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Secondary Metabolites and Biological Activities of *Allium porrum* L. in Attacking Ehrlich Ascites Carcinoma in Mice

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ALLIUM vegetables have been recorded to be used in many medical remedies for protection and fighting diseases. The aim of the study is to investigate the phenolic compounds of methanol extract of *Allium porrum* L. aerial parts (MEAP) and introduce the methanolic extract as a candidate for the treatment of cancer *in vivo* as well as explore its antitumor mechanisms in attacking tumor cells at the molecular level. Isolation of phenolic and flavonoid compounds by column chromatography has been carried out. They were purified by Sephadex LH-20, and the structures were established with the aid of spectroscopic methods (UV, EI/MS, ESI/MS, ^{1}H-NMR, and ^{13}C-NMR spectroscopy). A total of 45 female Swiss albino mice were divided into five groups. One group represents the normal control. The four remaining groups were divided as follows: two groups were injected with Ehrlich ascites carcinoma (EAC) cells intraperitoneally and the other two were injected intramuscularly (to form solid tumor), representing the positive nontreated groups. One group from each model was administrated orally the extract at a dose of 300 mg/kg body weight 3 days weekly for 21 days after 24 h from the intraperitoneal or intramuscular injection of EAC cells. The influence of the extract on the apoptotic proteins were tested in EAC cells and in solid tumor of the treated groups compared with the nontreated ones. Eleven phenolic and flavonoid compounds were isolated from the aerial parts of methanol extract of *A. porrum* L., and characterized as follows: quercetin-3-O-*β*-glucopyranoside-7-O-*α*-rhamnopyranoside, quercetin-3-O-*α*-rhamnopyranoside, quercetin-4'-O-*β*-glucopyranoside, kaempferol-3-O-*β*-glucopyranoside, kaempferol-7-O-*β*-glucopyranoside, quercetin, isorhamnetin, kaempferol, *p*-coumaric acid, ferulic acid, and gallic acid. The gene expressions of caspase-3, Bax, and Bcl2 proteins and DNA fragmentation test were performed in the EAC cells. Hematological profile was conducted on mice's blood in addition to the Immuno-histochemical determination of P53 protein in solid tumors. MEAP has abundant phenolic compounds. It exhibits tumor suppressing mechanisms through the coordination between the apoptic proteins and DNA fragmentation in the EAC or solid cancer cells. MEAP is suggested to be a potent anticancer agent.

Keywords: Phenolic and flavanoid compounds, *Allium porrum* L., Ehrlich ascites, Cell apoptosis, Immunohistochemistry.

Introduction

Allium vegetables have been recorded for many years, to be one of the cheapest and available plants that are used in many medical remedies

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for protection and fighting diseases, including microbial infections, cardiovascular diseases and cancer diseases [1]. Nearly 500 species are included in the Allium genus, the most species that have gotten much of fame are the garlic (*Allium sativum*), onions (*Allium cepa*) and leeks (*Allium porrum*). Many studies recorded the relationship between *Allium* vegetables (especially garlic and onion) consumption and the decrease in the incidence of tumors [2]. Their large content of organosulpher compounds and antioxidants, such as phenolics and flavonoids, are responsible for their anticancer properties [3,4]. *Allium porrum* belongs to the family Liliaceae. It contains large amounts of sulpher compounds. *Allium porrum* consumption reduces the incidence of colorectal, stomach and prostate cancers [5].

One of the important mechanisms for the existence of cancer cells is suppressing their apoptosis. Apoptosis is defined as a mechanism that occurs spontaneously in humans and animals to control the populations of tissue cells. The human body is programmed to remove undesirable cells or damaged cells from the tissues during their growth and metabolic process by apoptosis [6]. Defects in the cell apoptotic mechanisms can lead to many diseases such as cancer disease [7]. Higher indices of cancer cases are reported in the developing countries more than that are found in the developed ones. New trends such as molecular biology and discovering genome's function are directed recently to understand cancer survival mechanisms at their molecular levels.

Molecular biology or genomics focuses on observing the pathogenic events at the genome level in living organisms. Recently, trends of structural and functional genomics are directed for discovering the genes and their structural alterations that are responsible for causing chronic and fatal diseases. Functional genomics are concerned with gene expression levels by applying PCR or sequence based technologies. The goal of performing these trends is translating their research findings into early diagnosis, improved therapy and expectations of human diseases [8].

Tumor cells are highly resistant to apoptosis and use a variety of molecular mechanisms to suppress their apoptosis. This can be achieved by down-regulation of the pro-apoptotic protein Bax, which in turn decreases the tumor suppressor protein P53 and increase the expression of the anti-apoptotic protein Bcl2 [9,10]. New cancer treatment strategies, including modern cytotoxic drugs are now available in the market and have an integrated role with the chemotherapy and induce tumor cell apoptosis and strengthen the phagocytotic mechanisms of the surrounding immune cells [11]. Unfortunately, cytotoxic drugs are accused of causing much harm to the patients as inducing toxicity and killing the normal cells in addition to affording their expensive prices. Accordingly, discovering natural sources that have the ability to induce and activate the cell apoptosis mechanisms against the different stages of cancer diseases is the main goal of cancer therapy nowadays. Nutrigenomics is the relation between diet and genes. It discusses the effect of food components on gene expression levels for achieving the best health effects [12]. A recent study on the methanol extract of the aerial parts of *Allium porrum* L. proved the presence of a large amount of kaempherol and its derivatives which are flavonoids that have the ability to inhibit cancer cell proliferation by cell apoptosis [13].

In this study, we introduce the methanol extract of (leek) *Allium porrum* L. (MEAP) as anti-cancer agent *in vivo* and investigate its phenolic compounds. The gene expression of caspase-3, Bax, Bcl2 proteins and DNA fragmentation test in addition to immunohistochemical determination of P53 protein were preformed in solid tumor of the MEAP treated and non treated groups. Blood picture was conducted examining the effect of the extract on the blood cells count in all groups.

Materials & Methods

Materials

Plant material

Fresh aerial parts of *Allium porrum* L. were obtained from Giza farm, Egypt in 2017 and identified by Mm. Tressa Labib, Taxonomist, at El-Orman Botanical Garden, Giza, Egypt.

Extraction and isolation

Fresh aerial parts of *Allium porrum* L. (3.5 Kg) were exhaustively extracted with 70% methanol, then the methanolic extract was dried under vacuum (giving 600 gm) and examined by both $AlCl_3$ and Shinoda's test which indicate the presence of compounds of strong phenolic and flavonoid nature. Its two-dimensional paper chromatography using the solvent systems BAW (*n*-BuOH/AcOH/H_2O, 4:1:5 upper layer); and 15% acetic acid (AcOH)/H_2O, respectively, revealed the presence of many compounds of flavonoids nature. The extract was defatted with petroleum

ether (40-60ºC), then the residue (480 gm) was slurred with water, mixed with a small amount of polyamide and subjected to a polyamide column chromatography (CC). Six main fractions (F1-F6) were collected, dried and subjected to repeated purification on columns using Sephadex LH-20 to give the isolated compounds (1-11). Identification of the isolated compounds was carried out through R_f values, color reactions, chemical investigations (complete and mild acid hydrolysis), physical investigations (UV, ^{1}H-NMR, ^{13}C-NMR, ESI-MS, EI/MS) and by comparing the spectral data with those previously published [14,15].

Compounds 1 (12 mg) and 2 (10 mg) were isolated from fraction F1, which eluted with 20% MeOH: H_2O, while, compound 3 (9 mg) was isolated from F2, eluted with 40% MeOH: H_2O. F3 eluted with 60% MeOH: H_2O yielded three compounds, 4 (15 mg), 5 (12 mg) and 6 (18 mg), on the other hand, elution of F4 with 80% Me OH: H_2O resulted in the separation of compounds 7 (15 mg) and 8 (10 mg). Finally, three compounds, 9 (8 mg), 10 (7 mg) and 11 (12 mg) were isolated from F5 and F6 eluted with 100% MeOH.

Structure elucidation of the isolated compounds

NMR experiments were recorded on a Joel Ex-500 spectroscopy: 500 MHz (^{1}H-NMR), 125 MHz (^{13}C-NMR). UV spectrophotometer (Shimadzu UV-240). EI-MS was determined on a Finnigan MAT-SSQ 7000 instrument. ESI/MS were recorded on a Waters-Micromass Quattro Premier Triple Quadrupole mass spectrometer. R_f values were measured on Polygram SILF/UV254 sheets (Merck precoated sheets). Column chromatography (CC) was performed using Polyamide 6S (Riedel, De Haen AG, Seelze Haen AG, Seelze Hanver, Germany), Sephadex LH-20 (Pharmazia) using MeOH/H_2O as eluent, Cellulose (Merck), and paper chromatography (PC): Whatman No.1 and preparative (PPC) on 3 MM paper using the following solvent systems: (1) BAW ; 15% AcOH/H_2O (2) and H_2O; (3).

Complete acid hydrolysis:

About 3 mg of the compound was dissolved in 10 ml methanol mixed with 10% HCl refluxed on a boiling water bath for 2 hrs. The solution was diluted with distilled water and extracted with ethyl acetate (3 x 50 ml). The ethyl acetate extract was washed with distilled water and evaporated in vacuum at 45ºC till dryness; the obtained residue was chromatographed on PC with the authentic aglycone sample. The aqueous acidic solution after separation of the aglycone was neutralized with barium carbonate, filtered and evaporated till dryness. The residue was dissolved in isopropanol and subjected to paper chromatography using ethyl acetate: pyridine: water 12: 5: 4 and benzene: n-butanol: pyridine: water 1: 5: 3: 3 as developing solvents with authentic references from different sugars. The chromatograms were visualized by spraying with aniline phthalate [16,17] and heated at 105ºC for a few minutes, their data were identical to those previously reported [18,19].

Animals

The Study was carried out using female Swiss albino mice weighing 25 g. which obtained from the animal house of National Research Centre, Cairo, Egypt. The mice were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions with dark/ light cycle.

They were allowed free access to standard dry pellet diet and water *ad libitum*. The mice were acclimatized to laboratory conditions for 10 days before commencement of the experiment.

Compounds characterizations

Quercetin-3-O-β-glucopyranosyl-7-O-α-rhamnopyranoside (1)

Yellow amorphous powder (12 mg), m.p.242-244ºC, R_f 0.45 (BAW), 0.18 (15%AcOH/H_2O). (+)ESI-MS: *m/z* 611 $[M+H]^+$(quercetin + 1mole rhamnose + 1mole glucose) and 302 $[M-308+H]^+$(quercetin). UV spectral data λ_{max}(nm): MeOH 256.78, 294sh, 357; +NaOMe 265, 404; +$AlCl_3$ 272, 299 sh, 339 sh, 425; +$AlCl_3$/HCl 268, 298sh, 358, 401; +NaOAc 258, 395 sh, 366, 415; +NaOAc/H_3BO_3 260, 294sh, and 377.

^{1}H-NMR (500 MHz, DMSO-d_6), at δ_{ppm}: 7.54 (1H, d, J = 2.5 Hz , H-2'), 7.65 (1H, dd, *J* = 2.5 Hz, 8 Hz, H-6'), 6.76 (1H, d, *J* = 8.5 Hz, H-5'), 6.2 (1H, d, *J* = 2.5 Hz, H-8), 6.42 (1H, d, *J* = 2.5 Hz, H-6), 5.24 (1H, d, *J* = 2.0 Hz, H-1''), 5.98 (1H, d, *J* = 7.5 Hz, H-1'''), 3.01-3.5 (m, sugar protons overlapped with –OH proton signals), 0.87 (3H, d, *J* = 6.0, CH_3 of rhamnose moiety). ^{13}C-NMR (125 MHz, DMSO-d_6), at δ ppm: 157.69 (C-2), 134.87 (C-3), 177.98 (C-4), 161.79 (C-5), 98.87 (C-6), 165.14 (C-7), 93.89 (C-8), 157.64 (C-9), 103.86 (C-10), 122.14 (C-1'), 115.26 (C-2'), 145.65 (C-3'), 149.34 (C-4'), 116.97 (C-5'), 122.56 (C-6'), 102.63 (C-1''), 70.86 (C-2''), 71.43 (C-3''), 72.12 (C-4''), 70.86 (C-5''), 18.32 (C-6''), 101.46 (C-1'''), 72.87 (C-2'''), 75.68 (C-3'''), 71.23 (C-4'''), 77.86 (C-5'''), 61.98 (C-6'''). Acid hydrolysis of compound **1** yields the aglycone and the two

sugars, which their R_f values were identical with quercetin (aglycone) and the standards, rhamnose and glucose (sugars). So, from the previous results, compound 1 is established as quercetin-3-*O*-*β*-glucopyranosyl -7-*O*- *α*-rhamnopyranoside

Quercetin3-O-α-rhamnopyranoside (2)

Yellow amorphous powder (10 mg), m.p. 316-318ºC C. R_f 0.43 (BAW), 0.16 (15%AcOH/ H_2O). (+)ESI-MS: *m/z* 449 $[M+H]^+$(quercetin + 1mole rhamnose) and 301 $[M-147+H]^+$(quercetin). UV-Spectral data λ_{max}(nm) MeOH: 258, 269sh, 360; NaOMe : 272, 328sh, 405; $AlCl_3$: 275, 305sh, 332sh, 435; $AlCl_3$/HCl: 275, 305sh, 361, 403; NaOAc: 269, 323sh, 380; NaOAc/H_3BO_3: 262, 300sh, 387; ^{1}H-NMR (500 MHz, DMSO-d_6): aglycone moiety: at δ_{ppm}: 7.6 (1H, d, *J*= 2.5 Hz, H-2'), 7.5 (1H, dd, *J*= 2.5 Hz and *J*= 8 Hz, H-6'), 6.77 (1H, d, *J*= 8Hz, H-5'), 6.24 (1H, d, *J*= 2.0 Hz, H-8), 6.04 (1H, d, *J*= 2.0 Hz, H-6) . Sugar moiety: 5.5 (1H, d, *J*= 2.0 Hz, H-1'' of rhamnose), 3.1 → 3.5 (m, sugar protons) and 0.98 (3H, d, J= 6.1 Hz, CH_3 of rhamnosyl) . ^{13}C-NMR (125 MHz, DMSO-d_6): at δ_{ppm}: 157.04 (C-2), 134.5 (C-3), 177.9 (C-4), 161.7 (C-5), 99.5 (C-6), 161.2 (C-7), 94.3 (C-8), 157.4 (C-9), 103.9 (C-10), 121.1 (C-1'), 115.9 (C-2'), 145.76 (C-3'), 149.1 (C-4'), 115.99 (C-5'), 121.52 (C-6'). Sugar moiety: 101.10 (C-1''), 70.61 (C-2''), 70.81 (C-3''), 71.66 (C-4''), 70.42 (C-5'') and 17.91 (C-6''). The acid hydrolysis of compound 2 yielded both quercetin and rhamnose, which were identified by Co-PC, using authentic in different solvents. Thus, the structure of compound 2 was proved as quercetin -3-O-*α*-rhamnopyranoside.

Quercetin-4'-O-β-glucopyranoside (3)

Yellow amorphous powder (9 mg), m.p.226-228ºC, R_f 0.44 (BAW), 0.17 (15%AcOH/ H_2O). (+)ESI-MS: *m/z* 465 $[M+H]^+$(quercetin + 1mole glucose) and 302 $[M-162+H]^+$(quercetin). UV spectral data λ_{max}(nm): MeOH 256.78, 294 sh, 357; +NaOMe 266, 405; +$AlCl_3$ 272, 299 sh, 339 sh, 426; +$AlCl_3$/HCl 268, 298 sh, 357, 400; +NaOAc 259, 395 sh, 365, 415; +NaOAc/H_3BO_3 260, 294 sh, 377. ^{1}H-NMR (500 MHz, DMSO-d_6), at δppm: 7.54 (1H, d, *J* = 2.5 Hz , H-2'), 7.65 (1H, dd, *J* = 2.5 Hz, 8 Hz, H-6'), 6.76 (1H, d, *J* = 8.5 Hz, H-5'), 6.2 (1H, d, *J* = 2.5 Hz, H-8), 6.42 (1H, d, *J* = 2.5 Hz, H-6), 5.24 (1H, d, *J* = 2.0 Hz, H-1''), 5.98 (1H, d, *J* = 7.5 Hz, H-1'''), 3.01-3.5 (m, sugar protons overlapped with –OH proton signals), ^{13}C NMR (125 MHz, DMSO-d_6), at δppm: 157.69 (C-2), 134.87 (C-3), 177.98 (C-4), 161.79 (C-5), 98.87 (C-6), 165.14 (C-7), 93.89 (C-8), 157.64 (C-9), 103.86 (C-10), 121.14 (C-1'), 115.26 (C-2'), 145.65 (C-3'), 151.34 (C-4'), 115.97 (C-5'), 121.56 (C-6'), 101.46 (C-1''), 72.87 (C-2''), 75.68 (C-3''), 71.23 (C-4''), 77.86 (C-5''), 61.98 (C-6''). Acid hydrolysis of compound 3 yields the aglycone and sugar, which their R_f values were identical with quercetin (aglycone) and the standard glucose (sugar). So, from the previous results, compound 3 is established as quercetin-*4'-O-β*-glucopyranoside.

Kaempferol-3-O-β-glucopyranoside (4)

Dull yellow powder (15 mg), m.p. 240-242ºC. R_f BAW:0.29, 15%AcOH/H_2O: 0.18; (+)ESI-MS: *m/z* 449 $[M+H]^+$(kaempferol + 1mole glucose) and 286 $[M-162+H]^+$(kaemperol). UV λ_{max} (nm) MeOH 264, (294), (320), 350; +NaOMe 271, 326 sh, 406; +$AlCl_3$ 273, 304 sh, 346, 401; +$AlCl_3$/HCl 273, 302 sh, 344, 401; +NaOAc 271, 301 sh, 371; +NaOAc/H_3BO_3 266, 292 sh, 321 sh, 351 nm. ^{1}H-NMR (500 MHz, DMSO-d_6), at δppm: 12.52 (1H, s, OH-5), 8.03 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.67 (2H, d, *J* = 8.9 Hz, H-3', H-5'), 6.34 (1H, d, *J* = 2.5 Hz, H-8), 6.15 (1H, d, *J* = 2.5 Hz, H-6), 5.32 (1H, d, *J* = 7.6 Hz, H-1''), 3.55-3.14 (m, sugar protons signals). ^{13}C-NMR (125 MHz, DMSO-d_6), at δppm: 156.12 (C-2), 134.43 (C-3), 177.26 (C-4), 161.52 (C-5), 99.81 (C-6), 167.14 (C-7), 94.23 (C-8), 157.42 (C-9), 103.98 (C-10), 121.73 (C-1'), 129.42 (C-2', 6'), 115.63 (C-3', 5'), 160.17 (C-4'), 102.62 (C-1''), 71.54 (C-2''), 73.65 (C-3''), 67.95 (C-4''), 75.89 (C-5''), 61.14 (C-6''). After acid hydrolysis of compound 4, R_f values of the aglycone and the sugar were identical with kaempferol (aglycone) and the standard, glucose (sugar). Compound 4 is established as kaempferol 3-O-*β*-glucopyranoside

Kaempferol-7-O-β-glucopyranoside (5)

Yellow amorphous needles (12 mg), m.p. 190-192ºC. R_f BAW:0.28, 15%AcOH/ H_2O: 0.17. (+) ESI-MS: *m/z* 449 $[M+H]^+$(kaempferol + 1mole glucose) and 286 $[M-162+H]^+$(kaemperol). UV spectral data λ_{max}(nm) MeOH: 253, 266, 300 sh, 366; NaOMe: 245, 266, 314, 424; $AlCl_3$: 267, 273, 301sh, 335sh, 248, 422; $AlCl_3$/HCl: 267, 300sh, 348, 424; NaOAc: 265, 396sh, 367, 415; NaOAc/H_3BO_3: 265, 295sh, 366;. ^{1}H-NMR (500 MHz, DMSO-d_6): at δppm: 12.60 (1H, s, OH-5), 8.01 (2H, d, *J* = 8.5, H-2',6'); 6.94 (2H, d, *J* = 8.5, H-3',5'); 6.31 (1H, d, *J* = 2.0, H-8); 6.05 (1H, d, *J* = 2.0, H-6). The sugar protons at 5.5 (1H, J= 7.5 Hz, H-1''), 3.1→ 3.6 (m, sugar protons signals).^{13}C- NMR (125 MHz, DMSO-d_6), at δppm: 159.1 (C-2), 133.6 (C-3), 177.4 (C-4),

162.2 (C-5), 98.6 (C-6), 165.6 (C-7), 94.4 (C-8), 159.1 (C-9), 104.6 (C-10), 121.1 (C-1'), 130.1 (C-2'/6'), 115.2 (C-3'/5'), 159.7 (C-4'), 122.7 (C-6'), 100.61 (C-1''), 74.31 (C-2''), 77.87 (C-3''), 70.22 (C-4''), 76.51 (C-5''), 61.84 (C-6''). The complete acid hydrolysis of compound 5 gave kaempferol and glucose by comparing with Co-PC with authentic samples. The compound 5 was identified as kaempferol-7-O-*β*-glucopyranoside.

Quercetin (6)

Yellow powder (18 mg), m.p. 322-323 ºC. R_f 0.59 (BAW), 0.08 (15%AcOH/ H_2O). EI/MS *m/z* 302 (100%) $[M]^+$. UV-Spectral data λ_{max}(nm) MeOH: 256, 268sh, 371; NaOMe: 249, 424; $AlCl_3$: 273, 305sh, 335sh; $AlCl_3$/HCl: 266, 303sh, 350, 414; NaOAc: 264, 325, 390; NaOAc/H_3BO_3: 262, 302sh, 386.^{1}H-NMR (500 MHz, DMSO-d_6), at δppm: 7.8 (1H, dd, *J*= 2.5 Hz and *J*= 8Hz, H-6'), 7.6 (1H, d, *J*= 2.5 Hz, H-2'), 6.88 (1H, d, *J*= 8.5 Hz, H-5'), 6.4 (1H, d, *J*= 2.5 Hz, H-8) and 6.2 (1H, d, *J*= 2.5 Hz, H-6).^{13}C-NMR (125 MHz, DMSO-d_6), at δppm: 147.25 (C-2), 136.18 (C-3), 176.29 (C-4), 161.17 (C-5), 98.63 (C-6), 164.35 (C-7), 93.81 (C-8), 156.59 (C-9), 103.46 (C-10), 122.40 (C-1'), 115.3 (C-2'), 154.51 (C-3'), 148.15 (C-4'), 115.9 (C-5'), 120.43 (C-6'). From the above data, compound 6 was identified as quercetin.

Isorhamntin (7)

Light yellow amorphous powder (15gm), m.p. 305-306ºC. R_f 0.56 (BAW), 0.08 (15%AcOH/ H_2O). EI/MS *m/z* 316, (100%) $[M^+]^+$. UV-Spectral data λ_{max}(nm) MeOH: 246, 295sh, 375; NaOMe: 266,328sh, 428; $AlCl_3$: 260, 278, 298, 305sh, 355, 384; $AlCl_3$/HCl: 260, 276, 297, 303sh, 352, 415; NaOAc: 265, 328, 394; NaOAc/ H_3BO_3: 265, 302sh, 381. EI/MS *m/z*: 316 $[M^+]^+$, (100%);^{1}H-NMR (500 MHz, DMSO-d_6), at δppm: 7.8 (1H, dd, *J*= 2.5 Hz and J= 8Hz, H-6'), 7.5 (1H, d, *J*= 2.5 Hz, H-2'), 6.91 (1H, d, *J*= 8.5 Hz, H-5'), 6.4 (1H, d, *J*= 2.5 Hz, H-8), 6.2 (1H, d, *J*= 2.5 Hz, H-6) and 3.83 (3H, s, -OCH_3). ^{13}C-NMR (125 MHz, DMSO-d_6), at δppm: 147.25 (C-2), 136.18 (C-3), 177.29 (C-4), 161.17 (C-5), 98.63 (C-6), 164.35 (C-7), 93.81 (C-8), 156.35 (C-9), 103.46 (C-10), 122.40 (C-1'), 115.3 (C-2'), 149.51 (C-3'), 149.15 (C-4'), 115.9 (C-5'), 120.43 (C-6') and 56.21 (OCH_3 -3'). From the above data, compound 7 was identified as isorhamntin.

Kaempferol (8)

Yellow needles (10 mg), m.p. 265-267ºC, R_f 0.58 (BAW), 0.09 (15%AcOH/ H_2O). UV spectral data λ_{max} (nm): MeOH 265, 291sh, 318 sh, 365; +NaOMe 275, 319 sh, 410; + $AlCl_3$ 268, 304, 349, 422; +$AlCl_3$/HCl 266, 304, 349, 423; +NaOAc 273, 305, 377; +NaOAc/H_3BO_3 264, 293, 318, 368. EI/MS: *m/z* 286 (100%)$[M]^+$.^{1}H-NMR (500 MHz, DMSO-d_6), δppm: 7.98 (2H, d, *J* = 8.5, H-2',6'); 6.91 (2H, d, *J* = 8.5, H-3',5'); 6.38 (1H, d, *J* = 2.0, H-8); 6.13 (1H, d, *J* = 2.0, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6), at δ ppm: 155.62 (C-2), 133.24 (C-3), 176.76 (C-4), 160.87 (C-5), 99.7 (C-6), 166.51 (C-7), 94.32 (C-8), 156.81 (C-9), 103.45 (C-10), 120.91 (C-1'), 129.14 (C-2', 6'), 114.98 (C-3', 5'), 159.89 (C-4'). Compound *8* was identified as kaempferol**.**

p-Coumaric acid (9)

A buff powder (8 mg), m.p. 212-214ºC UV spectral data λ_{max} (nm) MeOH: 245 and 325 nm. EI-MS *m/z***:**164(100%) $[M^+]^+$.^{1}H-NMR (500 MHz, DMSO-d_6) at δppm: 12.46 (s, 1H, carboxylic proton), 9.31 (s, broad, 1H, H-2), 7.64 (1H, d, *J*=15.8, H-1'), 7.51 (1H, d, *J*=7.7Hz, H-3), 6.85 (1H, *J*= 2.2Hz, H-6), 6.83 (1H, dd, *J*=2.2, 7.7Hz, H-5), 6.37 (1H,d, *J*=15.8Hz, H-2').^{13}C-NMR (125 MHz, DMSO-d_6) at δppm:168.41 (C-3'), 160.13 (C-1), 146.08 (C-6), 137.21 (C-1'), 133.71 (C-4), 126.21 (C-3), 121.62 (C-2'), 116.24 (C-2), 115.71 (C-5). So, compound 9 was identified as *p***-**coumaric acid

Ferulic acid (10)

Colorless powder (7 mg), m.p. 169-170 ºC. EI/MS, *m/z* 194 (100%) $[M]^+$, 100%. ^{1}H-NMR (500 MHz, DMSO-d_6), at δppm: 7.67 (1H, d, *J* = 2.0 Hz, H-2), 9.12 OH, s, H-4), 6.42 (1H, d, *J* = 8.0 Hz, H-5), 6.65 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.98 (1H, d, *J* = 16.0 Hz, H-7), 6.12 (1H, d, *J* = 16.0 Hz, H-8), 3.60 (3H, s, O-Me). ^{13}C-NMR (125 MHz, DMSO-d_6), at δppm: 127.1 (C-1), 110.8 (C-2), 148.7 (C-3), 150.0 (C-4), 115.7 (C-5), 123.5 (C-6), 146.2 (C-7), 116.1 (C-8), 170.8 (C-9), 56.2 (O-Me). Compound 10 is confirmed by comparison with literature data which is established as ferulic acid.

Gallic acid (11)

White amorphous powder (12 mg), m.p. 258-260ºC. UV spectral data λ_{max}(nm) MeOH: 272 nm. EI/MS *m/z* 170 $[M^+]^+$, 100%. ^{1}H-NMR (500 MHz, DMSO-d_6) at δ ppm: 6.83 (2H, s, H-2 and H-6). ^{13}C-NMR (125 MHz, DMSO-d_6), at δppm: 120.6 (C-1), 108.8 (C-2 and C-6), 145.5 (C-3 and C-5), 138.1 (C-4), 167.7 (C-7). So, compound 11 was identified as gallic acid

All compounds were identified by a combination of spectroscopic methods (UV, EI/

MS, (+)ESI-MS and ^{1}H-NMR,^{13}C-NMR) and comparison with literature data. This is first report of phenolic and flavonoid compounds in this plant.

B-Methods

Methods of phytochemical study

Phenolic and flavonoid compounds are separated by column chromatography, purified by sephadex LH-20 and established with the aid of spectroscopic methods (UV, EI/MS, ESI-MS,^{1}H-NMR and^{13}C-NMR spectroscopy).

Methods of biochemical analysis in mice

Experimental design.

Forty five healthy female albino mice weighing about 25g were injected with Ehrlich ascites carcinoma cells ($2.5x10^{6}$ cells/mouse) interperitoneally or in the hind limb (solid tumor). The mice groups were divided as follows:

Group 1: Ten mice were injected by (EAC cells) interperitoneally (positive control).

Group 2: Ten mice were supplemented orally with MEAP (300 mg/kg) [20] after 24 hours of the injection of EAC cells interperitoneally.

Group 3: Ten mice were injected with EAC cells in the hind limb (solid tumor) representing the positive control groups.

Group 4: Ten mice were supplemented orally with MEAP (300 mg/kg) after 24 hours of the injection of EAC cells in the hind limb.

Group 5: Five mice representing the normal control group.

In both treated and non treated interpreitonial groups, part of the mice was dissected after two or three weeks and the other part was left for determining the lifespan. The body weight of mice was measured every two days for observing the difference and the change in the weight.

Preparations of blood samples

Blood was collected from retro-orbital plexes in EDTA containing tubes and was prepared for counting red and white blood cells in addition to examining the whole blood picture.

Preparation of tumor cells

The transplantable murine tumor cell line, namely EAC cells were obtained from the National Cancer Institute in Cairo, Egypt. The EAC cells were maintained in the ascitic form *in vivo* in Swiss mice by means of serial intraperitoneal transplantation of $2.5X10^{6}$ cells/mouse after every 10 days. Ascitic fluid was drawn out from EAC bearing mouse 8 days after transplantation. The freshly drawn fluid was diluted with ice-cold sterile isotonic saline and the tumor cell count was adjusted to 2.5 $X10^{6}$ cells/mouse by sterile isotonic saline.

Tumor cell count

The ascitic fluid was taken and diluted 10 times. Then one drop of the diluted suspension was placed in the Neubauer counting chamber and the numbers of cells in the 64 small squares were counted.

Viable and non-viable tumor cell count

The cells were then stained with trypan blue (0.4 % in isotonic saline) dye (Trypan blue dye exclusion assay). The cells that did not take up the dye were viable and those took the dye were non-viable. The viable cells were counted.

Cell count = No. of cells × dilution factor

Mean survival Time (MST) and Increase in life span (ILS %).

The animals were observed for their mortality daily until their death. The mortality was monitored by recording MST and % ILS by the following formulae:

MST= [First death + Last death]/2

T=Time denoted by the number of days.

% ILS = [(MST of treated group / MST of EAC control group) − 1)] × 100 [21]

Solid Tumor volume

The tumor volume was measured in cm by using a caliper**.** The volume of the tumor was measured at the end of every week for three weeks.

Determination of the apoptotic parameters in the EAC cells and solid tumor in treated and non treated mice.

i-Detection of BAX, BCL-2, caspase-3 gene expression in Ehrlich and solid tumors by real-time RT-PCR .

Total RNA was isolated from 500 µl of Ehrlich samples and 100 mg of solid tumor tissues by the standard TRIzol extraction method (Invitrogen, Paisley, UK), In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase treatment and removal reagent kits (Ambion, Austin, TX, USA) following the manufacturer's protocol and recovered in 100 µl molecular biology grade water and stored at -20°C. The

RNA concentration and purity were determined by Nanodrop Spectrophotometer absorption (Thermo Scientific, USA) at 260 nm [22].

First-strand cDNA synthesis was performed with the Super Script Choice System (Life Technologies, Breda, Netherlands). For real time quantitative PCR, 5 µl of first-strand cDNA in a total volume of 25 ml containing 12.5 ml 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and 200 ng of each primer. The sequences of specific primer of the genes used are listed in (Table 1). , Thermal cycling conditions 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of (*β*- Actin) as housekeeping gene by the ΔΔCt method [22,23].

ii- DNA fragmentation

DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described according to Lu et al. [24]. Ehrlich tumor samples and solid tumor tissues were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer, centrifuged at 10 000 r.p.m. for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 0.5 ml of 25% trichloroacetic acid (TCA) was added and incubated at 4°C for 24 h. The samples were centrifuged at 10 000 r.p.m. for 20 min at 4°C and the pellets were suspended in 80 ml of 5% TCA, followed by incubation at 83°C for 20 min. Subsequently, to each sample, 160 ml of DPA solution was added and incubated at room temperature for 24 h.37. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm.

Histopathological Studies

Solid tumor sections were excised, washed with normal saline and processed separately for histopathological observation. Initially samples were fixed in 10% buffered neutral formalin and paraffin sections were taken at 5 µm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathology changes. Immunohistochemical dye for observing the amount of P53 in the solid tumor tissues of treated and non treated groups was used.

Statistical analysis.

Data were analyzed by comparing values for treatment groups with the values for individual control. All values were expressed as the mean ± SE. Significant differences between the groups were statistically analyzed using a one- way analysis of variance (ANOVA), followed by a non-parametric post hoc test (LSD). *P* value of 0.05 or less was considered statistically significant.

Results and Discussion

Phytochemical results:

Identification of separated flavonoid and phenolic constituents:

The methanol extract of the aerial parts of *Allium purrom* L. was separated and purified through chromatographic techniques yielding eleven flavonoid and phenolic compounds Fig.(1). The structures were identified by physical (UV, EI/MS, ESI/MS, ^{1}H-NMR and ^{13}C-NMR) and chemical methods (mild and complete acid hydrolysis) [14, 25, 26]. These compounds were identified as quercetin-*3-O-β*-glucopyranoside-*7-O-α*-rhamnpyranoside (1), quercetin-*3-O-α*-rhamnopyranoside (2), quercetin-*4'-O-β*-glucopyranoside (3), kaempferol*3*-

TABLE 1. The sequence of specific primer of the genes used.

Gene		Primer sequence (5′-3′)	Gene bank accession no.
Bcl2	F	GGA CTT GAA GTG CCA TTG GT	AA867214.1
	R	CAG GCT GGA AGG AGA AGA TG	
Bax	F	CTG CAG AGG ATG ATT GCT GA	L22472.1
	R	GAT CAG CTC GGG CAC TTT AG	
Caspase3	F	CTA GCA GGA TCC AGC AGT CC	AK014231.1
	R	CCC CTA TTC CAC CCA ACT TT	
β-actin	F	GCT ACA GCT TCA CCA CCA CA	AY618569.1
	R	AAG GAA GGC TGG AAA AGA GC	

O-β-glucopyranoside (4), kaempferol-7-*O-β*-glucopyranoside (5), quercetin (6), isorhamnetin (7), kaempferol (8), *p*-coumaric acid (9), ferulic acid (10) and gallic acid (11). The spectral data of the isolated compounds were consistent with those previously published [27-34].

Biochemical and molecular results:

Effect of extract on body weight, life span and EAC cell count.

Injecting EAC cells intraperitonially to female mice caused an observed increase in weight in the positive non treated group. This increase started from the 6th day from injection and remained till the 14th day, which lead to the death of all the mice. This increase represented the rapid multiple reproduction of EAC cells in the ascitic fluid as shown in Fig (2).

Supplementation of MEAP in mice after 24 hrs from injecting EAC cells affected greatly the reproduction of the tumor cells from the 6th day to the 14th (Fig (2)). Stability in weight followed by a normal increase was established. An increase in life span by 34.61% was observed in the MEAP treated group as shown in Table (2) & Fig (2). The decrease in the body weight was accompanied by a decrease in counted of EAC cells in the MEAP treated mice as shown inTable (2).

Table (3) showed significant decrease in RBCs count in the MEAP treated group in case of intraperitonial injection of EAC. Normal leukocytes count was observed in the treated group compared to normal control and the positive non treated groups. The data reflected the anti-inflammatory impact of the *Allium* extract.

ii- Effect of the aerial parts of Allium porrum L. extract on solid tumor volume.

Figure (3) showed the increase in solid tumor volume in the positive non treated group. The therapeutic effect of the MEAP and its anti-tumor effect was detected from the first week from the EAC cells injection.

Table (4) showed the normal significant levels of RBCs, HB content versus non-significant decrease in total leukocyte in the MEAP treated group compared to the non treated one in case of intramuscular injection of EAC which confirmed the anti-inflammatory effect of the extract.

Figure (4 a, b, c) illustrated the anti-tumor activity of MEAP against anti-apoptotic properties of EAC cells. The extract was able to increase significantly the DNA damage and caspase -3 protein in the EAC cells intraperitonially injected or as a solid tumor. Also, an increase in Bax protein was accompanied by a decrease in BCl2 protein in EAC cells in both treated cases.

Histological and immunohistochemical examination results.

Figure 5: Micrographs of histopathological and immunohisto-chemical studies

Micrograph (1): Normal histological structure of skeletal muscle showing myofibers peripheral nuclei, intact sarcolemma and non-fragmented sarcoplasm (H&E x 40)

Micrograph (2): Micrograph showing tumor infiltration and necrosis between fragmented muscles in control non treated mice with solid Ehrilch tumors (H&Ex40).

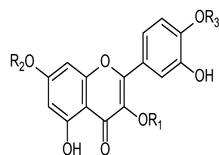
Micrograph (3): Micrograph showing necrotic muscle is identified by the presence of infiltrating inflammatory cells, hyper contracted myofibers and degenerating myofibers with fragmented sarcoplasm, decreased muscle size (atrophy) in non treated mice (H&E,x40).

Micrograph (4): Micrograph is showing

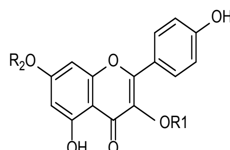
TABLE 2: Effect of methanolic extract of the aerial parts of *Allium porum* L. on body weights and life span and tumor cell count in mice intraperitonially injected Ehrlich ascites carcinoma cells.

	EAC Control	EAC treated with MEAP (300mg/kg)
Initial b.wt	28.5 ± 1.02	27.33 ± 1.66
Final b.wt after 2weeks	37.18 ± 2.91	24.35 ± 1.45*
Mean survival time in days (MST)	13 ± 1.5	17.5 ±2.5
Increase in life span (%) (ILS)	_	34.61
Viable tumor cell count	$2.5x\ 10^{6}$	$1.99x\ 10^{6}$*

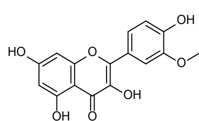
Data are represented as Mean ±SE. P^* Significant at $P \leq 0.05$



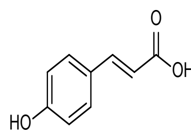
Compound (1) R_1 = Glucose, R_2= Rhamnose, R_3= H
Compound (2) R_1 = Rhamnose, R_2 = H, R_3 = H
Compound (3) R_1 = H, R_2 = H, R_3 = Glucose
Compound (6) R_1 = H, R_2 = H, R_3 = H



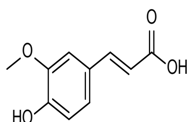
Compound (4) R_1 = Glucose, R_2 = H
Compound (5) R_1 = H, R_2 = Glucose
Compound (8) R_1 = H, R_2 = H



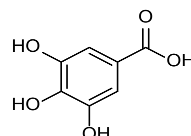
Compound (7)



Compound (9)



Compound (10)



Compound (11)

Fig. 1. Chemical structures of the isolated compounds (1→11) from the aerial parts of Allium porum L.

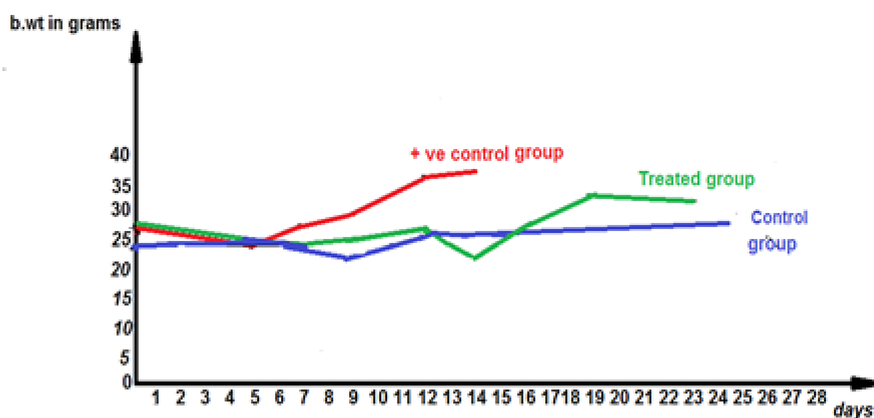


Fig. 2. A diagram showing the change in body weight in mice of normal control, treated and non treated (+ve control) intraperitonially injected EAC cell groups

TABLE 3. Effect of methanol extract of the aerial parts of *Allium porum* L. on hematological parameters in mice intrapritonially injected EAC cells.

	EAC treated with MEAP(300mg/kg) Group 1	EAC control Group2	Normal Control Group 3
RBCs count x 10^{12}	5.77 ±1.11 [2,3]	8.83 ± 0.26 [1]	8.63 ± 0.20 [1]
HB	9.60 ±1.96	11.80 ± 0.20	13.14 ± 0.23
HCT	25.26 ± 4.80 [2,3]	40.16 ± 0.94 [1]	43.66 ± 5.32 [1]
MCV	43.33 ± 0.75	45.26 ± 1.84	50.43 ± 5.46
MCH	16.43 ± 0.20 [2]	14.1 ± 1.13 [1]	15.12 ± 0.25
MCHC	37.93 ± 0.50 [2,3]	29.56 ± 0.23 [1]	31.64 ± 1.61 [1]
Total leukocytes x10^9	6.86 ± 0.94 [2]	12.42 ± 0.60 [1,3]	6.60 ± 1.05 [2]
Platelets	1001.33 ± 50.99	755.00 ± 241.87	701.33 ± 47.93

Data are represented as Mean ±SE.
$P^{1,2,3}$ Significant at $P \leq 0.05$

TABLE 4. Effect of methanol extract of the aerial parts of Allium porum L. on hematological parameters in mice intramuscularly injected EAC cells.

	EAC treated with MEAP(300mg/kg) Group1	EAC Control Group 2	Normal Control Group 3
RBCs count x 10^{12}	8.00 ± 0.33 [2]	8.73 ± 0.23 [1]	8.59 ± 0.14
HB	10.6 ± 0.50 [2,3]	11.88 ± 0.43 [1,3]	13.14 ± 0.23 [1,2]
HCT	38.7 ± 2.04	40.56 ± 0.83	41.2 ± 3.28
MCV	48.36 ± 0.54	44.56 ± 1.60	48.26 ± 3.29
MCH	13.18 ± 0.09 [3]	13.60 ± 0.37 [3]	15.12 ± 0.14 [1,2]
MCHC	27.42 ± 0.19 [3]	29.28 ± 0.65	31.64 ± 1.61 [1]
Total leukocyte x10^9	14.67 ± 3.46	21.45 ± 7.52 [3]	6.60 ± 0.58 [2]
platelets	1190.33 ±118.92 [2,3]	1185. 33 ±117.5 [1,3]	701.33 ±47.9 [1,2]

Data are represented as Mean ±SE. $P^{1,2,3}$ Significant at $P \leq 0.05$

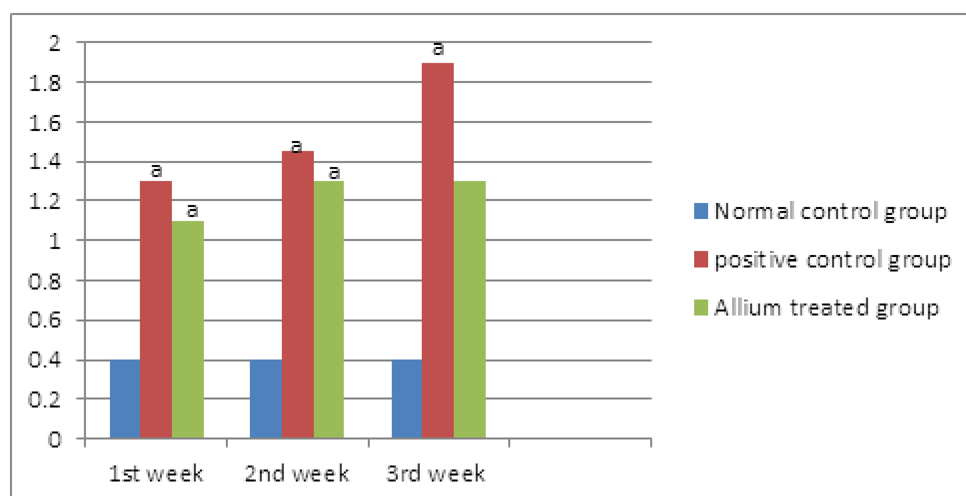


Fig. 3. A diagram showing the change in solid tumor volume in treated and nontreated mice.
$P^a \leq 0.05$ compared to control

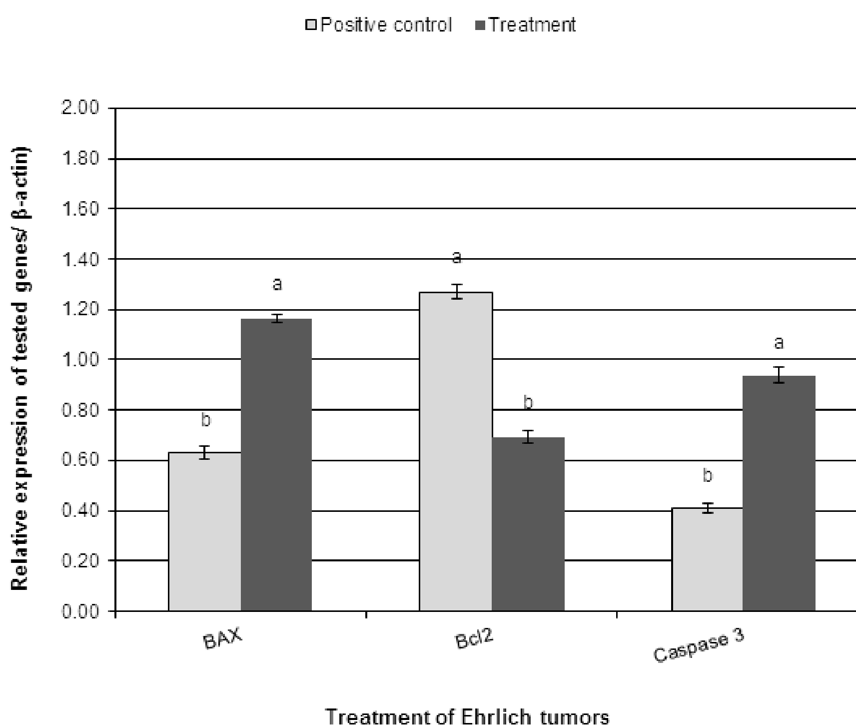


Fig. 4a. quantitative RT-PCR of Bax, Bcl2 and Caspase-3 genes in Ehrlich tumor and treated Ehrlich tumor samples.

Data are presented as mean ± SEM. [a,b] Mean values within columns for each gene with unlike superscript letters were significantly different ($P \leq 0.05$).

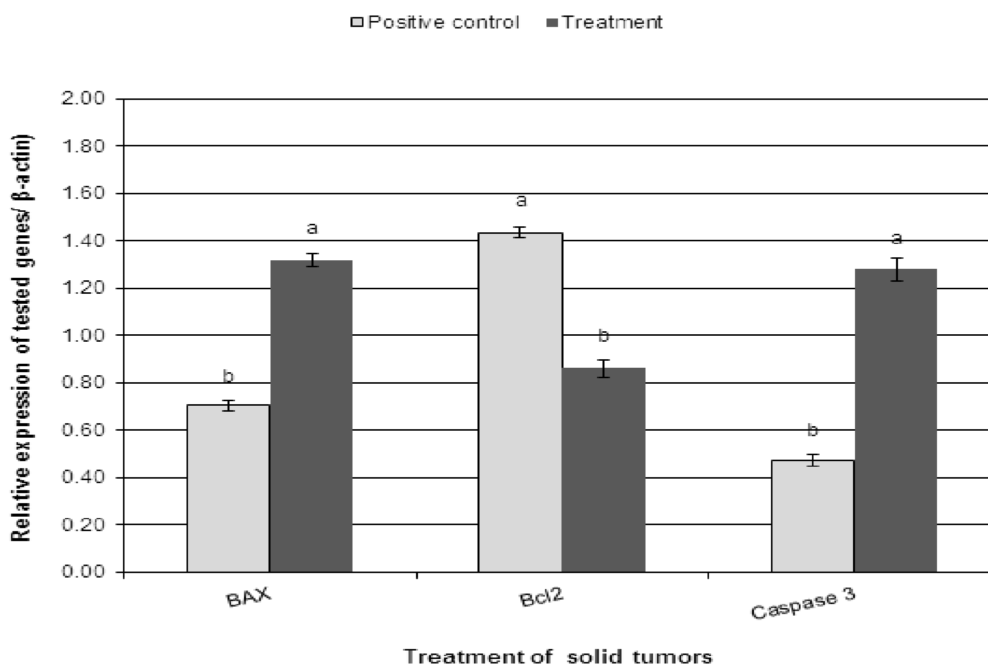


Fig. 4b. quantitative RT-PCR of Bax, Bcl2 and Caspase-3 genes in solid tumor and treated solid tumor samples
Data are presented as mean ± SEM. [a,b] Mean values within columns for each gene with unlike superscript letters were significantly different ($P \leq 0.05$).

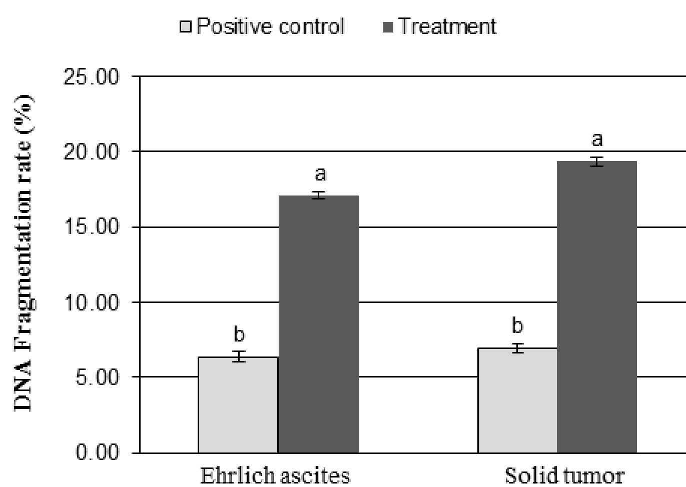


Fig. 4c. DNA fragmentation detected in solid and Ehrlich tumor tissues.
Data are presented as mean ± SEM. Data are presented as mean ± SEM. [a,b] Mean values within columns with unlike superscript letters were significantly different ($P \leq 0.05$).

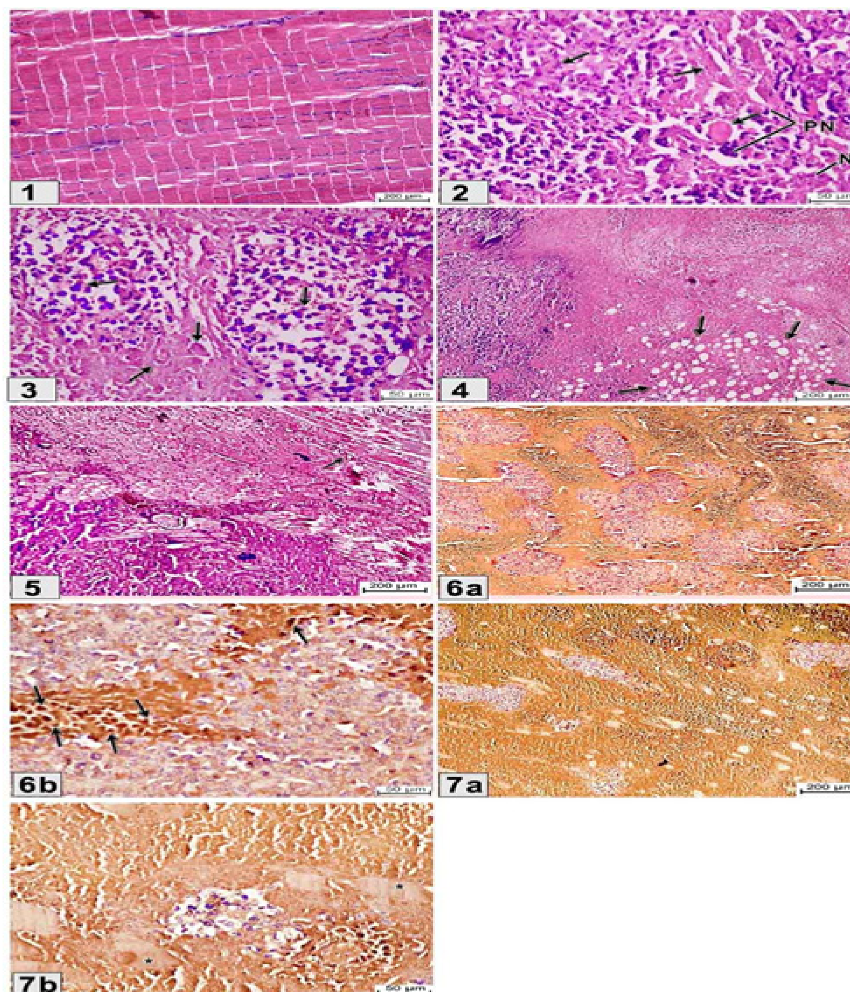


Fig. 5. Micrographs of histopathological and immunohistochemical studies.

aggregates of foamy macrophages in between degenerated muscles in non treated mice (H&E, x10).

Micrograph 5: Micrographs showing reduced mitotic figure and regenerated myofibers in treated group, indicated by large plump mature dystrophies myofibers with central nuclei.

Micrograph 6 (a, b): Immunohistochemistry in tumor sections of the muscle (a&b) showing the intensity of p53 reaction in the tumor of non treated mice (H&E x 400).

Micrograph7 (a, b): Immunohistochemistry in tumor sections (a&b) Mice were treated with methanolic extract of *Allium porrum* showing reduction of p53 reaction (H&E x 400).

Discussion

Leeks are among the most studied vegetables in the last few years and made a great interest in pharmacy industries due to their wide diversity of bioactive compounds [35]. *Allium porrum* (leek) is available in abundance in Egypt and exists as basic ingredient in some of the Egyptian food. The plant contains many vital components such as active sulphur components represented in allicin, aliin, diallyl sulphide and diallyl disulphide [36]. It also contains important flavonoids and phenolics such as kaempherol and quercetin which are considered powerful antioxidants and have a variety of anti- cancer activites [13, 37].

EAC cells in ascitic fluids of mice are considered a practical way to examine many anti-tumor agents *in vivo* [38]. In this study, intraperitoneal injection, of EAC cells leads to a rapid increase in the volume of the ascitic fluid in the non treated group which was shown clearly in the body weight increase of mice. This can be explained by the utilization of the EAC cells to the ascitic fluid as a source for its nutrition to meet its growing criteria. On the other hand, there was a slight and a slow increase in the ascitic fluid in mice treated with MEAP. The anti-tumor effect of the extract appeared to the naked eye when observing the body weights of the treated group during the 14 days after injection of the EAC cells compared to the non treated one (Figure 2). Our data are in accordance with Gupta et al. [21] who discussed the relation between the body weight gain in EAC bearing mice and the EAC cells growing intraperitonially.

Our results also revealed the increase in life span of the treated mice with 34.6% more than the non treated mice. The data indicated greatly the efficacy of the extract in preventing the progression of the tumor and enhancing the life span of the EAC bearing mice. These results achieved; the fact demonstrated by Andreani et al. [39] who approved that ≥ 25% increase in lifespan of EAC bearing mice treated by a substance is considered an indication of its antitumor activity.

In the EAC cells intramuscularly injected groups (representing the existence of the solid tumor in the mice legs, there was an observed decay in tumor volume in the MEAP treated group compared to the non treated one from the beginning of the first week (figure 3). This action reflected the antitumor impact of the extract on the solid tumors. An *in vitro* study of the *Allium* extracts cytotoxic effect against cancer cells was performed by Pan et al. [40]. They attributed this cytotoxic effect to the presence of high phenolic compounds such as quercetin glucosides. Many recent studies attributed the anticancer activity of the plant due to the kaempferol existence and the organosulpher compounds [41-43].

Our data proved the effect of the plant in attacking EAC cells in a short time with certain mechanisms. For exploring these mechanisms on the EAC cells at their molecular level, five important parameters were chosen in this study.

These parameters are represented in testing the gene expression levels of the pro-apoptotic protein Bax, the anti-apoptotic protein Bcl2, the death protein caspase-3, DNA fragmentation and evaluating the P53 protein amount in the tumor cells.

A high significant increase in the DNA fragmentation was observed in both of the MEAP treated groups (intraperitonially & intramuscularly injected EAC cells) compared to the non treated ones. A significant increase in Bax and caspase -3 were accompanied by a significant decrease in Bcl2 in the MEAP treated groups compared to the non treated ones.

The anti-tumor efficacy of the MEAP can be attributed to inducing the apoptotic mechanisms in the EAC cells. The extract played a role in decreasing Bcl2 and increasing Bax amounts in the EAC cells, which may lead to an induction of the mitochondrial mediated apoptosis by changing the mitochondrial permeability and releasing of some apoptotic proteins such as cytochrome C [44]. This in turn increases the caspase cascade in the EAC cells, especially

caspase -3 which is known as death protein. Ahmed & El Menoufy [45] and Farhadi_et al [6], approved these mechanisms in executing tumor cells *in vitro* in human colon cancer cells and in human oral squamous cell carcinoma respectively. Also, our data are in accordance with Jin et al.[46] who denoted a significant apoptotic effect on colorectal cancer cells *in vitro and in vivo* which was accompanied with the Bcl-2/Bax/caspase-3 signaling pathway. These observations were confirmed by the experiment applied by Russo et al [47] who approved these apoptotic mechanisms in human prostate cancer cells *in vitro*.

The extract also affected the p53 amount in the EAC cells. P53 gene is known as the tumor suppressor gene and is responsible for the repair or the damage of the DNA in the cells [48]. In case of tumor cells, the p53 responsible gene is subjected to mutation and p53 proteins are changed to mutant p53 proteins that supply the cancer cells with the energy and food antioxidants, making the cancer cells more resistant to the chemotherapeutic drugs and increases their proliferation [49]. Mutant p53 proteins greatly accumulate in tumor cells, this accumulation is detected by immunohistochemical staining and can unveil the existence in mutation of the p53 gene [50].

The immunohistochemical results by our study revealed the decrease in the p53 amount in the tumor cells of the treated groups compared to the non treated one. So, we can conclude that the extract was able to decrease the mutant p53 amount leading to its apoptosis. The extract also succeeded in decreasing the activity of Bcl2, which promoted the increase in Bax and caspase cascade, especially caspase-3 which caused the execution of the tumor cells. These data may discuss the mechanism by which the *Allium porrum* execute the tumor cells.

With respect to the hematological parameters there was a significant increase in the total WBCs and platelets count in both of the positive groups. The increase in WBCs count is considered the first defense mechanism of the body against invader cells. The increase in the WBCs, which were produced from the bone marrow was reported in cancer cases accompanied with metastasis [51]. Their high count is considered as indicator for inflammation, trauma and leukemia. Wang et al. [51] observed also in their experiment that the implantation of 4T1 breast cancer cells into BALB/c mice led to thrombosis as well as high WBC count, high platelet count, high platelet crit and low blood perfusion.

The safety of the extract on the normal body cells was demonstrated in the normal significant levels in the RBCs count and haemoglobin found in the case of solid tumor MEAP treated groups compared to the control group. The data also revealed a remarked but non-significant decrease in total leukocyte count in the treated groups compared to the non- treated group (Table 4).

In case of the heamatological results in the i.p injected EAC cells and treated with MEAP, there was a significant decrease in RBCs count compared to the positive and the normal control groups in spite of restoring the normal level of the total leukocytes count (table 3). These data indicated the low cytotoxic effect of the extract on blood picture in the treated groups. The cytoprotective effect of the plant to the normal cells was attributed to the presence of the flavonoid kaempferol as denoted by Di Donna et al. [13]. They reported the ability of kaempferol in neutralizing the toxicity of 7-beta-hydroxycholesterol in rat smooth muscle cells and avoiding its death.

The clear effect of the extract in decreasing total leukocytes confirmed the anti-inflammatory effect of the plant. This data is in accordance with Adao et al. [52] who proved the anti-inflammatory and anti-ulcerogenic properties of *Allium ampeloprasum* var. porrum bulbs. They attributed these findings to the presence of a new steroidal saponin in the plant.

Conclusion

It was concluded that the methanol extract of the aerial parts of *Allium porrum* L. (MEAP) is a potential anti-cancer agent performing its influence through inducing the apoptotic mechanisms in the tumor cells. Its activity may be due to the presence of bioactive phenolic and flavanoid compounds and these compounds were isolated for the first time from this plant.

Financial support and sponsorship: Nil

Compliance with ethical standards: All procedures described were reviewed and approved by the Animals Ethical Committee of the National Research Centre under the ethics number **(18035)**.

*Contributions:*A.H. Mahmoud suggested the idea and the experimental design. A.H.Mahamoud, D.Foda. and N.E.Ibrahim made the practical work and collect the scientific subject. D. Foda interpreted the biochemical data analysis and

wrote the manuscript and All authors revised the final manuscript. A.M. El-Hagrassi. made the plant extract and the phytochemical constituents. O.Yousf made the histopathological studies.

Conflicts of Interest

The authors have declared that the present study was performed in absence of any conflict of interest.

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