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Ameliorative Effects of Spirulina and Chamomile Aqueous Extract against Mice Bearing Ehrlich Solid Tumor Induced Apoptosis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AEA and ET designed the study, performed the statistical analysis and wrote the protocol. Authors EEE, AM and MAZ the first draft of the manuscript, managed the literature searches and managed the analyses of the study. 'All authors read and approved the final manuscript.

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ABSTRACT

Aims: Cancer is caused by an imbalance in the rate of proliferation and apoptosis and the positive effect of anticancer therapy is taken into consideration when its ability to initiate apoptosis in cancer cells**.** In vivo; several experimental models established in experimental animals besides the Ehrlich solid tumor (EST), derived from the mouse breast adenocarcinoma. This study aims to determine the curative role of spirulina or/ and chamomile to acting effectively in inhibition of Ehrlich cells growth and tumor development, in addition to the therapeutic activity of spirulina and chamomile aqueous extract on Ehrlich tumor in the solid forms induced apoptosis in solid tumor.

Study Design: A total of 80 mice were equally divided into eight groups (Group 1, Control group; Group 2, spirulina group; Group 3, Chamomile group; Group 4, spirulina and chamomile group; Group 5, Ehrlish solid tumor group; Group 6: Mice Ehrlish solid tumor treated orally with spirulina; Group 7, Mice Ehrlish solid tumor treated orally with chamomile; Group 8, Mice Ehrlish solid tumor treated orally with both spirulina and chamomile for 2 weeks).

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Results: In the current study; a significant decrease in tumour volume, an decrease in apoptotic P53, Bax and pro apoptotic Caspase 3 expressions and increased in Bcl2 expressions was detected after the treatment of EST with extract of chamomile and spirulina together and spirulina extract only as compared with the tumour volume of EST group. In conclusion, the present study demonstrated that the combination of spirulina and chamomile has a better effect than each of spirulina or chamomile alone against Ehrlich solid tumor in mice.
Conclusion: Ehrlich solid tumor induced apoptosis by increased P53, Bax, Caspase 3 and decrease Bcl2; treatment of Ehrlich solid tumor with combination of spirulina and chamomile improved these alterations in apoptosis markers.

Keywords: Ehrlich solid tumor; spirulina; chamomile; apoptosis; mice.

1. INTRODUCTION

Cancer is the largest single cause of death in humans and is a cellular malignancy that results in the loss of normal cell-cycle control, such as unregulated growth and the lack of differentiation, can develop in any tissue of any organ, and at any time [1-3]. Cancer, the most awful disease found among people, is a class of diseases designated by uncontrolled cell growth. More than 100 different types of cancer exist, and each is categorized by the type of cell initially affected. In many countries, the second most common cause of death after cardiovascular diseases is cancer [4].

Chemotherapy and radiation are the most approaches used in cancer treatment, to kill cancer cells by inducing apoptosis; however, cancer cells often develop resistance to these types of therapies and induced various side effects of vital organs [5-12]. Apoptosis has been accepted as a fundamental component in the pathogenesis of cancer and the origin of cancer involves deregulated cellular proliferation and the suppression of apoptotic processes, ultimately leading to tumor establishment and growth [13-16]. Cancer is caused by an imbalance in the rate of proliferation and apoptosis and the positive effect of anticancer therapy is taken into consideration when its ability to initiate apoptosis in cancer cells [17,18].

Apoptosis, also defined as programmed cell death, is a mechanism that plays a role in immune regulation and normal tissue homeostasis, cellular differentiation, and development [10,19,20]. Apoptosis is a mode of cellular death based on a genetic mechanism that induces a series of cellular, morphological and biochemical alternations leading to cell suicide [19,21-24]. Apoptosis usually takes place at a specific moment in normal embryonic development to allow the definitive form of tissues and in adult life to discard cells that no longer have a function or have an altered function [25].

Furthermore, many cancer therapies indirectly activate apoptosis by chemically or physically damaging DNA. Breast cancer is the most public cancer amongst women world-wide (1.38m new cases/year, 23% of all cancers). Graded as fifth cause of death (the first in women) from cancer overall (45800 deaths). In Egypt, it represents almost 37% of cancer in women (18% overall) [1,26]. Breast cancer is the most prevalent cancer between women it ranks second cause of death [27]. In vivo; several experimental models established in experimental animals besides the Ehrlich solid tumor (EST), derived from the mouse breast adenocarcinoma which is an aggressive and fast growing carcinoma capable of develop both in the solid or in the ascetic form depending whether inoculated subcutaneously or, intraperitoneously respectively [3,28,29]. The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma [2]. Recently, a number of plants have been revealed to have important antioxidant action which may be a significant property of pharmaceutical plants related with the treatment of numerous ill-fated diseases [18,24,30-34]. Phytochemicals exist as natural compounds that have antioxidant, antiinflammatory and it have been accepted as one of the main source of cancer chemoprevention drug discovery and development due to their diverse pharmacological properties including cytotoxic and cancer chemo preventive effects [11,35,36]. A number of mechanisms exist by which phytochemicals aid in the prevention of cancer.

Spirulina is a natural “algae” (cyanbacteria) powder that is incredibly high in protein and a good source of antioxidants, B-vitamins and other nutrients [37,38]. Spirulina is rich in protein, vitamins, minerals, carotenoids, and antioxidants that can help protect cells from damage [39]. It

contains nutrients, including B complex vitamins, beta-carotene, vitamin E, manganese, zinc, copper, iron, selenium, and gamma linolenic acid (an essential fatty acid). Kornhauser et al. [40] find that; Beta-carotene may help to protect the skin against the damaging effects of sunlight and help to prevent skin cancers. Ziegler [41] has shown that people whose diets are high in betacarotene have a lower incidence of various cancers. Palan et al. [42] shown that beta-carotene exerts a protective effect against the development and progression of cervical cancer.

Chamomile (*Matricaria chamomilla*) has been one of the most widely used and well-documented medicinal plants for centuries [43]. As a traditional medicine, it is used to treat wounds, ulcers, eczema, gout, skin irritations, neuralgia, sciatica, rheumatic pain, hemorrhoids, mastitis, and other ailments [44].

This study aims to determine the curative role of spirulina or/ and chamomile to acting effectively in inhibition of Ehrlich cells growth and tumor development, in addition to the therapeutic activity of spirulina and chamomile aqueous extract on Ehrlich tumor in the solid forms induced apoptosis in solid tumor.

2. MATERIALS AND METHODS

2.1 Chemical and Reagent

Chamomile: Dry chamomile flowers were purchased from Baroody Imports Inc. (Clifton, NJ).

Preparation of Extracts: Dry chamomile flowers was weighed and crushed to powder with a marble pestle and mortar, and a 5% (w/v) suspension was prepared in a flask by adding hot boiled water. The flask was then placed on a shaker (200 rpm) for 4 h, and the temperature was maintained at 37ºC. After being shaken, the flask was brought to room temperature, and the suspension was filtered through a series of Whatman filters and finally passed through a 0.22 µm filter (Millipore, Billerica, MA). The filtered aqueous extract was freeze-dried and stored at -20ºC until it was used [45].

Spirulina: Spirulina tablets, containing 100% *Spirulina platensis* microalgae powder, were obtained from Allcura Naturheilmittel (Wertheim, Germany). Spirulina tablets were manually crushed, ground, and then suspended in 1% gum acacia in distilled water just before administration according to Barakat et al. [46].

2.2 Transplantation of Tumor Cells and Induction of Ehrlich Solid Tumor (EST)

The Egyptian National Cancer Institute (NCI; Cairo University, Egypt) supplied the mice which had been injected with Ehrlich ascites carcinoma (EAC). These were utilized as the source of EAC cells. 0.2 ml of acitic fluid was aspirated from each EAC bearing mice and diluted with diluted with physiological saline.

Between 2.5 and 3 million EAC cells were injected beneath the skin on the left thigh of each mouse. The presence of the tumor was confirmed by scarfing a select number of mice and the Ehrlich solid tumor was exposed and its size was measured.

2.3 Animals

A total of 80 female CDI mice (aged between ten to twelve weeks old and weighing between 23-25 kg each) were performed for the experiments. They had been obtained from the breeding unit at the Egyptian Organization for Biological Products and Vaccines, Abbassia, Cairo. Free access to normal diet and water supplies was granted to all mice.

2.4 Experimental Design and Animal Groups

The mice were equally divided into eight groups:

Group 1: Control group in which mice did not receive any treatment.

Group 2: spirulina group in which mice received spirulina (300 mg/Kg body weight/ day) orally by stomach tube for 2 weeks according to Barakat et al. [46].

Group 3: Chamomile group included mice that received chamomile (50 mg/kg B.W/2 day) orally by stomach tube for 2 weeks according to Srivastava and Gupta [45].

Group 4: spirulina and chamomile group included mice that received both spirulina and chamomile orally by stomach tube for 2 weeks

Group 5: Ehrlish solid tumor group with induced breast cancer tumor, Ehrlish solid tumor subcutaneous.

Group 6: Mice Ehrlish solid tumor treated orally with spirulina for 2 weeks.

Group 7: Mice Ehrlish solid tumor treated orally with chamomile for 2 weeks.

Group 8: Mice Ehrlish solid tumor treated orally with both spirulina and chamomile for 2 weeks.

2.5 Tumor Sizes

Tumor sizes were determined in all mice, the radii of the developing tumors (EST) were measured every 3rd day from day 8 to day 14, using vernier calipers and the tumor volume was estimated using the formula: $V = 4/3\ r1\ 2\ r2$, where $r1$ and $r2$ represent the radii from two different sites.

The percentage of inhibition of tumor volume in animals = Tumor volume of Control on 14th Day – Tumor Volume of Treated on 14th day / Tumor volume of Control on 14th Day x 100

2.6 Sample Collection

By the end of the experiment, mice were euthanized with intraperitoneal injection with sodium pentobarbital and then underwent total necropsy. Blood samples from each mouse were obtained from the vena cava and gathered in non-heparinised glass tubes before being left for thirty minutes to clot at room temperature prior to their being subject to 5000 rpm centrifugal for ten minutes [47,48]. Sera were separated and stored in aliquots at −80ºC until required. In addition solid tumor of each mouse was removed, weighed and measure volume after labelling samples; they were fixed in 10% buffer neutrals formalin for histology and immunohistochemistry analysis.

2.7 Histological Investigation

Ehrlich tumours removed immediately following necropsy were fixed in 10% neutral buffered formalin. Following the protocol of Tousson [49], fresh isolated tumour from each of the experimental groups was stained by standard haematoxylin and eosin counterstain techniques [50].

2.8 Immunohistochemical Investigation

The rest of tumor sections from different groups were subjected to antigen retrieval by boiling the slides in 500 mL of 9 mmol/L citrate buffer (PH 6) (Invitrogen, CA, USA) for 25 min. Adjacent sections were examined for expression of p53 proteins; anti-apoptotic Bcl2 proteins (Bcl2), and pro-*apoptotic Bax* in solid tumor sections were distinguished using avidin Biotin Complex (ABC; Elite–ABC, Vector Laboratories, CA, USA).

2.8.1 Immunohistochemical detection for apoptotic p53 and caspase 3 markers

Sections were incubated overnight at 4°C after the application of the appropriate primary antibody. Sections incubated with anti-rabbit p53 monoclonal antibody for P53 expression and with anti-rabbit casp3 monoclonal antibody for caspase 3 expression (dilution 1:80; and 1:100 respectively; DAKO Japan Co, Ltd, Tokyo, Japan) according to Tousson et al. [14] and Hafez et al. [51] respectively.

2.8.2 Immunohisto chemical detection for pro-*apoptotic* bax markers

Sections were incubated overnight at 4ºC after the application of the appropriate primary antibody. Sections were incubated with rabbit antimurine/human Bax polyclonal antibody (dilution 1:50; Santa Cruz Biotechnology, Santa Cruz, CA, USA) according to Kondo et al. [52].

2.8.3 Immunohistochemical detection for anti-apoptotic bcl2 markers

Sections were incubated overnight at 4°C after the application of the appropriate primary antibody. Sections incubated with anti-rabbit Bcl-2 monoclonal antibody and with (dilution 1:2000; DAKO Japan Co, Ltd, Tokyo, Japan) according to Tousson et al. [14].

Wholly stained tumor slides were observed by using Olympus microscope and descriptions were captured by digital Cannon 620 camera. Contrast and brightness were attuned using software (Adobe Photoshop; version 7.0; Adobe Systems; Mountain View; CA).

2.9 Statistical Analysis

The analysis was done using the Statistical Package for the Social Sciences (SPSS software version 16). Data were presented as the mean± standard error of the mean (SEM) and statistically analyzed by one-way ANOVA (Analysis of Variance) followed by the Least Significant Difference (LSD) tests. Significance at $P<0.05$ was considered statistically significant. LSD comparisons were performed to assess the significance of differences between groups.

3. RESULTS

3.1 Effect of Chamomile and Spirulina on Ehrlich Tumour Volume

The influence of chamomile and spirulina treatment on the growth and proliferation of Ehrlich cells is determined by comparing the growth-dependent change in tumour volume for the various experimental groups 14 days after subcutaneous injection of the cells. A significant decrease in tumour volume was detected after the treatment of EST with extract of chamomile and spirulina together and spirulina extract only as compared with the tumour volume of EST group (Fig. 1).

3.2 Effect of Chamomile and Spirulina on the Histopathology of Ehrlich Solid Tumours

Histopathological examination of Ehrlich solid tumors (EST) under light microscope revealed that, tumor exhibits compact and aggregation of the tumor tissue cells (polymorphonuclear lymphocytes and neoplastic cells) spread within the muscular tissues and the tumor was surrounded by a well-defined capsule composed by connective tissue (Fig. 2a-c). Tumor sections in treated EST with chamomile showed minimal improvement and depression in tumor structure and large zones of apoptotic cells and small necrotic zones (Fig. 2d-e).

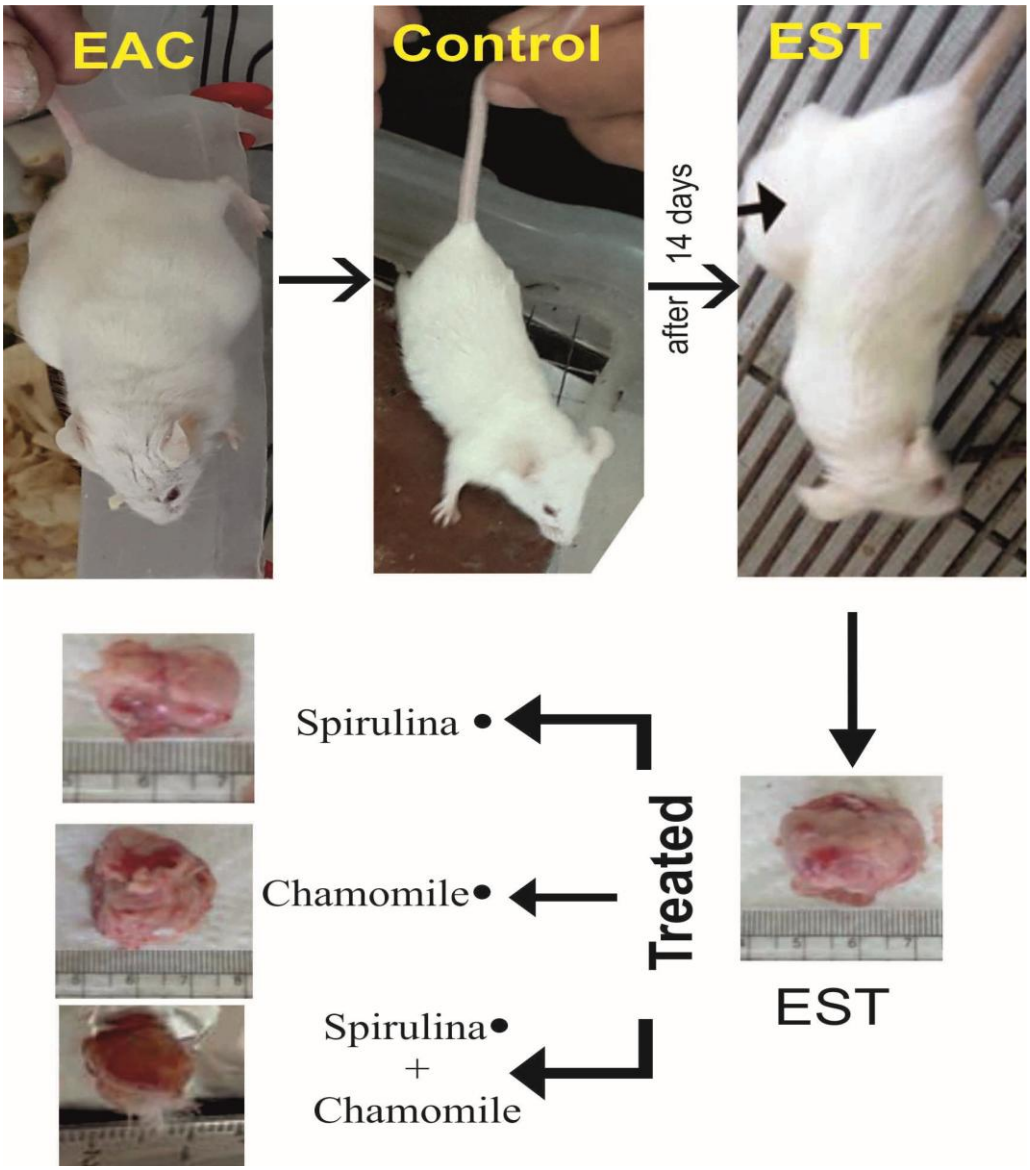

Fig. 1. Effect of chamomile and spirulina on the development of Ehrlich solid tumours volume

In contrast; tumor sections in treated EST with spirulina revealed good improvement and depression in tumor structure and small zones of apoptotic cells and an increase in many necrotic zones (Fig. 2f-h). On the other hand; tumor sections in post treated EST with chamomile and spirulina exhibits a tumor regression with high and wide zones of necrotic cells and other many zones of tumor cells remnants (Fig.2i-l).

3.3 Effect of Spirulina and Chamomile Extract on Apoptotic P53 Expression

Tumor sections in Ehrlich solid tumor (EST) group showed strong positive reaction for P53 expressions (Fig. 3a&b). In contrast, tumor sections in treated EST with chamomile group revealed moderate positive reaction for P53 expressions while tumor sections in treated EST with spirulina group revealed mild positive reaction for P53 expressions (Fig. 3c-f). On the other hand; tumor sections in treated EST with chamomile and spirulina group revealed faint to mild positive reaction for P53 expressions (Fig. 4g&h).

3.4 Effect of Spirulina and Chamomile Extract on Apoptotic Bax Expression

Tumor sections in Ehrlich solid tumor (EST) group showed strong positive reaction for Bax expressions (Fig. 4a&b). In contrast, tumor sections in treated EST with chamomile group revealed strong to moderate positive reaction for P53 expressions while tumor sections in treated

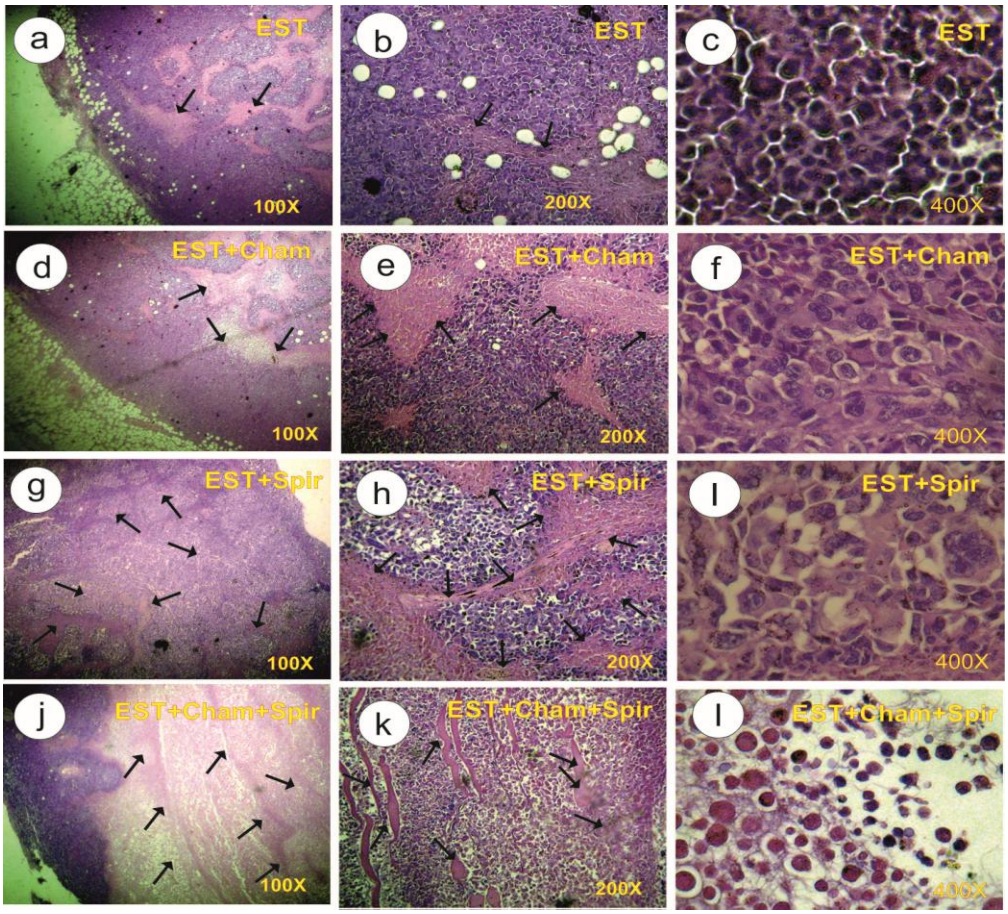

Fig. 2a-l. Photomicrographs of tumour sections stained with haematoxylin and eosin. a-c: untreated EST exhibits compact and aggregation of the tumor tissue cells (polymorphonuclear lymphocytes and neoplastic cells spread within the muscular; d-e: treated EST with chamomile showed minimal improvement, depression in tumor structure and small necrotic zones (arrows). f-h: Tumor sections in treated EST with spirulina revealed small zones of apoptotic cells and an increase in many necrotic zones (arrows). j-l: Tumor sections in post treated EST with chamomile and spirulina exhibits a tumor regression with high and wide necrotic zones (arrows)

EST with spirulina group revealed moderate positive reaction for Bax expressions (Fig. 4c-f). On the other hand; tumor sections in treated EST with chamomile and spirulina group revealed mild positive reaction for Bax expressions (Fig. 4g&h).

3.5 Effect of Spirulina and Chamomile Extract on Pro-apoptotic Caspase 3 Expressions

Tumor sections in Ehrlich solid tumor (EST) group showed strong positive reaction for Caspase 3 expressions (Fig. 5a&b). In contrast, tumor sections in treated EST with chamomile group revealed strong positive reaction for Caspase 3 expressions while tumor sections in treated EST with spirulina group revealed moderate positive reaction for Caspase 3 expressions (Fig. 5c-f). On the other hand; tumor sections in treated EST with chamomile and spirulina group revealed mild positive reaction for Caspase 3 expressions (Fig. 5g&h).

3.6 Effect of Spirulina and Chamomile Extract on Anti-apoptotic Bcl2 Expressions

Tumor sections in Ehrlich solid tumor (EST) group showed mild positive reaction for Bcl2 expressions (Fig. 5a&b). In contrast,

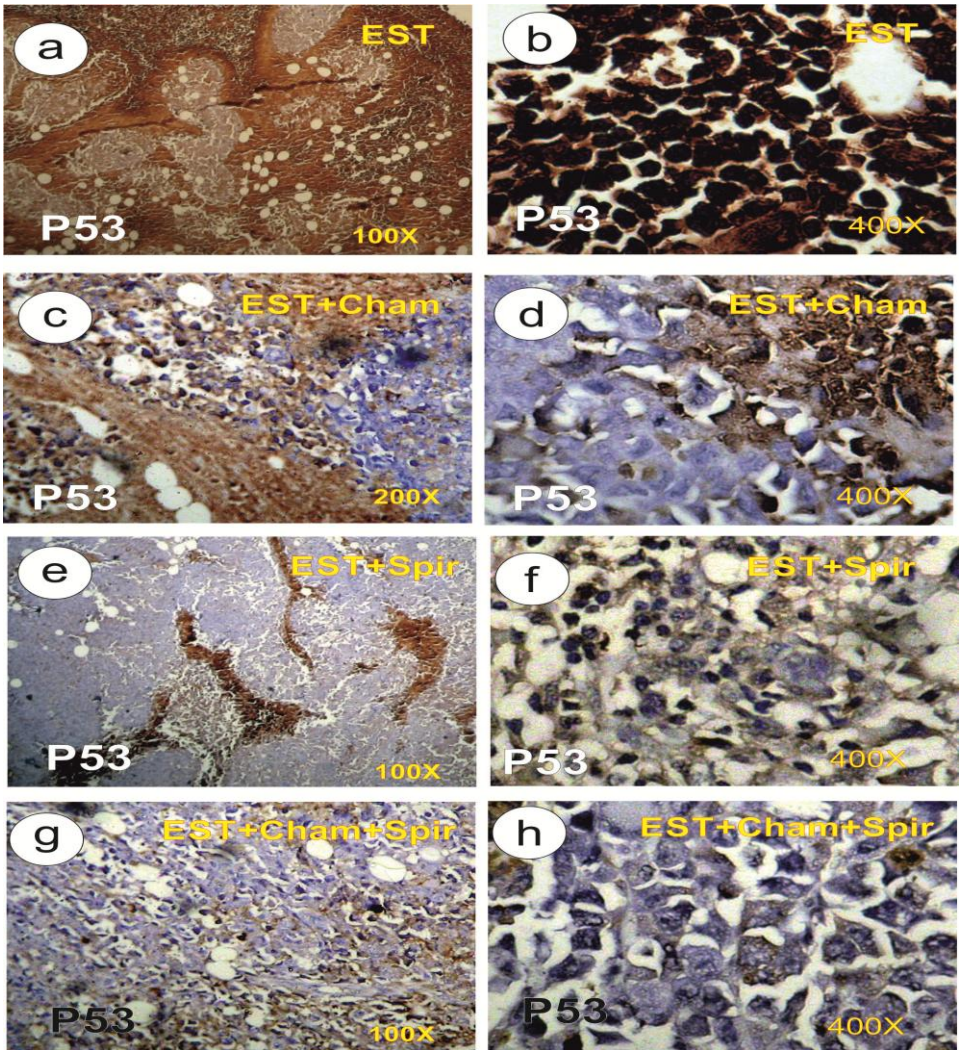

Fig. 3(a-h). Photomicrographs of Tumor sections in different groups stained with P53 expressions. a&b: In EST group revealed strong positive for P53 expression. c&d: In treated EST with chamomile group revealed moderate positive for P53 expression. e&f: In treated EST with spirulina group revealed mild positive for P53 expression. g&h: In treated EST with chamomile and spirulina group revealed mild positive for P53 expression

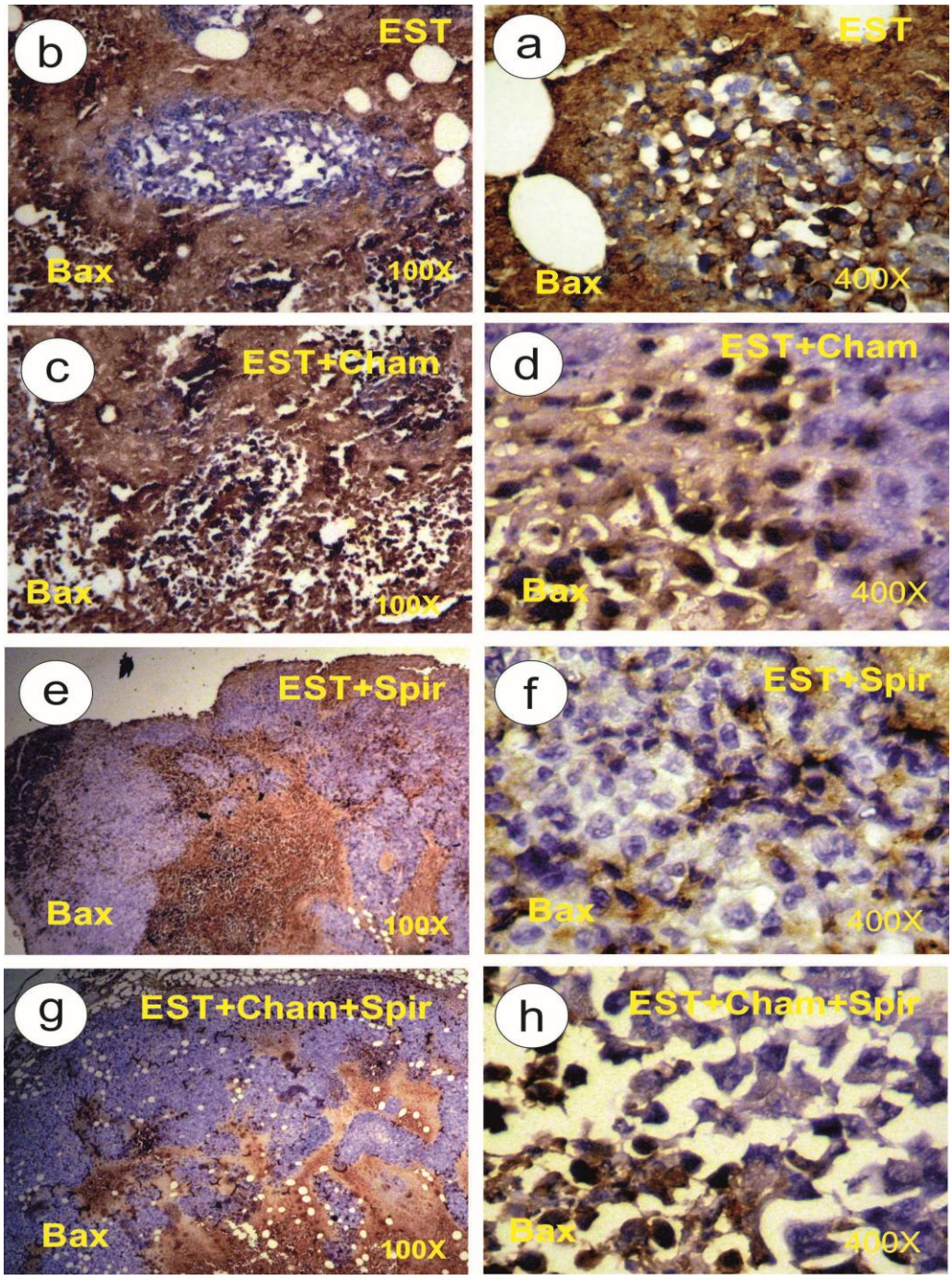

Fig. 4(a-h). Photomicrographs of Tumor sections in different groups stained with Bax expressions. a&b: In EST group revealed strong positive for Bax expression. c&d: In treated EST with chamomile group revealed strong positive for Bax expression. e&f: In treated EST with spirulina group revealed moderate positive for Bax expression. g&h: In treated EST with chamomile and spirulina group revealed mild positive for Bax expression

tumor sections in treated EST with chamomile group revealed mild to moderate positive reaction for Bcl2 expressions while tumor sections in treated EST with spirulina group revealed moderate positive reaction for Bcl2 expressions (Figs. 5c-f). On the other hand; tumor sections in treated EST with chamomile and spirulina group revealed strong positive reaction for Bcl2 expressions (Fig. 5g&h).

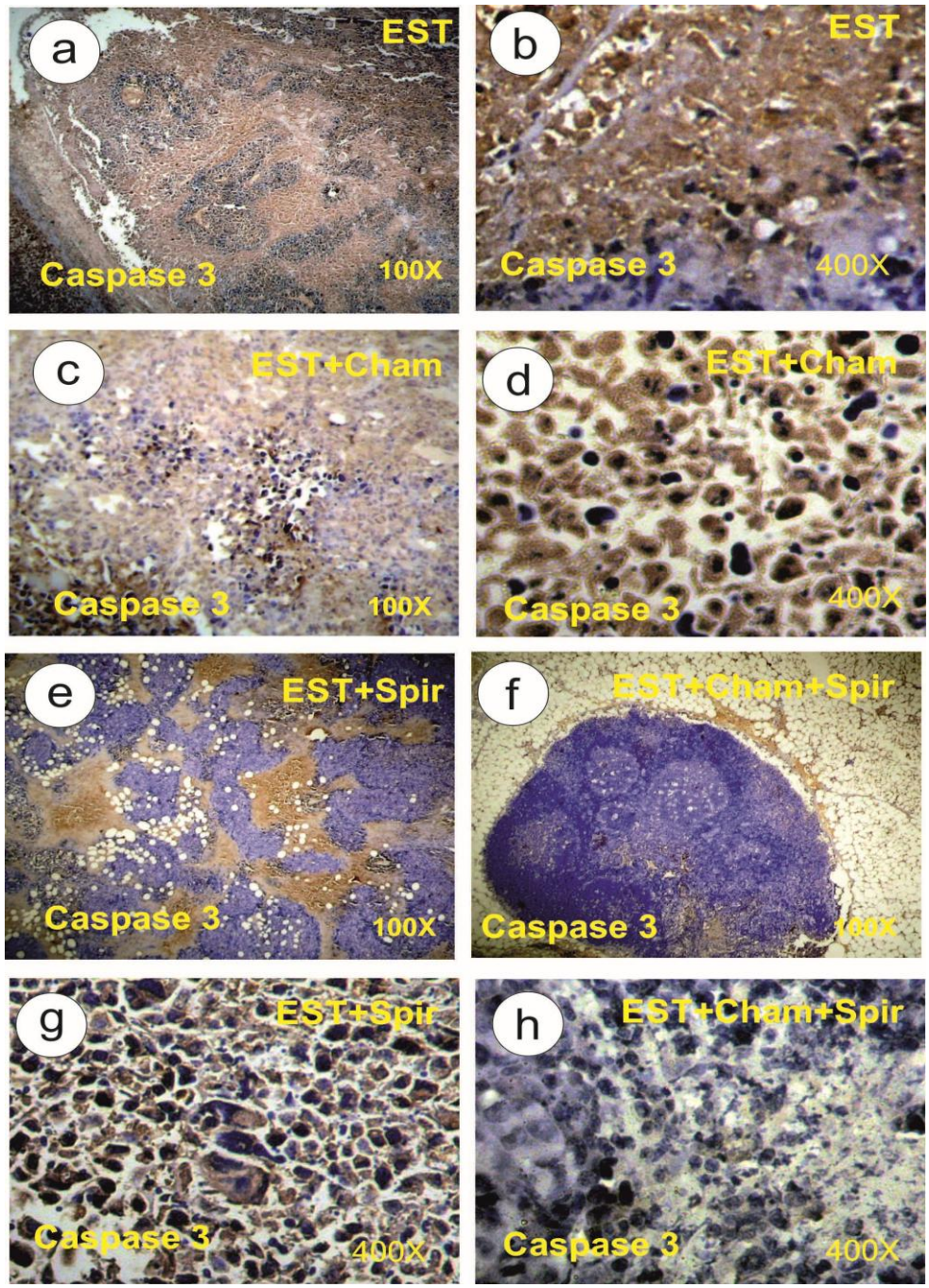

Fig. 5(a-h). Photomicrographs of Tumor sections in different groups stained with Caspase 3 expressions. a&b: In EST group revealed strong positive for Caspase 3 expression. c&d: In treated EST with chamomile group revealed moderate positive for Caspase 3 expression. e&f: In treated EST with spirulina group revealed moderate positive for Caspase 3 expression. g&h: In treated EST with chamomile and spirulina group revealed mild positive for Caspase 3 expression

4. DISCUSSION

Cancer is one of the leading causes of mortality worldwide, according to WHO, more than 10 million new cases of cancer are diagnosed every year, and the statistical trends indicate that this number would double by 2020. Cancer is the uncontrolled growth and spread of abnormal cells, associated with dysregulation of apoptosis, a programmed cell death. Most of the anti-cancer

drugs are derived from plant sources, which act through different pathways aiming to activation of apoptosis in cancer cells.

The treatment of cancer involves surgery, chemotherapy, radiation therapy, hormonal therapy and biological therapy. Thus, efforts have been made to identify both natural and synthetic anticancer and antioxidants. In particular, after the recommendations of the World Health Organization that investigations of antitumor and antioxidants agents from medicinal plants of interest has arisen in plant sources such as vegetables, fruits, and medicinal plants for use as more effective and safe anticancer and antioxidant agents.

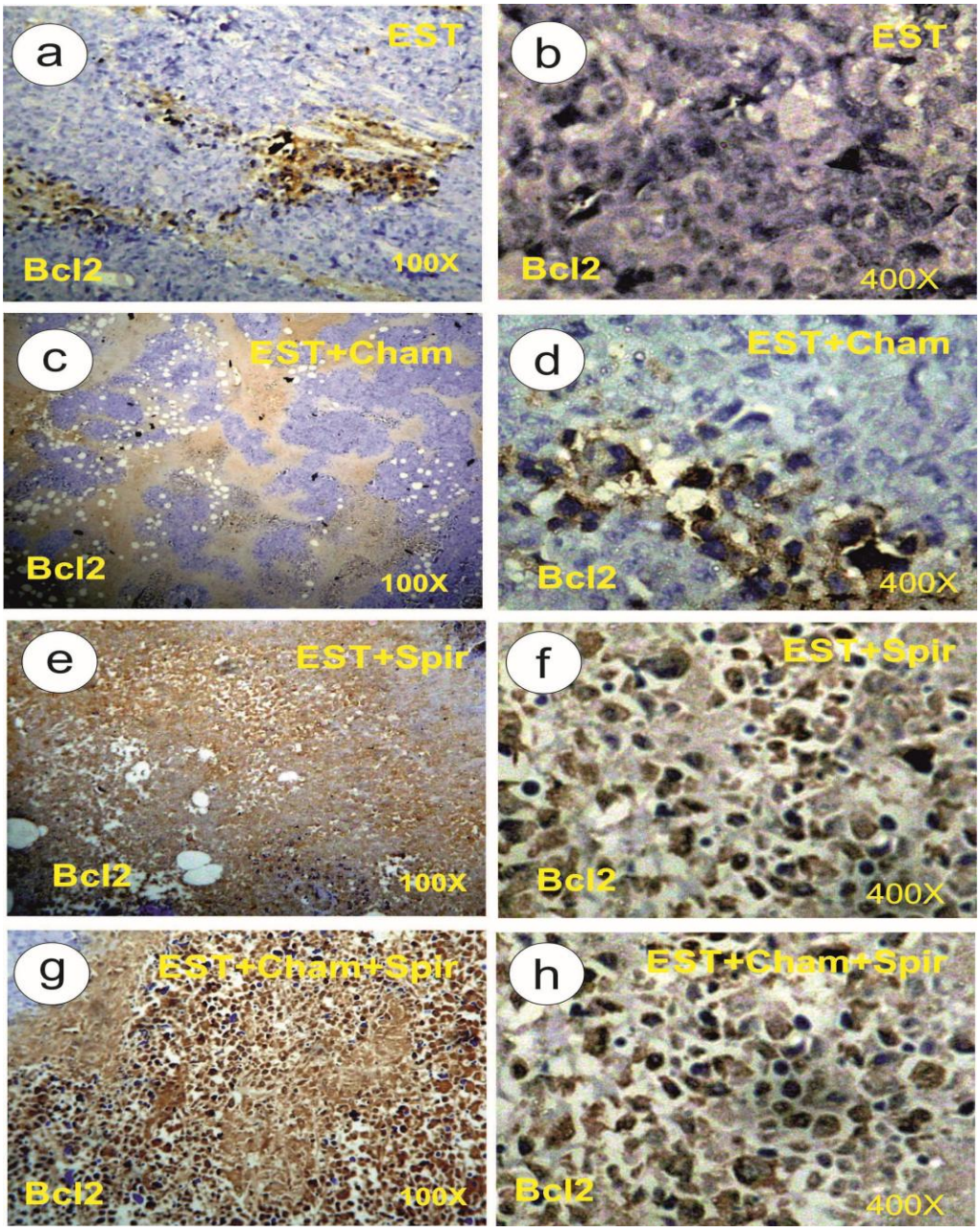

Fig. 6(a-h). Photomicrographs of Tumor sections in different groups stained with Bcl2 expressions. a&b: In EST group revealed mild positive for Bcl2 expression. c&d: In treated EST with chamomile group revealed moderate positive for Bcl2 expression. e&f: In treated EST with spirulina group revealed strong positive for Bcl2 expression. g&h: In treated EST with chamomile and spirulina group revealed strong positive for Bcl2 expression

The use of medicinal plants to inhibit carcinogenesis and to treat cancer is an important and rapidly growing field of cancer research due to less toxicity of natural products compared with the modern chemotherapy [53-55]. Chamomile has been used for centuries as antioxidant, anti-inflammatory, antibacterial and curative medicine [56]. Different kinds of bioactive compounds are existing in chamomile, including phenolic compounds as flavonoids, proved to have the potency to regulate proliferation and cell death pathways leading to cancer via various mechanisms including cell growth inhibition and apoptosis induction [57-59].

Spirulina is a widespread dietary supplement imitative from blue-green algae (cyanobacteria) that naturally grows and is commercially formed in alkaline water pools in tropical and subtropical areas of Asia, America, and Central Africa [60]. Spirulina intake has been linked to hypolipidemic and antioxidant effects as well as neuroprotective and immunomodulatory activity [39,61]. Furthermore, *Spirulina* extracts have been defined as antimicrobial and anticancer agents [62].

Ehrlich tumour first arose spontaneously in the form of breast cancer in mice and has since become the most frequently used transplantable experimental cancer model. Control or untreated mice inoculated with EAC cells intramuscularly in the right thigh of the hind limb developed a palpable solid tumor in two weeks following inoculation, these results are consistent with the reduction of the thickness of the tumor mass observed in these groups. This is consistent with other previous studies that used the same model [18,63]. Vaupel and Mayer [64] who reported that; necrosis is directly associated with tumor progression, increased aggressiveness and metastatic potential. Tumor cell morphology was similar in all groups, with a pronounced pleomorphism, slightly basophilic cytoplasm and oval nucleus, with several evident nucleoli.

There was a marked reduction in tumor size after the treatment of Ehrlich solid tumor with spirulina or/and chamomile the tumor was found to be discontinuous and appeared growing slow and fragmented with best results for the combination of spirulina and chamomile and then for spirulina. This indicates a partial prevention of the effects of EAC cells by spirulina.

Our histopathological results supports these results were minimal improvement and depression in tumor structure and large zones of apoptotic cells and small necrotic zone after the treatment of EST with chamomile in contrast; tumor sections in treated EST with spirulina revealed good improvement and depression in tumor structure and small zones of apoptotic cells and an increase in many necrotic zones. These results indicated that treatment of EST with spirulina more effective than the treatment of EST with chamomile.

On the other hand; treatment of EST with chamomile and spirulina together exhibits a tumor regression with high and wide zones of necrotic cells and other many zones of tumor cells remnants. Our results revealed that spirulina exhibited antitumor activity against Ehrlich solid tumor, presented by the decrease in tumor volume mice. Following treatment of the EST with spirulina, a significant decrease in tumour size was noted. Moreover, the slow-growing, discontinuous and fragmented appearance of the treated tumour, along with the decrease in tumour weight and volume demonstrated the antitumor activity of spirulina against EST in the mice.

These results support the conclusions of Barakat et al. [46] who find that spirulina platensis (200 or 800 mg/kg) inhibit the Solid Ehrlich Carcinoma in Female Mice. The current results agree with Yogianti et al. [65] showed that spirulina exerts antitumor effects against UVB-induced skin tumor development in mice. In addition, it has been reported that spirulina exerts a chemopreventive effect against 7,12-dimethylbenz[a]anthracene-induced breast carcinogenesis and against dibutyl nitrosamine-induced liver cytotoxicity and carcinogenesis in rat [66,67]. Schwartz and Shklar [68] reported that beta carotene in spirulina was found to inhibit both the imitation and promotion of oral carcinogenesis in an initiation-promotion hamster buccal pouch system. Chamorro et al. [69] showed that; Spirulina reduced the size of the tumors and to reduce the incidence of tumors in both skin and stomach tumors.

Apoptosis is a specific mode of cell death recognized by a characteristic pattern of morphological, biochemical, and molecular changes. Most of the existing cancer drugs target apoptosis. Apoptosis plays a very important role in regulating a variety of diseases that have enormous social impacts. For example,

schizophrenia is a neurodegenerative disease that may result from an abnormal ratio of pro- and anti-apoptotic factors. Numerous studies, including El-Masry et al. [25], Tousson et al. [14] and El Barbary et al. [21], have indicated the central role of apoptosis in controlling a range of socially significant diseases. For instance, Tousson et al. [14] noted the key influence of apoptosis upon tumour progression, citing it as the cause of tumour cell death during chemotherapy, immunotherapy and radiation therapy.

In addition, a study by Campisi [70] has correlated enhanced rates of apoptosis with growth inhibition of potentially oncogenic cells. Apoptosis has been accepted as a fundamental component in the pathogenesis of cancer and the origin of cancer involves deregulated cellular proliferation and the suppression of apoptotic processes, ultimately leading to tumor establishment and growth.

Cellular proliferation and apoptosis are important events in carcinogenesis, since tumor growth is related to the balance resulting from the sum of these two events [71]. Furthermore, many cancer therapies indirectly activate apoptosis by chemically or physically damaging DNA. There is some evidence that this defective apoptosis may result from abnormal expression of Bcl-2 and increased expression of caspase-3 [72]. When the cells expose to the external damage stimuli they activate the regulation of expression of these genes. P53 tumor suppressor protein is a transcription factor that regulates the transcription rate of several genes involved in the regulation of cells cycle, DNA repair and apoptosis [5,14].

In the current study; tumor sections in Ehrlich solid tumor (EST) group exhibited strong positive reaction for apoptotic P53, Bax and pro apoptotic Caspase 3 expressions in contrast; mild positive reaction on tumor sections for Bcl2 expressions. Our results decide with Aldubayan et al. [18] who reported that Ehrlich tumor-induced a significant increase in P53 immunoreactivity. Additionally, Eldaim et al. [29] who reported that; EST induced apoptosis and DNA damage in renal tissues.

The pro-apoptotic activity is a very important feature of anticancer agents. It has been shown that phycocyanin may induce apoptosis. Such C-PC activity has been described by Roy et al. [73] in human hepatocellular-carcinoma HepG2. Furthermore, chamomile exposure resulted in differential apoptosis in cancer cells but not in normal cells at similar concentrations [45]. Our results decide with De Heer et al. [74] who found that; the activity of caspase-3 was significantly higher in carcinomas tissue than in the corresponding normal tissue. Also, the activity of caspase-3 is increased in tumor cells due to the inactivation of p53 (tumor suppressor protein), which is responsible for protecting cells from tumorigenic alterations [75]. Increased rates of apoptosis have been associated with inhibition of growth of potentially oncogenic cells [76]. Apoptosis is an important pathway in antitumor drug response [77]. Apoptosis plays an important role in tumor progression, and is reported as the cause of death of tumor cells during chemotherapy, radiation therapy and immunotherapy. For instance, Eldaim et al. [29] and Tousson et al. [10] noted the key influence of apoptosis upon tumour progression, citing it as the cause of tumour cell death during chemotherapy, immunotherapy and radiation therapy.

In the current study; treatment of EST with chamomile or/and spirulina revealed significant decrease in apoptotic P53, Bax and pro apoptotic Caspase 3 and significant increase in Bcl2 expressions in tumor sections. These results indicated that; treatment of EST with chamomile or/and spirulina induced apoptosis. The present study is in line with Baojiang [78] who reported that; inhibition activity of spirulina proteins photo immobilization biomaterial on proliferation of cancer cells.

The present study is in line also with Roy et al. [73] who reported that; C-PC (C-phycocyanin) induces apoptosis in R-HepG2 cells and its potential as an anti-HCC agent. Liu and Zhang [79] reported the effect of polysaccharide from Spirulina indirectly upregulated Bcl-2 expression of hematopoietic cells by promoting endogenous cytokines secretion which may be one of the mechanisms, by which PSP enhanced hematopoietic cell proliferation and inhibited its apoptosis in mice bearing tumor treated with chemotherapy.

5. CONCLUSION

The present study demonstrated that the combination of spirulina and chamomile has a better effect than each of spirulina or chamomile alone against Ehrlich solid tumor in mice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that; all experiments were conducted according to guidelines issued by the Ethical Committee of Faculty of Science at Tanta University and subject to approval by the Institutional Animal Care and Use Committee (IACUC-SCI-TU-0041).

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