Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 25/06/2023;
Date Revised: 11/08/2023;
Date Published Online: 20/10/2023;

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How to Cite:
Aziz DZ, Hadi MA, Al-Kelaby KKA, Abdullah ZA, Hamza A, Altaee AA, AL-Qaraawi HAH (2023). Cytotoxic and Antioxidant Activity of Linseeds Against Rhabdomyosarcoma Cell Line. Adv. Life Sci. 10S(1): 67-72.

Keywords:
Linseeds; Cytotoxicity; MTT Assay; Rhabdomyosarcoma Cell Line

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Cytotoxic and Antioxidant Activity of Linseeds Against Rhabdomyosarcoma Cell Line

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Abstract

Background: Flaxseeds (*Linum usitatissimum L.)* are rich in phytochemicals and antioxidants that may have anticancer properties.

Methods: The anticancer activity of the hydro-methanolic extract of linseeds (*Linum usitatissimum* L.) on the Rhabdomyosarcoma (RD) cell line had been evaluated through determination of phytochemical content, anti-oxidative potential by the free. radical-scavenging capability of 2, 2, diphenyl_1_picrylhydrazyl (D.P.P.H) and cytotoxic effects which were carried out by utilizing M.T.T assay.

Results: Phytochemical analysis recorded various phytochemical compounds, with antioxidant activity of hydro-methanolic extract of linseeds. Furthermore, linseeds hydro-methanolic extract has a significant cytotoxic influence on the RD cell line in concentration ranged 0.05-80 mg/ml by MTT assay with an IC_{50} value of 0.07 mg/ml.

Conclusion: From the performed assays, the hydro-methanolic extract of linseeds shows greater activity on the RD cell line which ensured the possibility of applying linseeds as anticancer that due to the anti-oxidative potential of its phytochemical compounds.

Introduction

The medicinal consequence of plants is connected to the influence of various amounts of their chemical compounds (phytochemical) which could yield a limited physiological activity on the body [1-3]. *Linum usitatissimum* L. (linseeds, or flaxseeds) is belongings to Linaceae family which found in West Asia and the Mediterranean. Linseeds have high value or quality of fat, proteins, and dietary fibers [4]. Linseeds have much potent advantages of health and therapeutic utilizes as a complete seed, precursors of lignan, oil from flaxseeds, and it has several potential benefits in the prevention or treatment of various disorders [5], like suppression of cardiovascular disease, cancer, diabetes, osteoporosis, arthritis, atherosclerosis, neurological and autoimmune diseases [6-9].

Rhabdomyosarcoma (RD) is considered as a malignant tumor of mesenchymal origin; it is one of the most frequent pediatric, soft tissue sarcoma, and rationale for approximately 5% of cancers in childhood [10]. This type of cancer can be found practically at any place in the body like striated muscle. Is not begin naturally and the peak occurrence can be noticed early in childhood with a mean of age (5 years) at diagnosis [11-12]. The current investigation plans to appraise the cytotoxic in addition to antioxidant activity of the linseeds hydro-methanolic extracts against Rhabdomyosarcoma cancer cells line.

Methods

Chemicals and Reagents

Rhabdomyosarcoma (RD) cell line was kindly gain from the (Central Public Health Laboratory/ Baghdad- Iraq), antibiotic (penicillin// streptomycin) purchased from (Gillingham, U.K). DMEM media powder (GIBCCO, U.S.A) supplemented by (10.0%) of fetal bovine serum (Capricorn Scientific, Germany)-heat inactivated-, M.T.T (3_4, 5_dimetthyl_2_thiazolyl_2,5_diphenyl_ 2H_tetrazollium bromide) purchased from (Bioworld U.S.A). Besides, 2, 2_Diphenyl_1_picrylhydrrazyl (D.P.P.H) was purchased from (Sigma, Aldrich, U.S.A). All different reagents utilized during this study were of analytical grade and consumed as taken.

Plant Identification

Linseeds were bought from-local Iraqi markets of herb and distinguish by Plant-Herbarium of the College, of Science/ Department of Biology/ University of Babylon.

Plant Extraction Procedure

One hundred gram of aground-dried sample extracted with 300 ml of organic extract (30.0 % methanol: 70.0 % distilled water V/V) then homogenized by electrical blender for a half hour at room temperature and filtered, concentrated to dryness at 40 ºC, weighed, and stored at 4 ºC till additional assays.

Phytochemical Analysis

The initial qualitative analysis of phytochemical was achieved by discriminating the secondary metabolites exist in this-extract. A particular way was applied for alkaloids previously depicted by [13] whereas the appearance of glycosides, resins, and determination the pH of the extract was employed like illustrated by [14]. Steroids and terpenes detection performed depending on [15]. Saponins were diagnosed by 2 various procedures illustrated by [14] and [16]. Moreover, coumarins, essential oils, flavones were detected depending on the consecutive references respectively [17-19] while detection of phenolic compounds and tannins were depending on [20].

Evaluation of Antioxidant Activity

The activity like antioxidant was assessed by 2, 2_Diphenyl_1_picrylhydrazyl (D.P.P.H) assay for scavenging of radical activity. After the scavenging of D.P.P.H, the diminishment in absorbance at 515nm that occur as a result of the minimizing by the-antioxidant was noticed. DPPH is highly employed to ensuring the capability, of compounds to achieve the donors of-hydrogen or free-radical-scavengers and to appraising the antioxidant activity in foods. Various graduate concentrations of extract were prepared for determination the inhibition concentration of extract that scavenges 50% of the oxidation state of D.P.P.H. The antioxidant activity of linseeds extract were calculated by mixing (100 µl) of the extract with, solution of ethanol of 0.0044 % D.P.P.H (30ml) then reading the absorbance at 515 nm after 30 min. Standard antioxidant (ascorbic-acid, 12mg// ml) was utilized for ,comparison as a positive-control [21]. Percentage-scavenging of the DPPH free radical was calculated by the equation:

$$\text{DPPH scavenging activity (\%)} = [Ac - As / Ac] \times 100$$

Where Ac refer to the control absorbance while As refer to the sample absorbance.

Cell Viability Assay

Cell line was grown as a monolayer culture in microtiter plate, dose-response curve is accomplish to notice the cytotoxic activities of linseeds on cancer RD cell line by M.T.T assay as follow, multiple doses of the extract are prepared in concentrations (0.001, 0.01, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8) mg/mL in media were added as (100µl) to the flat-bottomed micro-culture plate wells, in triplicate and incubated for 24 h. After that, (10 µl) of 5 mg/ml MTT in PBS solution was added to the wells with incubation for another 4 hr at 37 ºC.

Then, the dead cells and extra MTT are removed and at last (200 µl) of DMSO: isopropanol (1:1) was added directly to every well and shaken for 5 min (the DMSO solution became purple). The optical density of each sample is recorded at a wavelength of 490 nm by ELISA-reader. The measurement of cell inhibition was carried out and the concentration that inhibits 500% of cell-viability (IC_{50}) was calculated. Half inhibitory-concentration was fitted by blotting the percentage of inhibition versus the concentration log of any-compound utilized.

Statistical analysis

All experiments were carried out, in any case in triplicate and for three times the outcomes were presented as mean± standard errors.(SE). Statistical tests (Mean SE, and one way A.N.O.V.A) were achieved utilizing I.B.M.-SPSS.-statistics version 21. For all assays, the level of significance was P,≤ 0.055.

Results

Analysis of Phytochemical in *L. usitatissimum* L. extract

After the extract subjected to chemical analysis to identify the phytochemical, compounds, the results were-shown in Table 1.

Phytochemical compound	Detection method		+/-
	Reagent	Positive result	
Coumarins	UV light	Yellow greenish color	+
Steroids	Chloroform + acetic acid+H_2SO	Blue color	+
Terpens	Chloroform + acetic acid+H_2SO4	Brown color	+
Resins	Ethanol + D.W containing HCI 4%	Turbidity	+
Flavones	Ethanol 50%+KOH 50%	Yellow color	+
Alkaloids	Mayer	White color	+
	Wagner	Brown color	+
Glycosides	Benedict	Red pellet	-
Essential oils	UV light	Bright pink color	+
Phenol compound	Ferric chloride 1%	Green-bluish color	+
Tannins	Lead acetate 1%	White gelatinous pellet	+
Saponins	Shaken	Formation of foam	+
	HgCl 1%	White color	+
PH	PH meter	----	7.3

+ Positive detection - Negative detection

Table 1: Detection of some phytochemical compounds in Linseeds extract.

DPPH Radical-Scavenger Activity

The results in Table 2 revealed the values of scavenging activity compared with ascorbic acid and noticed that radical-scavenging-activity of linseeds which scavenge half of D.P.P.H. radicals (IC_{50}) was 1.5 compared with IC_{50} of ascorbic- acid which was 1.02.

Cytotoxicity Assay

The results of the cytotoxicity dose-response-curve were indicated the inhibitory activity with statistical accepted differences (P≤ 0.05) between untreated cells of RD as compared with those taken multiple concentrations of linseeds-extract. Linseeds-extract was toxic to the RD cells as shown by nearly complete cell death at the 0.8 mg/mL dose after 24 hr and it's IC_{50} about 0.07 mg/mL as shown in Table 3 and Figure 2.

Concentration of extract (mg/ml)	Scavenging of DPPH radical(%)	
	Linseeds	Ascorbic acid
2	60.75± 1.33*	85.29± 0.78
5	64.53± 0.67*	90.22± 0.33
8	68.31± 0.71*	95.14± 2.8
10	68.67± 10.48*	97.1± 0.67
20	70.72± 4.41*	98.55± 3.41
40	80.63± 3.11*	99.81± 0.45
IC_{50}	1.5	1.02

*Refer to significant-differences compared to ascorbic-acid.

Table 2: Percentage of scavenging of 2,2 -Diphenyl -1 -Picrylhydrazyl radical.

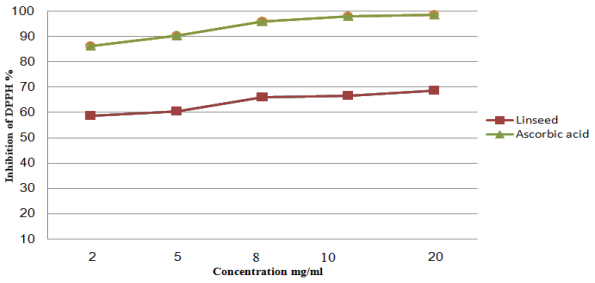

Figure 1: percentage of inhibition of DPPH by hydro-methanolic extract of Linseeds.

Concentration (mg/mL)	Growth inhibition± S.E	IC_{50}
0.001	0^{a}	0.07 mg/mL
0.01	10.97^{b}± 0.008	
0.025	21.01^{b}± 0.017	
0.05	43.85^{b}± 0.024	
0.1	50.42^{c}± 0.005	
0.2	74.74^{d}± 0.012	
0.4	83.12^{e}± 0.011	
0.8	91.09^{f}± 0.01	

S.E: standard error, similar letters indicate no significant-differences, *different letters indicate significant-difference (P≤0.05).

Table 3: Cytotoxic activity of linseeds extract at different concentration against Rhabdomyosarcoma(RD).

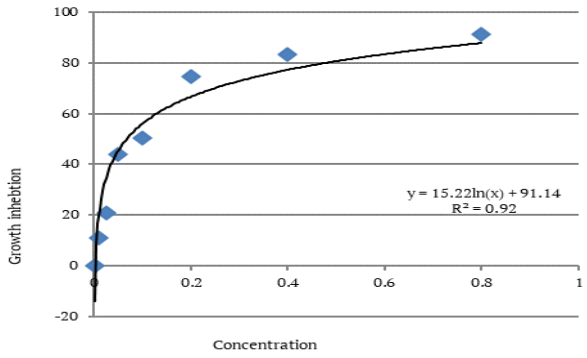

Figure 2: Dose-response curve of growth inhibition of linseeds extract on the RD cells line represented by plotting of concentration 'log' versus GI% values.

Furthermore, it is noticed that RD cells have endure particular morphological alterations, like shrinkage of cells and blubbing (Figure 3), which are property feature of apoptosis.

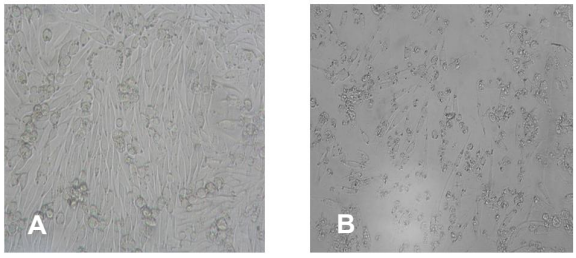

Figure 3: Morphological alteration of RD cell line after linseeds extract treatment A) Untreated RD cells B) linseed extract treated RD cells. Inverted microscope magnification power 40X.

Discussion

Antioxidant compounds had an influential function in minimizing some chronic disorders like cardiovascular disorders and cancer. Extracts of plant are projected to cell lines to conclude their effectiveness as a possible alternate of drugs and.to checkup their effectiveness in the medical application [22]. Plants regarded as main resources for natural antioxidants like carotenes, phenolic acids, etc. The analysis of phytochemical of linseeds extract was in consistence with previous-studies mentioned that linseeds had alkaloids, essential oils, flavones, resins, saponins, phenolic compounds, steroids, and terpenes [13, 23,24].

According to DPPH assay performed in the current study to survey the activity of radicals-scavenging and comparing the antioxidant activity of hydro-methanolic extract of linseeds and ascorbic-acid, the results recorded that the IC_{50} value of hydro-methanolic extract of linseed was 1.5 mg/ml. This scavenging- activity was inconsistent with results reported by [25]. In general, the antioxidant impact may be connected to the presence of phytochemical-compounds noticed that had antioxidant activity or may be from its other phytochemical compounds as linseed lignans which significantly enhanced the capability of chemotherapeutic agents to cause cytotoxicity and inhibited the proliferation of breast cancer cells *in vitro* [26].

Antioxidant potential of linseeds phenolic constituent have been measured, in both *in-vivo* and *in-vitro* models. The previous result noticed significant dose-dependent inhibition against D.P.P.H radical-scavenging, superoxide-anion and hydroxyl radicals scavenging, reducing-power, hydrogen peroxide scavenging, metal chelating in consequence of phenolic component which notice to be the principal constitutes responsible for the remarked antioxidant activity [27]. Moreover, another study conducted by [24] explore the antioxidative properties of flaxseed 20% methanolic extract in male rats against oxidative stress induced by cyproterone acetate (CPA) drug. They conclude that the flaxseeds methanolic extract possess a cytogenetic activity to inhibit genotoxic and mutagenic influences of CPA caused by its antioxidant features. In another study, it was exhibited that supplementation with linseeds in diet raise antioxidant defenses through both raise detoxification of ROS and minimized generation of ROS [28].

Results of the phytochemical and antioxidant tests have additional guided these investigations towards assay of cytotoxicity; hence RD cells line was selected for the cytotoxic activity. The outcomes of M.T.T assay presented in Figure 3 and Table 3 revealed that the hydro-methanolic extract of linseeds has cytotoxic activity toward this cancer cells line which caused growth inhibition (91.09 %) at concentration 0.8 mg/mL. In earlier reports, various extracts of linseeds have exhibited cytotoxic properties on various cancer cell lines.

These results inconsistent with earlier reports which proved that various extracts of linseeds have displayed cytotoxic properties in another cell lines as the study by (34) who observed hormone-decreasing and anti-proliferative influences on the chorion carcinoma cells line (jeg3) induced by the flaxseed crude extract. In another study, a fraction rich with lignan in the purified flaxseed hydrolysate (PFH) employs its anticancer-activity on a breast-cancer cells line (T.47.D) from human and in mice suffering tumor and PFH -G9 appeared the foremost significant cytotoxic-activity toward breast cell lines T47D and MCF7 that had ER-receptor positive with IC_{50} 15.8 and 13.88 µg/ml , respectively. Additionally, P.F.H-gG9 minimize the expression of metastasis marker 1-α, metalloproteinases, and vascular endothellial-growth factor (V.E.G.F), which is the mainly potent stimulators of angiogenesis, despite it raised apoptosis dependent on the caspase-3. Also, the supplement of 10% of Giza Flaxseeds (FS), flax meal or fixed oil two times in day for three weeks in mice- suffering Ehrlich ascites carcinoma (E.A.C) brought about inhibition the volume of tumor, the estrogen expression, MMP-2, insulin growth factor, progesterone and VEGF but elevated caspase-3 expression [30].

Preceding studies hypothesize that some phenolic substances in linseeds extract, like lignans, isoflavones, and monolignols, may have an influential impact in these inhibitory actions which capable of making alteration in the genes expression that is influential for the survival of cell [31] or cycle of cell [32]. Genistein, that is one of the isoflavones can enhance tumor suppressor genes.(p.21, p16) expression and diminished the tumor-promoting genes c-MYC and

BMI1 expression as described by [33]. Similarly, these results in concurrence with *in vivo* studies documented the possible protective influences of 200% methanolic extract of linseeds against CPA in adult albino rats and proved that supplement of the flaxseeds extract orally at 2 doses (250 & 500 mg/kg) with C.P.A have the ability to minimize the inhibitory impact of C.P.A in the weight of the body [34]. Furthermore, [16] recorded a diminished level of DNA fragmentation of testicular tissue and white blood cells in groups taken both flaxseeds with CPA compared with group taken CPA alone. At last, the extract showed a potent antioxidant activity, and prevented the toxicity and abnormalities of the cells [35]. The current investigation revealed that linseeds extract an effective inhibitor of the growth of the RD cell line through its antioxidant activity. Also, suggest that linseeds can perform as a dietary-supplement during treatment against cancer and can enhance chemotherapeutic efficacy. Further investigations of this plant are required to determine the active compound responsible of their cytotoxic activity on other cancer cells lines and there is a requisite to achieve *in vivo* investigation to additional confirm the antioxidant and anti-mutagenic potentials of linseeds.

Acknowledgment

The thankful produce by the authors to the department of Clinical and Laboratory Sciences // College, of Pharmacy-.University of Kufa.

Author Contributions

Dhifaf Zeki Aziz: Made the research article proposal, and perform the experiments.
Maysaa Adil Hadi: Drafted the manuscript and helped in Phytochemical analysis.
Khalida Kadhim Abbas Al-Kelaby: Designed the experiment.
Zahraa Ali Abdullah: Formatted the document.
Azhar Hamza: Helped in microscopy.
Amel Ali Altaee: Reviewed the document.
Hanan Ahmed Hadi AL-Qaraawi: Made statistical analysis.

Competing Interests

The authors declare that there is no conflict of interest.

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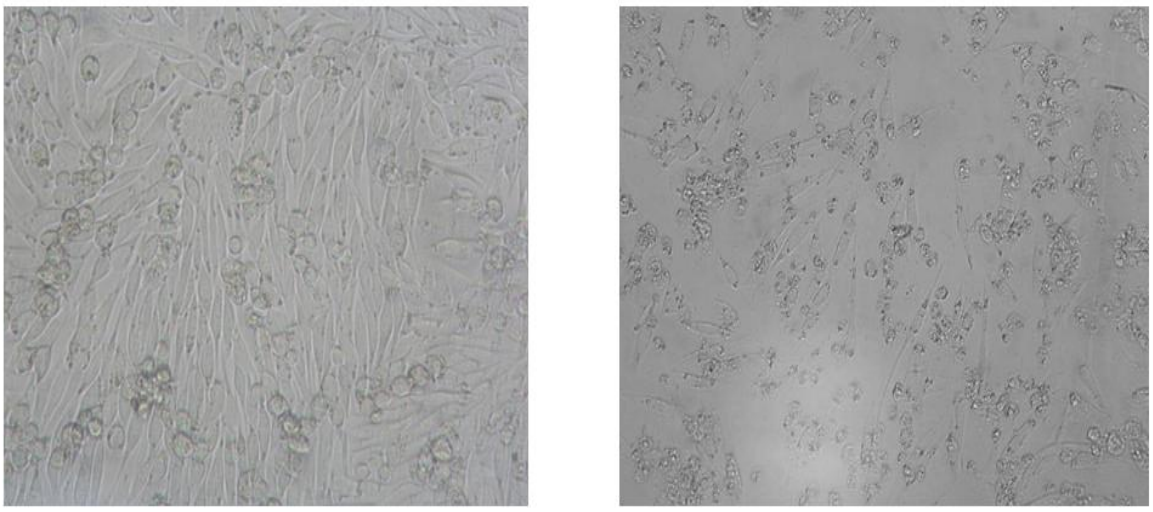

(A) (B)

Figure 3: Morphological alteration of RD cell line after linseeds extract treatment A) Untreated RD cells B) linseed extract treated RD cells. Inverted microscope magnification power 40X.

Editorial Note: Figure 3, in higher resolution, is re-produced here for better visibility.