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Cytotoxic effect of*Cladophoraglomerata*crude extracts andphytolin bone marrow of mice

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Abstract: *Cladophoraglomerata contain different active compounds like flavonoids, alkaloids, phenols and tannins, fatty acids, sterols, terpeneswhich could be used as a novel source of natural agents for pharmaceutical industries. Some Studies that determined the activity of immune system and the effects of different agents depended on the cells division in bone marrow as it is the source of all blood cells. Two different doses (50,150 mg/ kg) from algal crude extracts and phytol were used in this study to determine the activity of these extracts on Mitotic index and Blast index in mice bone marrow.The resultsrevealed that the Blast index (BI) of bone marrow had a significant differences and increased in the higher dose(15 mg/kg) of aqueous extract and phytolas compared with control group while in ethanolic extract no significant differences appeared in both doses. The decline in Mitottic index (MI) was evident on the cytotoxicity of the lower dose from aqueous extract.The results alsoshowed that the influence on increment of (MI) in ethanolic extract, indicated to the anticytotoxic effect of this extract. On the other hand, this study suggested, that the activity of ethanolic extract in these two parameters may be belonged to phytol, which did not show any significant differences with crude ethanolic extract when was used as a pure compound.*

Keywords: *algae, Cladophoraglomerata, ethanolic extract, mitotic index, phytol,*

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I. Introduction

Cladophora is a genus of reticulated filamentous green algae. It contains many species that are very hard to differentiate and classify mainly because of the great variation in their appearance which is affected by habitat, age and environmental conditions [1].

C. glomerata is the most widely distributed macroalga throughout the world's freshwater ecosystems [2].This alga contain different active compounds like flavonoids, alkaloids, phenols and tannins[3],fatty acids[4], sterols, terpenes[5],whichcould be usedas a novel source of natural antimicrobial and antioxidant agents for pharmaceutical industries [6].This alga could be also used in cosmetic preparations because itcontains fatty acids , polyphenols, macro- and microelements and terpenoids like phytol which is regenerating and rejuvenating the skin[7]. Phytol(3,7,11,15-tetramethyl-2-hexadecen-1-ol) is one of the most important and simplest of the diterpenes, which forms the lipophilic side-chain of the chlorophyll [8], and it is a precursor for vitamins E and K. This compound is converted to phytanic acid after absorption [9] and it is highly significant among algal terpenes, as it has a definite role to play in the metabolism of algae [10].Algae have been at the origin of only few studies focused on immunological applications. Thebiological properties of immunological interest have been demonstrated in 140 species of algae, and they have been found to have useful applications in human health, particularly in the fields of oncology and immunology[11].Studies that determined the activity of immune system and the effects of different agents, depended on the proliferation of lymphocytes in lymphoid organs, or on the cells division in bone marrow as it is the source of all blood cells [12],for this reason study the activity of *C. glomerata*crude extracts and phytol on Mitotic index and Blast index is the aim of this research.

II. Material And Methods

2.1. Sample collection

C. glomerata was collected from the fresh water in the canal around University of Baghdad campus and stored in plastic bags prior to transport to the laboratory.

2.2. Isolation of macro algae

Algal samples were identified with the help of classical algal classification reference[13].These samples were cleaned from epiphytes, necrotic parts and then washed with freshwater to remove sand, and any adhering substances. Samples were rinsed with sterile water to remove any associated debris and dried in oven with 38- 40℃ and then these samples were stored in the refrigerator.

2.3. Preparation of alcoholic extract of *C. glomerata*

The dried algal samples were powdered with the help of theblender. The powder (10 gm.) was filled in the thimble andextracted with ethanol 70% by usinga soxhlet apparatus atthe temperature of 60℃ for 9 h [14]. The extract was driedin oven at 38-40 C˚. The dry extract was collected and weighted then preserved at 4ºC in refrigerator untilrequired. A known weight of this extract was dissolved inPhosphate buffer saline (PBS) to make a stock solution forfurther concentrations

2.3. Preparation of aqueous extract of *C. glomerata*

Dried algal samples were weighed followed by boiling at 70℃ for one hour. The extract was then filtered through filter paper (Whatman no.1). The filtrate was evaporated and then algal extract was stored at 4℃ [15]. A known weight of this extract was dissolved in PBS to make a stock solution for further concentrations. Furthermore 0.3 gm. ofcrude extracts was dissolved in 2 ml ethanol. The solution was analyzed using Shimadzu GCMSQP2010Ultra.

2.4. Preparation of phytol stock solution

Phytol with purity (97%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Phytol was emulsified in 0.05% Tween 80, dissolved in 0.9% saline [9]. To make a stock solution for further concentrations.

2.5. Laboratory animals

Albino male mice in age 8-10 weeks and weight 23-25 gm were divided into three groups; each group was kept in a separate plastic cage. Mice were maintained at a temperature of 23 -25 C˚, and they had free excess to food (standard pellets) and water. Mice of the first group were injected intraperitoneal with crude extracts (aqueous and alcoholic) in two doses (50 and 150 mg/kg), while the second one injected with pure phytol (50 and 150 mg/kg) and in the control group, mice were injected with normal saline.

2.6. Metaphase Index Assay

Metaphase index was determined for cells obtained from bone marrow of treated mice with aqueous and ethanolic extract as well as to phytol by following the procedure of [16],each animal was injected intraperitoneal with 0.3 ml of colchicine solution and after 1.5-2 hours, the animals was sacrificed by cervical dislocation and then dissected to obtain femur bone which was cut from both ends,and its cellular contents were collected in a test tube using a disposable insulin syringe (1 ml) and Phosphate buffer saline (5 ml). The slide was examined under a high power lens (100x),and at least 1000 cells were examined. The percentage of blast index and metaphase cells (metaphase index) were recorded using the equations (1) and (2) respectively.

$$\textbf{(1)Blast index}(\%) = \frac{\textbf{Numbers of blast cells}}{\textbf{Total count}} \times \mathbf{100}$$

$$\textbf{(2)Mitotic index}(\%) = \frac{\textbf{Numbers of metaphase cells}}{\textbf{Total count}} \times \mathbf{100}$$

2.7. Statistical analysis

The results of a different parameters for the treatments (control, aqueous extract, alcoholic extract and phytol) in a current study, were analyzed by using the analysis ofvariance (ANOVA), Ftest and Ttest, carried out incomplete randomized design (CRD). Difference betweenmeans of treatments were analyzed using least significantdifferences (LSD) at ($P \leq 0.05$), and expressed as (mean ±Sd). Programming excel application and SPSS program(2010) was used to find the results and draw the figureswith some effects to explain the statistical difference.

III. Results And Discussion

3.1. GC-MS analysis of *C. glomerata* aqueous extract

Thirty four chemical compounds were presence and identified in aqueous extract of *C. glomerata*. The highestpeak of the screened componentsbelonged to formic acid, butyl esterThe other compounds represented alcohols, acids, monoterpene(Eucalyptol), hydrocarbons (undecane) and aromatics like (Pxylene and benzene 1,2,3 trimethyl).

3.2. GC-MSanalysis of *C. glomerata*alcoholic extract

In this study thirty-seven constituents were identified in alcoholic extract of *C. glomerata*. The highest peak of thescreened Compoundsbelonged to phytol. This compound is the common occurrence diterpene alcohol in the green algae. The other compounds represented phenol, sterol, acids,alcohols, hydrocarbons (like hexadecane) and fatty

3.3. Blast index

Table (1) revealed that the Blast index (BI) of bone marrow, had a significant differences in the higher dose(15 mg/kg) of aqueous extract and phytolas compared with control group, and the BI was increased as shown in " fig.1" ,while inethanolicextract no significant differences appeared in both doses.

Table (1): Effect of different doses from aqueous, ethanolic extracts and phytol on the Blast index in mice. Capital letters refer to comparison in rows and small letters refer to comparison in columns ,similar letters refer to non-significant differences between means at ($p \leq 0.05$),using (LSD test).

Blast index % Mean ± SE			
Doses / Treatments	Control	150 mg / kg	50 mg / kg
Aqueous extract	38.32 B ± 0.71 a	44.49 A ± 1.05 a	40.62 B ± 0.76 a
Ethanolic extract	38.32 A ± 0.71 a	40.77 A ± 0.77 b	38.64 A ± 1.05 a
Phytol	38.32 B ± 0.71 a	41.26 A ± 0.43 b	39.80 AB ± 0.98 a
LSD $P \leq 0.05$	2.57		

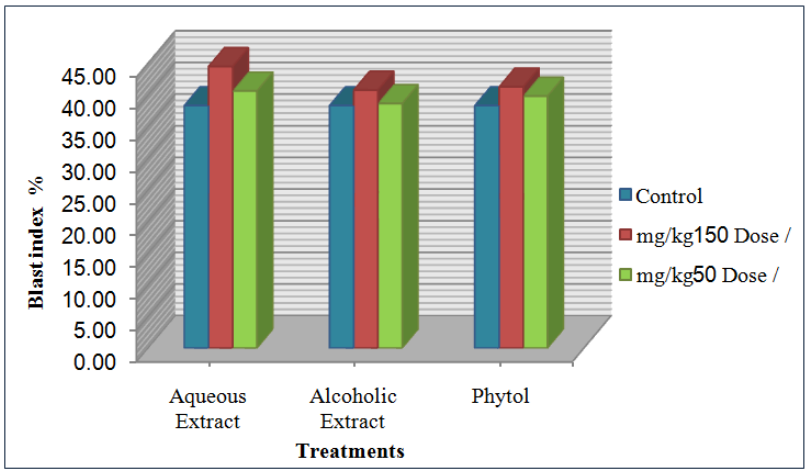

Figure 1: Blast index% in each treatment for two doses

3.4. Mitotic index

The results showed that mitotic index (MI) had a significant differences in both doses of aqueous extract Table(2), as compared with control group and showed also elevation in the higher dose and decreasing in the lower dose(Fig.2). This decline in MI was evident on the cytotoxicity of the lower dose from aqueous extract, different finding was shown by[17], who illustrated that the general use of *C. glomerata*in daily meals is safe for humans.[18] referred that mitotic index can be affected negatively or positively by chemicals, drugs, and medicinal plants .It is notable, that the decreasing in MI or the inhibition of the DNA synthesis, may be explained by the decreasing ATP levels, or by decreasing in number of cells moving into prophase from G2, as has been reported by [19]and[20].Ethanolic extract had a significant differences only in the higher dose which increased the MI. In the group of mice which were treated with higher dose of phytol, the results showed that there were a significant differences in MI as shown in Table (2) This dose also appeared to increase MI (fig.2) as compared with control group.

Table (2): Effect of different doses from aqueous, ethanolic extracts and phytol on the Mitotic index in mice

Mitotic index % Mean ± SE			
Doses / Treatments	Control	150 mg / kg	50 mg / kg
Aqueous extract	17.19 B ± 0.72 a	19.21 A ± 0.59 a	14.18 C ± 0.65 b
Ethanolic extract	17.19 B ± 0.72 a	19.19 A ± 0.37 a	17.65 B ± 0.64 a
Phytol	17.19 B ± 0.72 a	19.42 A ± 0.77 a	18.79 B ± 0.43 a
LSD $P \leq 0.05$	1.846		

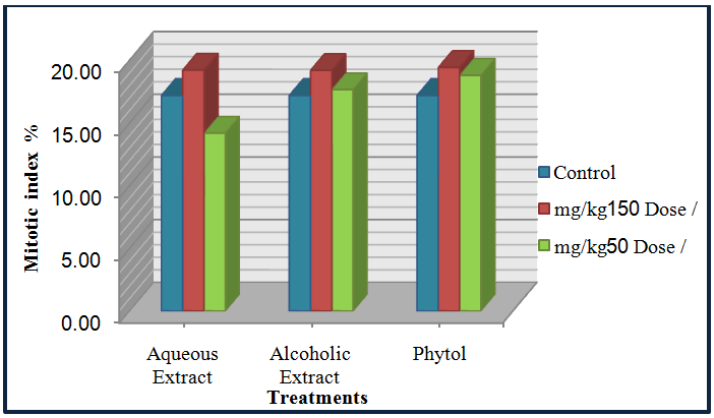

Figure 2: Mitotic index% in each treatment for two doses

IV. Conclusion

According to the results of mitotic and blast indexes, the influence on increment of (MI) in ethanolic extract, indicated to the anticytotoxic effect of this extract. On the other hand, this study suggested, that the activity of ethanolic extract in these two parameters may be belonged to phytol, which did not show any significant differences with crude ethanolic extract when was used as a pure compound.However, the concentration of doses in all treatments had main role in increasing MI as compared with control group ,while in blast index the concentration role appeared only in the aqueous extract.

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