **Aim:** Evaluate the antitumor activities of extracts of medicinal plants including green tea, chamomile, black cumin seeds, and wheat bran, as well as their capability to lower the toxicity induced by the conventional chemotherapeutic drugs cisplatin (CIS). **Materials and Methods:** The antitumor effects of these extracts were evaluated in *in vitro* and *in vivo* assays using Ehrlich ascites carcinoma (EAC) cells. *In vitro* studies, the cell viability and the expression of apoptosis-related genes Bad, Bax, Bcl-2, and Bcl-xL were analyzed by polymerase chain reaction technique. EAC cells were injected into mice followed by treatment with injection of CIS and oral administration of each extract or in combination. **Results:** The content of polyphenolic compounds in green tea, chamomile, black cumin, and wheat bran were 1200, 315, 35, and 20 mg/100 g of dry weight, respectively. Their 50% of inhibitory concentration values were 13.5, 30, 350, and 1060 mg/mL, respectively. The polyphenolic compounds suppressed EAC viability *in vitro* associated with up-regulation of Bad and Bax and down-regulation of Bcl-2 and Bcl-xL. Treatment of EAC tumor-bearing mice with these extracts associated with increases in the mean survival time of mice as well as increases and decreases in the numbers of dead and live EAC cells, respectively. The antitumor effect of a combinatorial treatment of the extracts together or with CIS was higher than those of single treatment. The hepatic and renal glutathione peroxidase and antioxidant capacity were significantly increased after treatment with the extracts. **Conclusion:** The extracts of these medicinal plants have potential antitumor effects when used in combination and can ameliorate the toxic effect of chemotherapy through antioxidant effects while enhance its antitumor effect through apoptotic effects.

KEY WORDS: Apoptosis, black cumin, chamomile, glutathione peroxidase, green tea, wheat bran

INTRODUCTION

Anticancer chemotherapeutic drugs - such as doxorubicin, 5-fluorouracil, cisplatin (CIS) - are commonly used to treat cancer. Unfortunately, however, treatment with these drugs associates with induction of significant toxicity [1] and as such limit their clinical application. One of the most effective chemotherapeutic agents is CIS which is used against a wide variety of cancers. The mechanisms of CIS-induced cytotoxicity involve inhibition of DNA synthesis, suppression of RNA transcription, and binding of this drug to DNA and non-DNA targets and subsequent induction of cell death through apoptosis, necrosis, resulting in cell cycle arrest. Unfortunately, however, these antitumor effects of CIS are associated with deleterious side effects such as leukopenia, peripheral neuropathy and nephrotoxicity, hepatotoxicity, ototoxicity, neurotoxicity, nausea and vomiting [2].

Because of serious side effects of CIS and other anticancer drugs, several studies have been evaluating the antitumor and antitoxic effects of medicinal plants which are commonly used in traditional medicine [3]. Medicinal plants which contain phytochemicals such as polyphenolic compounds and dietary polyphenols have received tremendous attention due to their recorded beneficial effects in human health. Indeed, polyphenols can induce prevention of degenerative diseases, particularly cancers, cardiovascular diseases, and neurodegenerative diseases [4]. In addition, polyphenols possess immunomodulatory and antioxidant properties, which can mediate anticancer activities. Polyphenols are strong antioxidants that complement and add to the functions of antioxidant vitamins and enzymes as a defense against oxidative stress caused by excess reactive oxygen species (ROS) [5]. Antioxidative effects of medicinal plants are important for prevention and improvement of various diseases relating to

evoking of oxidative stress accompanied by the generation of ROS such as hydroxyl radical, hydrogen peroxide, and superoxide anion changes biological functions for the worse [6].

Among the medicinal plants which have been found to induce the antitoxic effects are *Camellia sinensis* (green tea) [7], *Nigella sativa* (black cumin), *Matricaria Chamomilla* (Chamomile) [8], and *Triticum aestivum* (wheat bran). These medicinal plants contain polyphenolic compounds with variety of active ingredients. Furthermore, these selective plants have a potency to modulate many biological functions, including protection against coronary heart disease by reducing blood glucose levels and body weight [9]. Furthermore, antioxidant-rich polyphenolic fractions isolated from these extracts have been reported to possess anti-inflammatory, antibacterial, and antiviral properties [10,11].

The aim of this study was to evaluate the antitumor activities of combination of four selective medicinal plants including *C. sinensis* (green tea), *M. chamomilla* (chamomile), *N. sativa* (black cumin), and *T. aestivum* (wheat bran), with distinct active ingredients and test whether their coadministration with conventional chemotherapy lower the required chemotherapeutic dose and as a consequence the associated with toxicity.

MATERIALS AND METHODS

Mice

A total of 168 adult male Swiss albino mice, weighing 20 g, were purchased from the breeding unit of Egyptian Organization for Biological Products and Vaccines (Abbassia, Cairo, Egypt). The animals were housed in steel mesh cages and maintained for 1 week acclimatization period on commercial standard and pellet diet and drinking water *ad libitum*. The housing cycle was 12:12 h light-dark cycle under controlled temperature (20-22°C). The use of mice in all experiments was according to the guidelines of Zoology Department, Faculty of Science, Tanta University, Egypt.

Chemical and Reagents

Folin–Ciocalteu reagent (2N) was purchased from Sigma Chemical Co., USA. Dimethyl sulfoxide, sodium carbonate (20%), stock biuret reagent, phosphate buffer (0.15 M, pH 7.4), bovine serum albumin, and 2, 2 diphenyl-1-picrylhydrazyl (DPPH) were purchased from Fisher Scientific UK. Complete RPMI-1640 with L-glutamine media supplemented with 10% heated-inactivated fetal bovine serum and 1% penicillin/streptomycin was purchased from Sigma Chemical Co., St. Louis, Mo., USA. Trypan blue dye (0.4%): 0.4 g was dissolved in 100 ml sterilized saline (Sigma Chemical Co., USA). CIS (1 mg/ml; cytoplatin-50) was purchased from Verna Industrial Estate (Goa, India). 2.2, 4, 6-tripyridyl-S-triazine (TPTZ), $FeCl_3.6H_2O$ (20 mmol/L), M.W = 270.3, 0.008 M glutathione (GSH), 0.03 M sodium azide, 0.009 M disodium ethylenediaminetetraacetic acid (EDTA), 0.0018 M H_2O_2 (30%), precipitation solution: 1.67 g glacial metaphosphoric acid, 0.2 g of disodium or dipotassium EDTA and 30 g of NaCl were dissolved in 100 ml distilled water, disodium phosphate solution, 5.5`dithiobis-2-nitrobenzoic acid, RNA purification kit (Fermentas) was purchased from Thermo Scientific Gene JET (Foster City, California, USA) First strand cDNA synthesis was assayed using RevertAid first strand cDNA synthesis kit (Fermentas).

Medicinal Plants

C. sinensis (green tea), *M. chamomilla* (chamomile), *N. sativa* (black cumin), and *T. aestivum* (wheat bran) were purchased from local Egyptian market.

Extraction of Medicinal Plants

Phenolic compounds of green tea and wheat bran were extracted using ethanolic method and methanolic extraction method in black cumin case and water extraction in chamomile case [12]. The polyphenolic compounds of these extracted plants were estimated by Folin–Ciocalteu method [13], in this method phenol react with phosphomolybdic acid in alkaline media, the blue complex (molybdenum blue) was measured against reagent blank at 650 nm. The antioxidant activities (AOA) were measured using (2, 2 DPPH) radical scavenging activity test [14].

In Vitro Study

Antitumor activities of the phenolic extracts against Ehrlich ascites carcinoma (EAC) cells were evaluated by trypan blue assay [15]. EAC cells were adjusted at 1×10^6 EAC cells/well in 6-well plate and then treated in triplicate with phosphate buffered saline (PBS) (control cells), CIS (10, 20, and 40 μg/ml) (reference drug), or phenolic compound (experimental cells) as a dose (1, 10, and 100 μg/ml) for each phenolic extract. Cells were incubated at 37°C in a 5% CO_2 with 95% humidity incubator for 4 h. Then, the viability of EAC cells was determined by trypan blue exclusion assay.

Tumor Model

EAC cell line was used in all experiments in this study. EAC cells were collected from a donor mouse obtained from Egyptian Organization for Biological Products and Vaccines (Abbassia, Cairo, Egypt) bearing EAC in ascetic form. The EAC cells were collected from the peritoneal cavity and suspended in PBS and then washed twice in PBS. Viable EAC cells were counted using trypan blue exclusion assay and then adjusted for the required concentration.

In Vivo Study

EAC cells were counted using trypan blue method and were adjusted at 2×10^6 EAC cells/mouse. Mice were then treated with PBS divided (as a negative control group), CIS (as a positive control group), combination of polyphenolic compounds

(experimental groups), or CIS in combination with polyphenolic combinatorial groups. Each group contained 12 mice. On day 14, 6 mice from each group were euthanized for evaluation of antitumor activities of by counting the total and viable cells by trypan blue exclusion assay. The remaining 6 mice in each of the groups were kept to check the mean survival time (MST) and percentage increase in life span % increase in lifespan (ILS) of EAC tumor-bearing hosts [16]. The MST was monitored by recording the mortality daily and percentage increase % ILS was calculated using the following equations:

$$MST = \frac{\text{Day of first mice death} + \text{Day of last mice death}}{2}$$

$$\%ILS = \left[\frac{\text{Mean survival time of treated group}}{\text{Mean survival time of control group}} - 1\right] \times 100$$

Determination of Hepatic and Renal Total Protein Concentration

Principle

The protein concentration was determined by the Lowry *et al*. [17] method as modified by Ohnishi and Barr [18].

Measuring Antioxidant Parameters

GSH peroxidase (GPx) activity was assayed according to the method of Gross *et al*. [19]. The method relies on the following reaction:

$$2\ GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2\ H_2O$$

The residual GSH concentration was calculated, and the amount of GSH consumed per unit time is a measure of the catalytic activity of GPx. In addition, total antioxidant capacity this method was measured the ferric reducing antioxidant power of liver or kidney homogenate. At low pH, when a ferric tri-pyridyl-triazine (Fe III-TPTZ) complex is reduced to the ferrous form (Fe II), an intense blue color with an absorption maximum at 593 nm develops, and the rate limiting factor of (Fe II-TPTZ), and hence color formation is the reducing ability of the sample according to Benzie and Strain [20].

Apoptosis Assay

The viable EAC cells were counted using trypan blue assay and adjusted at 8×10^6 EAC cells/mL. Cells were seeded in 6-well plate at 1×10^6/mL cells and then incubated with the phenolic extracts as mentioned above. RNA extraction was assayed using Thermo Scientific Gene JET RNA Purification kit (Fermentas) according to the method described by Chomczynski and Sacchi [21], Boom [22], then first strand cDNA synthesis was assayed using RevertAid first strand cDNA synthesis kit (Fermentas) according to the method described by Wiame *et al*. [23]. The reverse transcription reaction product can be directly used in polymerase chain reaction (PCR) applications. After that PCR amplification were performed using AmpliTaq Gold 360 Master Mix. The primers sequences of Bad gene, Bax gene, Bcl-2 gene, and Bcl-Xl gene as following in Table 1.

In a thin-walled PCR tube on ice 2 μl of cDNA from RT reaction (1:1000 dilution) were added to 5 μl of ×10 AmpliTaq Gold 360 buffer then 3 μl of 25 mM $MgCl_2$ were added and 1.5 μl forward primer and 1.5 μl reverse primer and 0.5 μl Taq DNA polymerase then added 360 GC enhancer after that complete volume to 50 μl with water, nuclease-free. A thin-walled PCR tubes were placed in thermal cycler and adjusted to four steps initial denaturation step at 94°C for 3 min one cycle then denaturation step at 94°C for 30 s and annealing step at 58°C for 30 s and extension step at 72°C for 45 s 35 cycles.

After PCR amplification agarose gel electrophoresis were carried out by preparing of agarose gels then melted in microwave 2 μl ethidium bromide was added to gel then agarose was cooled. The combs were inserted, and the agarose gels were poured in gel tray 5-7 mm thick and allowed the gel to harden. Buffer was added to both reservoirs and cover then the comb out of the gel. The real time-PCR products were loaded in agarose gel and electrodes were attached and the power supply was turned on. The gel was lay on the transilluminator.

Statistical Analysis

Data were analyzed using GraphPad InStat software (San Diego, CA, USA). The experimental data were expressed as mean ± standard error mean. The significance of difference among the various treated groups and control were analyzed by means of one-way ANOVA followed by Tukey: Compare all pairs of columns. Acceptable significance was recorded when $P < 0.05$.

RESULTS

Contents of Polyphenolic Compounds in Medicinal Plant and their AOA

The results showed the total concentrations of polyphenolic compounds of green tea, chamomile, black cumin, and wheat bran were 1200, 315, 35, and 20 mg/100 g dry weight gallic acid equivalents (GAE), respectively. The polyphenolic contents were found in green tea > chamomile > black cumin > wheat bran. In addition, the phenolic compounds of these extracts showed potential antioxidant and radical scavenging activities as screened by DPPH assay. The 50% of inhibitory concentration (IC_{50}) values were 13.5, 30, 350, and 1060 mg/mL for green tea, chamomile, black cumin, and wheat bran, respectively. The green tea showed the lowest IC_{50} one Table 2.

Antitumor Activities of Phenolic Extracts against EAC cells

As shown in Figure 1a, the treatment of EAC cells with 10, 20, and 40 μg/ml CIS-induced 88%, 87.5%, and 75% decreases, respectively, in the number of viable cells, indicating that the

Table 1: The primers sequences of Bad gene, Bax gene, Bcl-2 gene, and Bcl-xL gene

Primer	Size	Forward	Reverse
Bad	340	CAGTGATCTGCTCCACATTC (612-632)	TCCAGCTAGGATGATAGGAC (952-932)
Bax	174	CTGCAGAGGATGATTGCTGA (225-245)	GATCAGCTCGGGCACTTTAG (399-379)
Bcl-2	472	GCTACGAGTGGGATACTGG (79-98)	GTGTGCAGATGCCGGTTCA (551-532)
Bcl-xL	417	AGGATACAGCTGGAGTCAG (77-97)	TCTCCTTGTCTACGCTTTCC (494-474)

antitumor effects of CIS against EAC cells is dose-dependent, We then tested the antitumor activities of the phenolic extracts of green tea, chamomile, black cumin and wheat bran against EAC cells at different concentrations ranged from 1, 10, and 100 μg/ml of the each extract. At these concentrations, treatment with green tea induced 83.3%, 80%, and 66.6%, respectively [Figure 1b], chamomile induced 83.3%, 83.3% and 60%, respectively [Figure 1c], black cumin induced 71%, 66.6% and 66.6%, respectively [Figure 1d], while wheat bran induced 87.5%, 71%, and 66.6%, respectively [Figure 1e] as compared with the control group.

Antitumor Effect of Polyphenolic Compounds against EAC Cells in Swiss Albino Mice

Treatment with CIS alone or in combination with green tea, chamomile, black cumin, or wheat bran resulted in significant decreases ($P < 0.001$) in the numbers of viable EAC cells as compared untreated EAC-bearing mice [Figure 2a and b]. These antitumor effects were associated with increases in the MST and in the life span (%ILS) as compared to that in EAC control group [Figure 3].

GPx Activity and Total Antioxidant Capacity

Treatment with CIS alone induced a significant decrease in the hepatic GPx activity ($P < 0.001$) as compared to control group. On the other hand, the hepatic GPx activity was significantly ($P < 0.001$) increased after treatment with combination with the phenolic compounds by 12%, 45%, and 65%, respectively, as compared to that in normal control group. Furthermore, the hepatic GPx activity decreased after treatment with CIS in combination with chamomile, black cumin or wheat bran by 22%, 30%, and 37%, respectively, as compared to that in normal control group [Figure 4].

In addition, treatment with CIS alone induced a significant decrease in the renal GPx activity ($P < 0.001$) as compared to control group. Furthermore, the renal GPx activity decreased in tumor-bearing mice and CIS treated mice groups by 16%, 33%, and 66%, respectively, as compared to that in normal control group. On the other hand, the renal GPx activity was significantly ($P < 0.001$) increased after treatment with combination with the phenolic compounds. Moreover, its activity increased after treatment with green tea and chamomile by 33% as compared to that in normal control group [Figure 5].

Treatment with CIS alone induced a significant decrease in the hepatic total antioxidant capacity ($P < 0.001$) as compared to the control group. In addition, hepatic total antioxidant capacity decreased in tumor-bearing mice and CIS treated mice groups by 24%, 53%, and 84%, respectively, as compared to that in normal control group. On the other hand, hepatic antioxidant capacity was significantly ($P < 0.001$) increased after treatment with combination with the phenolic compounds. Moreover, hepatic total antioxidant capacity increased after treatment with green tea and black cumin by 10% as compared to that in normal control group [Figure 6].

Table 2: Polyphenolic compounds concentration of green tea, chamomile, black cumin, and wheat bran (mg/100 g d. wt) and (IC_{50}) of these compounds

Medicinal plant	Concentration of polyphenolic compounds (mg/100 g d. wt) *	IC_{50} (mg/ml)**
Green tea	1200	13.5
Chamomile	315	30
Black cumin	35	350
Wheat bran	20	1060

*d. wt: Dry weight, **IC_{50}: 50% of inhibitory concentration

Furthermore, treatment with CIS alone induced a significant decrease in the renal total antioxidant capacity ($P < 0.001$) as compared to the control group. In addition, renal total antioxidant capacity decreased in tumor-bearing mice and CIS-treated mice groups by 15%, 43% and 60%, respectively, as compared to that in normal control group. On the other hand, renal antioxidant capacity was significantly ($P < 0.001$) increased after treatment with combination with the phenolic compounds. In addition, the renal total antioxidant capacity level increased after treatment with chamomile and black cumin by 5% as compared to that in normal control group [Figure 7].

Apoptotic Effect of Polyphenolic Extracts

The results showed to increase the expression of Bad and Bax genes as pro-apoptotic gene in EAC cells that treated with phenolic compounds (green tea, black cumin, chamomile, and wheat bran) extracts [Figure 8a and b]. However, the results showed decrease the expression of Bcl-2 and Bcl-xL genes as antiapoptotic gene in EAC cells that treated with phenolic compounds (green tea, *N. sativa*, chamomile and wheat bran) extracts [Figure 8c and d].

DISCUSSION

Conventional anticancer chemotherapy is limited due to the associated with toxicity. Exploring new agents which can lower toxicity and enhance efficacy of anticancer drug is of a paramount significance. With this goal in mind, this study was designed to compare the antitumor activities of extracts of medicinal plants including green tea, chamomile, black cumin seeds and wheat bran, individually or in combination as well

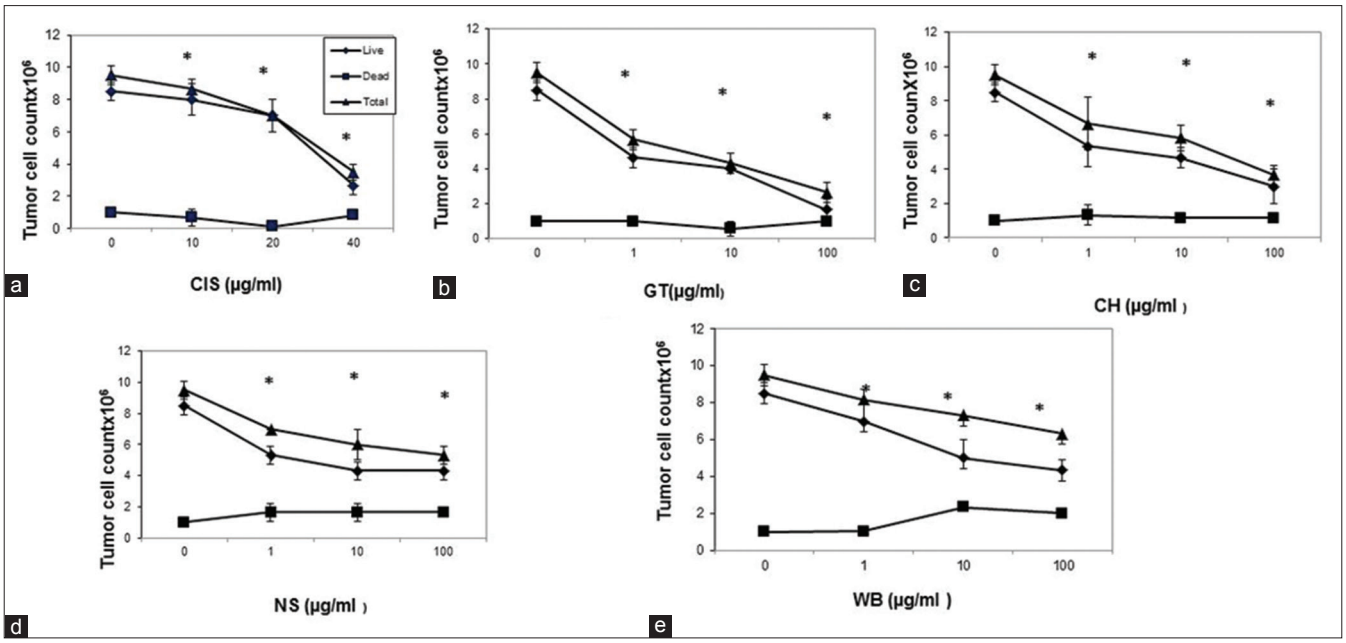

Figure 1: (a-e) Anti-tumor effect of different dose of (a) cisplatin, (b) green tea, (c) chamomile, (d) Nigella sativa, (e) wheat bran against EAC cells. EAC cells were cultured with different doses of extracts and cisplatin and incubated for 4 hr and evaluated by trypan blue method. *: p<0.001: versus EAC control group

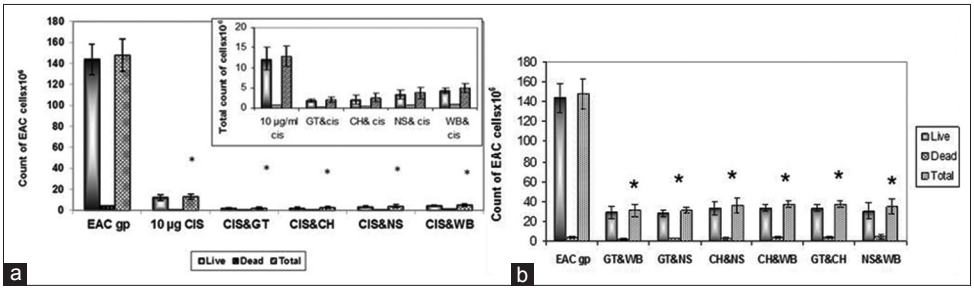

Figure 2: (a) Anti-tumor effect of phenolic extracts (green tea &chamomile&Nigella sativa and wheat bran, at the dose of 100 mg/kg body weight combined with 10 µg/mouse cisplatin. (b) Antitumor effect of phenolic extracts (green tea & chamomile&Nigella sativa and wheat bran combinatorial at the dose of 100 mg/kg body weight combined with each other. *: p<0.001: versus EAC control group.

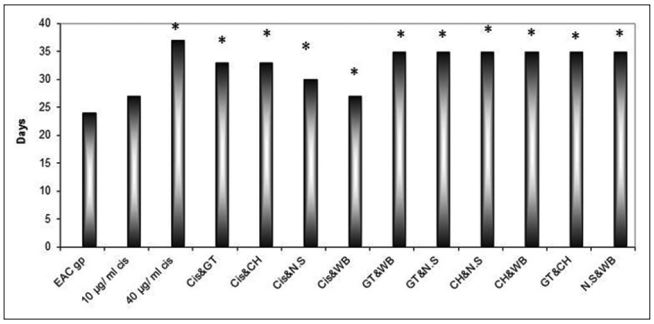

Figure 3: (a) Anti-tumor effect of phenolic extracts (green tea &chamomile&Nigella sativa and wheat bran, at the dose of 100 mg/kg body weight combined with 10 µg/mouse cisplatin. (b) Antitumor effect of phenolic extracts (green tea & chamomile&Nigella sativa and wheat bran combinatorial at the dose of 100 mg/kg body weight combined with each other. *: p<0.001: versus EAC control group.

as their capabilities to lower the toxicity induced by CIS. We have chosen these medicinal plants since their tested parts are known to contain polyphenolic compounds even though they have different active ingredients. In addition, these plants were selective based on their reported beneficial effects to several biological functions *in vitro* and *in vivo*.

Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials through induction of AOA [24]. As such, we first measured the AOA of these extracts by using DPPH radical scavenging activity. We found that total polyphenolic compounds in the tested plant parts, we found that green tea showed the highest value than the other three plants. Precisely, the total phenolic content of green tea was 1200 mg relative to the total phenolic extracts GAE/100 g of dry weight of these plants. This value is higher than the previous studies which reported that the total phenol content of green tea is 769.8 mg equivalent to GAE/100 g dry weight [25]. The total phenolic content of chamomile, black cumin and wheat bran were 315, 35, and 20 mg, respectively.

To understand whether the phenolic contents of these compounds correlate to their antitumor effects, we first

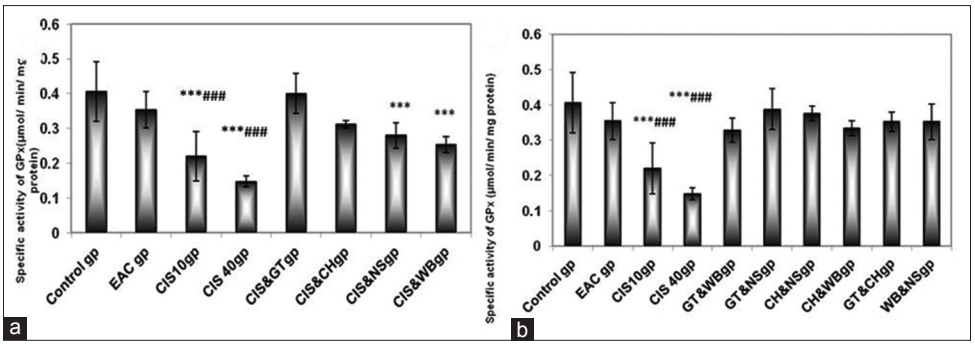

Figure 4: A- Hepatic glutathione peroxidase (GPx) activityof cisplatin with phenolic compounds groups, B- Glutathione peroxidase (GPx) activity in phenolic compound combinatorial groups,*: p<0.05: versus normal control group,***: p<0.001: versus normal control group, ###: p<0.001: versus EAC control group

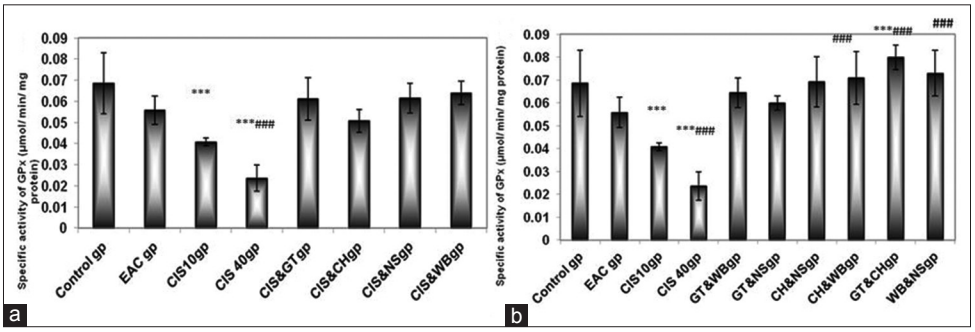

Figure 5: A- Hepatic glutathione peroxidase (GPx) activityof cisplatin with phenolic compounds groups, B- Glutathione peroxidase (GPx) activity in phenolic compound combinatorial groups,*: p<0.05: versus normal control group,***: p<0.001: versus normal control group, ###: p<0.001: versus EAC control group

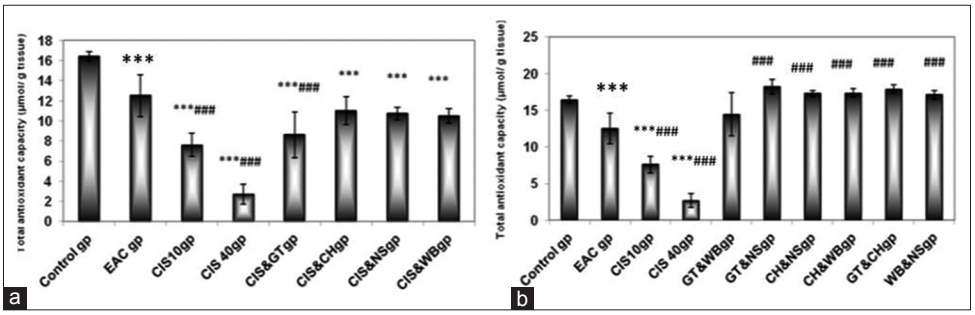

Figure 6: A- Hepatic total antioxidant capacity of cisplatin with phenolic compounds groups, B-Total antioxidant capacity in phenolic compound combinatorial groups, ***: p<0.001: versus normal control group, ###: p<0.001: versus EAC control group.

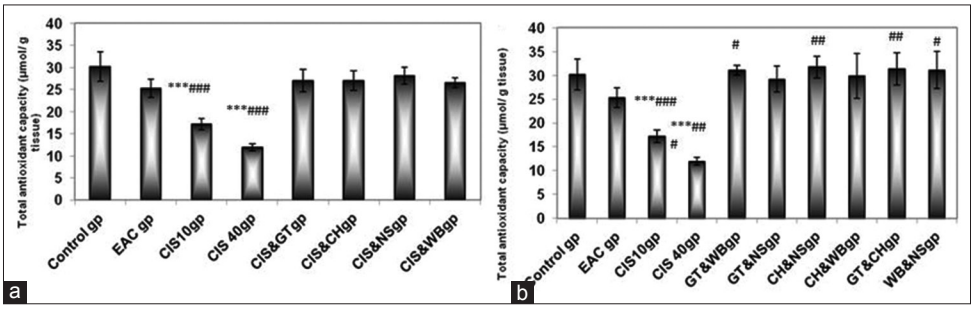

Figure 7: A- Renal total antioxidant capacity of cisplatin with phenolic compounds groups, B- Total antioxidant capacity in phenolic compounds combinatorial groups, #: p<0.05: versus control EAC group, ##: p<0.01: versus EAC control group, ***: p<0.001: versus normal control group, ###: p<0.001: versus EAC control group.

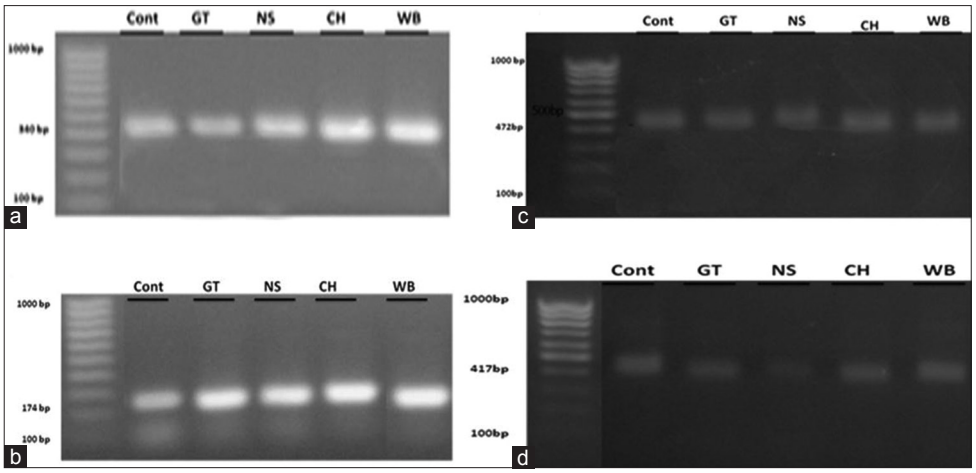

Figure 8: Detection of the effect of four phenolic compounds on expression of (a) Bad (b) Bax (c)Bcl-2 and (d) Bcl-xL gene in EAC cells.

compared their IC_{50} against Ehrlich ascites carcinoma cell line. Consistent with its highest total phenolic content and the greatest free radical scavenging activity, green tea also showed the lowest IC_{50} as compared to other extracts where it reached values of these 13.5 mg/ml versus 30 mg/ml, 350 mg/ml and 1060 mg/ml for chamomile, *N. sativa* and wheat bran, respectively. These results indicate that the total phenolic content correlates well with the antioxidant activity. One reason behind this correlation could be due to the fact that the mechanism of the antioxidant action of phenolic compounds resides mainly in their ability to donate electrons or hydrogen atoms. Polyphenols possess ideal structural chemistry for this activity a catechol group and the number and position of the hydroxyl group in the molecules which consider effective scavengers [26].

The present investigation then compared the antitumor activity of the phenolic extracts on EAC cells *in vitro* and *in vivo* studies. First, *in vitro* it was found that the numbers of viable EAC cells treated with 100 μg/ml of the each extract decreased as compared to control group. Interestingly, the total numbers of EAC cells significantly decreased even after treatment with 1 μg/ml of green tea and chamomile; their antitumor activity was higher than the suboptimal concentrations of CIS including of 10 and 20 μg/ml.

Although the antitumor effects of the phenolic compounds have not been well determined, it is suggested that they lyse the cells by direct cytotoxic mechanism [27], and induce chemopreventive effects against tumor through their effects on signal transduction in cell proliferation and angiogenesis and induce apoptosis [28]. *In vivo* studies, the total numbers of tumor EAC cells decreased in mice treated with any of the tested extracts at dose 100 mg/kg body weight which when combined with the suboptimal dose of CIS (10 μg/mouse) as compared to CIS alone. The reliable criteria for judging the value of any anticancer drugs are prolongation of the life span of animals. With this regard, mice treated with the phenolic extracts plus CIS and in combination of different phenolic extracts increased the MST and life span of the tumor-bearing mice this could be suggested due to the ability of the phenolic extracts to decrease the nutritional fluid volume and arresting the tumor growth. The decreases and increases in the numbers of viable and non-viable tumor cells, respectively, in the tumor-bearing host suggest indicate to the antitumor effect against EAC cells *in vivo*. With this regard, we found in this study that the phenolic extracts of green tea, chamomile, black cumin, and wheat bran increased the numbers of nonviable cells. These data are in line with those reported by Khatune *et al*. [29] who found that the compound that has the ability to suppress the growth of tumor cells more that 75% is considered to be highly anticarcinogenic.

The major intracellular antioxidant enzyme is GPx which catalyzes the reaction of hydroperoxides with reduced GSH to form GSH disulfide (GSSG) and detoxifies hydrogen peroxide to water. Our results showed that hepatic and renal GPx activity in tumor-bearing mice and CIS treated mice significantly decreased as compared to normal control. These results may be explained based on that CIS exerts its toxic effects by inducing the generation of ROS which can interact with cellular membranes to cause lipid peroxidation, and thereby alter the structure and function of membranes [30].

On the contrary, the present finding showed a significant increase in the hepatic and renal GPx activity in mice treated with polyphenolic compounds alone or in combination with CIS. These results are in line with a previous study [31] which reported increases in the hepatic GPx activity in wheat bran treated animals as associated with recovery of the antioxidant status. Our results are in accordance with Dayem *et al*. [32] who reported that the level of GPx back to normal in doxorubicin plus catechin group due to normalization of lipid peroxidation and catechin scavenges hydroxyl radicals which may initiate lipid peroxidation.

This study indicated that the hepatic and renal total antioxidant capacity in tumor-bearing mice treated with CIS significantly decreased as compared to those in control. In contrast, the total antioxidant capacity was significantly increased in mice treated with polyphenols alone or in combination with CIS. These results are consistent with Mahgoub *et al*. [33] who reported

that *N. sativa* and its active ingredient thymoquinone inhibited the non-enzymatic lipid peroxidation in liposomes and have appreciable antioxidant and free radical scavenger properties The antioxidant action of *N. sativa* and/or thymoquinone may explain the protective effect of these agents against various hepatotoxic and nephrotoxic models *in vivo* and *in vitro*, as well as liver fibrosis and cirrhosis. With this regard, previous clinical studies showed.

Significant plasma uptake and urinary excretion of polyphenolic in human after consumption of wheat bran where the plasma total antioxidant status was significantly increased Wang *et al*. [31]. Interestingly such study reported also that wheat bran polyphenolics were relatively well absorbed and showed enhanced antioxidant status in human as compared to the other polyphenolic-rich foods.

In vitro study, the results confirmed the antitumor effect of the phenolic extract against EAC cells. In this study, CIS was used as reference drug which has cytotoxic effect and induced apoptosis. It directs apoptosis through a mitochondria-mediated signaling pathway in human oral squamous carcinoma cells, where exposure of cancer cells to CIS leads to the activation of apoptosome complex, which in turn causes the activation of procaspase-9 and procaspase-3; the activated caspase-3 cleaves poly (ADPribose) polymerase-1, resulting in the induction of apoptosis [34].

This study explained the apoptotic effect of phenolic extracts on EAC cells; the results indicated that treatment with phenolic compounds induced apoptosis of tumor cells by stimulated the expression of Bad and Bax genes as apoptotic genes. On contrary, these phenolic compounds downregulated the expression of Bcl-2 and Bcl-xL which are antiapoptotic genes. These results agree with Nishikawa *et al*. [35] study which indicated that oral administration of green tea induces apoptosis of tumor cells *in vivo*, probably by down regulation of Bcl-2 and Bcl-xL in HLE cells. Furthermore, this study suggested that activation of caspase-9 by inhibition of Bcl-2 and Bcl-xL is a major mechanism for induction of apoptosis. In conclusion, phenolic compounds of green tea, chamomile, black cumin, and wheat bran extracts have potent AOA with high activity of GPx. Furthermore, these phenolic compounds have the ability to arrest the tumor growth by a direct cytotoxic effect by inducing apoptosis machinery of tumor cells apoptotic index. Together, they obtained data indicate that these four phenolic extracts are potential anticancer drugs in particular as adjuvant therapy.

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