Biochemistry Letters,21 (1) 2025, pages :161-173

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Ameliorative effect of Flaxseed on Ehrlich Carcinoma-bearing mice.

Soha M. Hamdy[(1)], Rania Salah Abdullah.[(2)], Heba Mohamed Rabie Mohamed Elesh [(3)], Ahmed Ismail[(4)], Amany M. Shabaan[(5)]

(1) Professor of Biochemistry, and Head of Chemistry department, Faculty of Science, Fayoum university
(2) Demonstrator, Chemistry department, Faculty of Science, Fayoum university
(3) Lecturer of histology, Zoology department, Faculty of Science, Fayoum university
(4) Lecturer of Pharmacognosy, Pharmacognosy department, Faculty of Pharmacy, Fayoum university
(5) Assistant professor of Biochemistry, Chemistry department, Faculty of Science, Fayoum university

ARTICLE INFO

Received : 5/8/2025
Accepted : 10/8/2025
Accepted to Online publish: 10/8/2025

Keywords:
Ehrlich carcinoma (EC) , Antioxidant, Anti-inflammatory, Anti-tumor

ABSTRACT

Objectives: Owing to the deleterious effects of treatment modalities currently in use for cancer treatment the need to develop safe cancer treatments with minimum or no side effects became a critical need to save lives of patients. This study was conducted to assess the efficiency of using Flaxseed as an anti-tumor agent on mice-bearing Ehrlich Carcinoma (EC).

Methods: Twenty four male swiss albino mice were divided into four groups: group1 was control normal group; group2 served as an untreated tumor control group; group3 was a tumor-bearing mice group treated with Flaxseed and group4 was Flaxseed protected tumor-bearing mice group that was administered with Flaxseed for 15 days before tumor inoculation. Flaxseed treatment started at day "0" of tumor inoculation and was continued for 30 days followed by evaluation of anti-tumor effects, liver and kidney function tests, total antioxidant capacity, oxidative stress and anti-inflammatory markers.

Results: Flaxseed showed potent anti-tumor effects by reducing tumor volume and weight significantly with tumor control group with ameliorative effects on liver and kidney function tests that were significantly elevated in tumor control group. Tumor lowering effect on Total antioxidant capacity (TAC) and elevation of oxidative stress markers were potently ameliorated by Flaxseed, restoring their values to ones close to normal control. Tumor-induced elevation of the inflammatory marker C-reactive protein (CRP) was also reduced by Flaxseed administration returning to levels near normal control.

Conclusion: Flaxseeds exhibited potent protective and therapeutic anti-tumor, antioxidant and anti-inflammatory properties with ameliorative effect on tumor-induced adverse effects on liver and kidney.

I. Introduction:

Cancer is a term known for a group of diseases affecting any body part characterized by uncontrolled rapid development and growth of abnormal

*** Corresponding author: Rania Salah Abdullah. Demonstrator, Chemistry department, Faculty of Science, Fayoum university. rsa14@fayoum.edu.eg.**

cells and their spread further than their normal boundaries which may lead to death if not treated (WHO, February 2025) [**1**]**.** Cancer is a global health problem ranking as the primary mortality cause globally; being about 10 million deaths cause in 2020 [**2].** In 2022, Northern Africa recorded 1, 185, 216 new cancer cases and 763, 843 cancer deaths [**3].**

Cancer models are used in cancer treatment discovery and are changing continuously extending to preclinical studies. Numerous studies were accomplished using cancer models to help in better understanding of cancer invasion, early detection and progression. These models give an insight into cancer molecular basis, etiology, tumor-host interaction, microenvironment role, and heterogeneity of tumors in metastasis [**4**]**.**

Ehrlich carcinoma (EC) is an easily transplantable, undifferentiated, and rapidly proliferating carcinoma which has short life span, no regression, no tumor-specific transplantation antigen and hundred percent malignancy [**5**]**.** Similar to human tumors ehrlich carcinoma is sensitive to chemotherapy due to its rapid growth rate and being undifferentiated [**6**]**.**

Common approaches used for treating cancer include, chemotherapy, photodynamic therapy, radiotherapy, surgery immunotherapy and other methods. These procedures have several drawbacks such as poor specificity, toxicity, rapid elimination, limited bioavailability and high cost. Contrariwise, plant-derived anticancer compounds display quite a lot of advantages and can overcome these inadequacies. Plant-based anticancer compounds are easily available, potent, safer, and cost-effective compared to other treatment procedures. Countless therapeutic potentials have been proven to be exhibited by these naturally occurring compounds demonstrating that they could be used in clinical applications as candidate drugs [**7].**

Flaxseed has been consumed by people for thousands of years. The seeds recently gained increasing attention because they are rich in lignans, proteins, omega-3 fatty acids and fibers. These constituents play important roles in cardiovascular health, cancer prevention and treatment. This is also achieved through lignans that possess antioxidant properties, anticancer effects, diabetes management properties, and gastrointestinal health enhancement properties [**8**]**.**The current study was performed to investigate protective and therapeutic potentials of Flaxseed on a model of Ehrlich carcinoma.

II.Material and Methods:

In vivo study

Ethical statement

This study was performed in line with Fayoum University's Committee for Institutional Animal Care and Use recommendations, where our study received an approval number of **AEC 2448-a** (27-7-2025) .

Tumor cells

EC cells were bought from the Egyptian Cancer National Institute. Cell line of Ehrlich tumor was preserved as ascites in female mice, where serial transplantation intraperitoneally was performed every 8-10 days by 200 µl of EC cells suspension in sterile saline containing 2 million tumor cells.

Plant material

Flaxseeds were obtained from a local herbarium. An amount enough for three days of mice doses was grinded in an electrical grinder to a fine powder and preserved at 4^{o}C till preparation for mice administration. Flaxseed powder was administered as a

suspension in distilled water which was prepared directly before administration to mice.

Experimental animals and experiment design

Twenty four male Swiss albino mice were obtained from breeding unit of VACSERA (Abbassia, Cairo, Egypt) with weights of 20-25 g. The animals were housed in plastic cages for two weeks to acclimatize with maintenance on commercial 21% protein diet and drinking water *ad libitum*. Throughout the experiment period mice of all groups were kept under the same conditions.

After the adaptation period, animals were divided into four groups, six mice each, along these lines:

Group1 (Normal control) mice with no treatments administered or tumor induced.

Group2 (Tumor control) mice were subcutaneously injected in the right thigh by 200µl of EC cells suspension containing 2 million tumor cells on day "0" to yield a solid tumor [**9],** with no treatments administered.

Group3 (Flaxseed treated tumor group) mice were subcutaneously injected in the right thigh by 200µl of EC cells suspension containing 2 million tumor cells on day "0", and administered with Flaxseed (8 g/kg body weight/daily, orally by oral gavage), for 30 days starting from day "0" [**10].**

Group4 (Flaxseed protected tumor group) mice were administered with Flaxseed (8 g/kg body weight/daily, orally by oral gavage), for 15 days before being subcutaneously injected by 200µl of EC cells suspension containing 2 million tumor cells in the right thigh on the 16^{th} day which was counted as day "0" of tumor inoculation, and administration of Flaxseed continued for 30 days starting from day "0"**.**

During the experiment after tumors have become measurable their lengths and widths were measured every five days using vernier calipers.

On day 30 mice were anesthetized followed by blood withdrawn from mice retro-orbital plexus on empty eppendorf tubes to harvest serum using the method of **Abd El-Dayem *et al.*, 2010 [11],** serum was then preserved at -20^{o}C until use in biochemical analysis. After blood withdrawal, animals were sacrificed under anesthesia by cervical dislocation and tumors were harvested, weighed and their length and width were measured also using vernier calipers.

Mice body weight change

Mice were weighed before the beginning of the experiment, on day 1, day of sacrifice and every 3 days and body weight change percent was calculated using the equation used by **Manjula & Mruthunjaya, 2014 [12]**

$$\%\textbf{Change in body weight} = ((\textbf{weight}_{\textbf{final}} - \textbf{weight}_{\textbf{initial}}) / \textbf{weight}_{\textbf{initial}}) * \mathbf{100}$$

Tumor volume

Tumor volume was calculated using the equation used by **Jaganathan *et al.*, 2010 [13]**

$$\textbf{Tumor volume} = \mathbf{1/2} * \mathbf{L} * \mathbf{W^2}$$

Biochemical analysis

Colorimetric measurements

Activities of serum Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined using the available commercial kits of Biodiagnostics, Egypt, which uses the method of **Reitman & Frankel, 1957 [14]**. Biodiagnostics kits were used also for Serum Creatinine and Urea levels and serum total antioxidant capacity (TAC) estimation, with methods of **Larsen, 1972 [15] and Bartels *et al.*, 1972 [16]** for creatinine, **Fawcett & Scott, 1960** [**17]** for urea

and **Koracevic *et al.*, 2001 [18]** for TAC, while the level of serum Malondialdehyde (MDA) was determined using **Buege & Aust, 1978** method **[19]**.

Turbidimetric measurements

C-reactive protein (CRP) was determined using the available commercial kit of Bioscien which uses the method of **Müller *et al.*, 1985 [20].**

Statistical analysis

IBM SPSS statistics 22 and Microsoft excel 2010 were used to accomplish Statistical analysis. Description of data was as Mean ± SE **[21;22]**. To compare all groups to normal and tumor control groups, Student's T-test was applied, where significance was at $p \leq 0.05$ [**23;24]**.

III.Results

Results of mice body weight and percent change in body weight

On the final experiment day, body weight of mice in all groups showed an increase compared to body weight at the beginning (Figure **1a**). An increase in percent body weight change was noted in EC-control group significant to normal control mice **($p < 0.001$)**. On the contrary, Flaxseed protected tumor group revealed a decrease in percent body weight change significant with both normal and tumor control groups. Treated Flaxseed group, however, showed reduction in percent body weight change non-significant with normal control, but significant with tumor control group **($p < 0.001$)** (Figure **1b**).

Results of Ehrlich tumor volume and weight

Significant diminishing of volume and weight of tumor was revealed in Flaxseed protected and treated tumor-bearing mice groups with respect to tumor-control mice group (**p≤0.001**) which is explained in figure **2 (a,b**). It's also worth noting that tumors became measurable starting from day 6 of the experiment on tumor control mice group, and starting from day 16 on Flaxseed treated and protected groups with a delay of 10 days in these groups.

Biochemical analysis results

Results of serum analysis

Mice serum ALT & AST activities results

Elevation in the activities of serum ALT and AST was revealed in tumor control mice group Significant with normal control (**p=0.01** and **p<0.001**, respectively). Flaxseed protected group caused lessening of serum ALT non-significant with normal control mice group, whereas Flaxseed treated group showed non-significant increase. On the other hand, both groups showed decrease in both enzymes significant with tumor control group.

For AST, protected and treated flaxseed groups showed increase in AST non-significant with normal control group (Figure **3 a,b**).

Mice serum Creatinine and Urea concentrations results

Tumor control group revealed an increase in serum Creatinine and Urea concentrations significant with normal control group (**p<0.001**). In addition, Flaxseed administration in protected and treated tumor-bearing groups led to reduction in both parameters in these groups significant with tumor control group (Figure **4**). Non-significant increase with normal control group in the kidney function tests was revealed in Flaxseed protected and treated mice groups.

Mice serum Total antioxidant capacity (TAC) results

EC- control mice group, caused a drop in TAC significant with normal control group (**p=0.005**). Compared to tumor control group both Flaxseed protected and treated mice groups

revealed a significant increase in serum TAC, whereas non-significant decrease in TAC was noted in these groups compared to normal control (Figure **5a**).

Results of mice serum MDA and CRP levels

Tumor control group illustrated an increase in serum MDA and CRP levels significant with respect to normal control group.

Protected and treated Flaxseed tumor-Bearing groups, on the other hand, showed significant decrease in these parameters with respect to EC-control group. Non-significant rise in both parameters with respect to normal control was noted in Flaxseed protected and treated mice groups (Figure **5 b,c**).

IV.Discussion.

According to the world health organization statistics for 2022, Egypt recorded 150, 578 new cancer cases and 95, 275 cancer deaths **[3].** Despite the efficiencies and applications of the common cancer treatments being used in the meantime, they are often obstructed by their severe side effects, which directly lower patients' quality of life of and may also lead to late side effects [**25**]**.** Ehrlich carcinoma is an undifferentiated, easily transplantable and rapidly proliferating tumor **[5]**, which is sensitive to chemotherapy similar to human tumors **[6].**

Over the past few decades, great importance has been laid on the development of novel cancer therapeutic strategies [**26].** Natural products along with their derivatives and analogues use as anticancer agents is satisfactory due to accessibility, applicability, and reduced cytotoxicity, also, they have been proven to be effective against numerous signalling pathways [**27].**

Our study was performed aiming to the evaluation of Flaxseed effectiveness as a protective and therapeutic antitumor therapy.

In the present study, it was shown that treatment and protection of EC-bearing mice with Flaxseed resulted in a very high drop in tumor weight and volume (***p*<0.001**) significant with tumor control. Also, treated and protected groups showed a delay of 10 days in tumor appearance compared to tumor control mice group. These results agree with previous studies that revealed that Flaxseed suppressed Ehrlich tumor growth [**28;29]**.

The body weight gain percent was also used as an indirect measure of tumor progression, where both treated and protected groups showed significant reduction in body weight change percent with respect to tumor control group (***p* <0.001**) due to reduced tumor weight. On the contrary, significant increase in body weight of EC control group was shown with respect to normal control **($p < 0.001$)** due to rapid tumor growth, this significant increase in body weight change percent in EC-control group was also shown by other previous studies **[30]** . Our results revealed that non- significant change in body weight with respect to control mice was noted when treating or protecting tumor bearing mice with Flaxseed which was also shown in many studies, coinciding with our results **[31;32]**. Many studies claimed that the antitumor properties of Flaxseeds are mainly due to the high content of bioactive compounds as essential omega-3 fatty acids, lignans, phytoestrogens, flavonoids, vitamins, and minerals that provide anti-cancer properties to Flaxseeds **[33]**

Regarding liver and kidney function tests the same results our study revealed were obtained in other experiments **[30;34;35;36]** including

significant elevation in liver function tests (AST and ALT activities) and kidney-function tests (Creatinine and Urea levels) caused by Ehrlich tumor. Liver injury that happened is probably contributed to tumor angiogenesis leading to hepatitis due to infiltration of inflammatory cells [**37]**. While kidney injury was illustrated to be induced by ehrlich solid tumor, contributing this to oxidative stress that is induced by tumor that interacted with DNA and damaged it [**38]**.

Flaxseed used in our study diminished these effects where obtained values were close to normal control. Many studies also reported Flaxseed to reduce both liver and kidney functions concurring with our study [**39;40;41].**

Field *et al*., 2008 [42] showed that some of chemotherapeutic agents currently in use for treating cancer might cause direct toxicity to the liver, in addition to direct and indirect effects of cancer on liver function. On the contrary, Flaxseed showed antitumor effects associated with no side effects on liver function tests. Studies also elucidated that chemotherapeutic drugs used by cancer patients are excreted by the kidneys and therefore may be nephrotoxic **[43]**, however, Flaxseed in our study revealed no side effects on kidney function tests.

Ehrlich tumor in our study caused significant reduction in TAC and significant elevation in oxidative stress markers including lipid peroxidation expressed as MDA level. It was revealed also in our experiment that Flaxseed returned all values to normal control levels. In harmony with our results**,** elevation in MDA levels in Ehrlich solid tumor-bearing mice significant with normal control ones, with significant drop in antioxidant enzyme levels in Ehrlich tumor group were reported **[30;37;44;45].**

Many human tumors produce high levels of reactive oxygen (ROS) species [**46**]**.** Oxidative metabolic processes occurring in the body lead to production of reactive oxygen species which is natural. Another natural source of ROS is immune system cells, where they play an important role as effectors in immune mechanisms [**47**]**.** Immune system activation results from inoculation of mice with EC results in the production of free radicals continuously due to immune system activation and excessive metabolic processes due to abnormally high number of tumor cells [**48**]**.** Oxidative degeneration forms free radicals which cause lipid peroxidation leading to the formation of Malondialdehyde [**49**]**,** which is known for being a lipid peroxidation indicator **[49].** Cancer tissues were reported to be higher in Malondialdehyde content than others [**50**]**.** Many studies established disruption of antioxidant system and elevation of lipid peroxidation as a consequence of growth of tumor [**51;52;53].**

Our study also revealed returning TAC and MDA values to normal. Harmonized with our experiment results, **Al Za'abi *et al*., 2021 [54]** showed that Flaxseed increases TAC. While, **Ezzat *et al*., 2018 [28]** results explained a decrease in MDA upon treating Ehrlich-tumor bearing mice with Flaxseed, which supports our findings on antioxidant effects of Flaxseed.

A significant elevation in serum CRP was noted in our experiment in Ehrlich tumor control group with respect to normal control group. In addition, serum CRP levels returned to values close to normal control group in

Flaxseed treated and protected tumor-bearing groups. Significant elevation in serum CRP in ehrlich group compared to normal control group was reported in other studies which agrees with our findings **[44;55;56;57;58]**.

Chronic inflammation facilitates tumor progression, which supports tumor growth and facilitates metastasis [**59**]**.** CRP is a systemic inflammatory biomarker produced in the liver and released to circulation in response to elevation of IL-6 level **[60].** CRP is a common sensitive prognostic and diagnostic marker for both solid-tumor response to chemotherapeutic agents and its progression [**61**]. Elevated CRP levels are reported to be concomitant with tissue damage, inflammation during cancer, cardiac stress and infection [**62;63;64**]**.** For this reason it was used to assess the effectiveness of the drugs used in this study. Compatible with our study noted significant reduction in CRP levels on treatment with Flaxseed was revealed by many studies either on animals or in clinical trials **[65;66;67]**.

V.Conclusion: In conclusion, Flaxseed proved to possess anticancer effects without any side effects contributing to their enrichment in bioactive compounds that possess antioxidant and antiinflammatory properties, proposing it as a promising effective natural cancer protective and therapeutic agent.

VI.References

[1] American Cancer Society. Cancer Facts & Figures 2025. Atlanta: American Cancer Society; 2025.

[2] Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global cancer observatory: cancer today. Lyon: International agency for research on cancer. 2020 Nov 25;20182020.

[3] Wéber A, Vignat J, Shah R, Morgan E, Laversanne M, Nagy P, Kenessey I, Znaor A. Global burden of bladder cancer mortality in 2020 and 2040 according to GLOBOCAN estimates. World journal of urology. 2024 Apr 16;42(1):237.

[4] Sajjad H, Imtiaz S, Noor T, Siddiqui YH, Sajjad A, Zia M. Cancer models in preclinical research: A chronicle review of advancement in effective cancer research. Animal models and experimental medicine. 2021 Jun;4(2):87-103.

[5] Kabel AM, Abd Elmaaboud MA. Murine models of cancer. J Cancer Res Treatment. 2014;2(1):16-8.

[6] Ozaslan M, Karagoz ID, Kilic IH, Guldur ME. Ehrlich ascites carcinoma. African journal of Biotechnology. 2011;10(13):2375-8.

[7] Ullah S, Khan T, Khan T, Ali M, Jan AK, Shinwari ZK, Khan A, Al-Farsi M, Waqas M. Phytochemicals for cancer treatment: An update on plant-derived anti-cancer compounds and their mechanisms of action. Current Topics in Medicinal Chemistry. 2025 Mar;25(8):871-90.

[8] Duarte S, Shah MA, Sanches Silva A. Flaxseed in Diet: A Comprehensive Look at Pros and Cons. Molecules. 2025 Mar 16;30(6):1335.

[9] Abouzed TK, Beltagy EE, Kahilo KA, Ibrahim WM. Molecular changes associated with the anticancer effect of sulforaphane against Ehrlich solid tumour in mice. Journal of Biochemical and Molecular Toxicology. 2021 Feb;35(2):e22655.

[10] Hernández-Salazar M, Guevara-González RG, Cruz-Hernández A, Guevara-Olvera L, Bello-Pérez LA, Castaño-Tostado E, Loarca-Piña G. Flaxseed (Linum usitatissimum L.) and its total non-digestible fraction influence the expression of genes involved in azoxymethane-induced colon cancer in rats. Plant foods for human nutrition. 2013 Sep;68:259-67.

[11] Abd El-Dayem SM, Fouda F, Helal M, Zaazaa A. The role of Catechin against doxorubicin-induced cardiotoxicity in Ehrlich ascites carcinoma cells (EAC) bearing mice. American Journal of Science. 2010;6(4):146-52.

[12] Manjula SN, Mruthunjaya K. A STUDY TO EVALUATE THE ANTICANCER ACTIVITY OF BARLERIA GRANDIFLORA DALZ (BG): AN IN VIVO STUDY.

[13] Jaganathan SK, Mondhe D, Wani ZA, Pal HC, Mandal M. Effect of honey and eugenol on Ehrlich ascites and solid carcinoma. BioMed Research International. 2010;2010(1):989163.

[14] Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology. 1957 Jul 1;28(1):56-63.

[15] Larsen K. Creatinine assay by a reaction-kinetic principle. Clinica chimica acta. 1972 Oct 1;41:209-17.

[16] Bartels H, Böhmer M, Heierli C. Serum creatinine determination without protein precipitation. Clinica chimica acta; international journal of clinical chemistry. 1972 Mar 1;37:193-7.

[17] Fawcett J, Scott J. A rapid and precise method for the determination of urea. Journal of clinical pathology. 1960 Mar 1;13(2):156-9.

[18] Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. Journal of clinical pathology. 2001 May 1;54(5):356-61.

[19] Buege JA, Aust SD. [30] Microsomal lipid peroxidation. InMethods in enzymology 1978 Jan 1 (Vol. 52, pp. 302-310). Academic press.

[20] Müller W, Mierau R, Wohltmann D. Interference of IgM rheumatoid factor with nephelometric C-reactive protein determinations. Journal of Immunological Methods. 1985 Jun 12;80(1):77-90.

[21] Sokal RR, Rohlf FJ. Biostatistics. Francise & Co, New York. 1987;10:2088-92.

[22] Chan YH. Biostatistics 101: data presentation. Singapore medical journal. 2003 Jun 1;44(6):280-5.

[23] Chan YH. Biostatistics 102: quantitative data–parametric & non-parametric tests. blood Press. 2003;140(24.08):79.

[24] GOSSETT W. Student's't'distribution'. Statistician to Guinness Brewery, UK. 1908.

[25] Liu YQ, Wang XL, He DH, Cheng YX. Protection against chemotherapy-and radiotherapy-induced side effects: A review based on the mechanisms and therapeutic opportunities of phytochemicals. Phytomedicine. 2021 Jan 1;80:153402.

[26] Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, Kitui SK, Manyazewal T. New approaches and procedures for cancer treatment: Current perspectives. SAGE open medicine. 2021 Aug;9:20503121211034366.

[27] Hashem S, Ali TA, Akhtar S, Nisar S, Sageena G, Ali S, Al-Mannai S, Therachiyil L, Mir R, Elfaki I, Mir MM. Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. Biomedicine & Pharmacotherapy. 2022 Jun 1;150:113054.

[28] Ezzat SM, Shouman SA, Elkhoely A, Attia YM, Elsesy MS, El Senousy AS, Choucry MA, El Gayed SH, El Sayed AA, Sattar EA, El Tanbouly N. Anticancer potentiality of lignan rich fraction of six Flaxseed cultivars. Scientific Reports. 2018 Jan 11;8(1):544.

[29] Chera EI, Pop RM, Pârvu M, Soriţău O, Uifălean A, Cătoi FA, Cecan A, Negoescu AG, Achimaș-Cadariu P, Pârvu AE. Flaxseed ethanol extracts' antitumor, antioxidant, and anti-inflammatory potential. Antioxidants. 2022 Apr 30;11(5):892.

[30] Hamdy SM, Moawad BM, Mohamed HR, Shabaan AM, Sayed ON. Anticancer Potential of Blue-Green Algae Extract (Spirulina Plantesis) and Curcumin Nanoparticles on Ehrlich Ascites Carcinoma–Bearing Mice. Egyptian Journal of Chemistry. 2024 May 1;67(5):335-46.

[31] Serraino M, Thompson LU. The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis.

[32] Thompson LU, Rickard SE, Orcheson LJ, Seidl MM. Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis.

[33] Stepień AE, Trojniak J, Tabarkiewicz J. Anti-Oxidant and Anti-Cancer Properties of Flaxseed. International Journal of Molecular Sciences. 2025 Jan 30;26(3):1226.

[34] Morsi DS, El-Nabi SH, Elmaghraby MA, Abu Ali OA, Fayad E, Khalifa SA, El-Seedi HR, El-Garawani IM. Anti-proliferative and immunomodulatory potencies of cinnamon oil on Ehrlich ascites carcinoma bearing mice. Scientific Reports. 2022 Jul 12;12(1):11839.

[35] El Behery M, Abo-Elmatty DM, Alsunbul M, El-Deen YI, Mohamed DI, Sindi ER, Hashem MH, Al-Olayan E, Ibrahim IA, Bamagous GA, Mali S. Exploration of the anticancer efficacy of a novel 1, 3-thiazole analog in an ehrlich ascites carcinoma model: in vivo and in silico insights into hepatorenal protective potentials via the modulation of apoptosis, oxidative stress and inflammation. RSC advances. 2025;15(25):20143-67.

[36] Al Abdan M. Alfa-Lipoic Acid Controls Tumor Growth and Modulates Hepatic Redox State in Ehrlich-Ascites-Carcinoma-Bearing Mice. The Scientific World Journal. 2012;2012(1):509838.

[37] Gowda NG, Shiragannavar VD, Prabhuswamimath SC, Tuladhar S, Chidambaram SB, Santhekadur PK. Ehrlich Ascites carcinoma mice model for studying liver inflammation and fibrosis. Advances in Cancer Biology-Metastasis. 2022 Jul 1;4:100029.

[38] Abd Eldaim MA, Tousson E, El Sayed IE, Abd El AE, Elsharkawy HN. Grape seeds proanthocyanidin extract ameliorates Ehrlich solid tumor induced renal tissue and DNA damage in mice. Biomedicine & Pharmacotherapy. 2019 Jul 1;115:108908.

[39] Abdrabou EA, Gouda DO. Effect of Feeding on Crackers Enrichment with Beetroot Powder and Flaxseeds in Obese Anemic Rats Induces by Phenylhydrazine. Alexandria Science Exchange Journal. 2025 Jun 30;46(2):303-18.

[40] Stuglin C, Prasad K. Effect of flaxseed consumption on blood pressure, serum lipids, hemopoietic system and liver and kidney enzymes in healthy humans. Journal of cardiovascular pharmacology and therapeutics. 2005 Jan;10(1):23-7.

[41] Al-Bishri WM. Favorable effects of flaxseed supplemented diet on liver and kidney functions in hypertensive Wistar rats. Journal of oleo science. 2013;62(9):709-15.

[42] Field KM, Dow C, Michael M. Part I: Liver function in oncology: biochemistry and beyond. The lancet oncology. 2008 Nov 1;9(11):1092-101.

[43] Hartlev LB, Boeje CR, Bluhme H, Palshof T, Rehling M. Monitoring renal function during chemotherapy. European journal of nuclear medicine

and molecular imaging. 2012 Sep;39:1478-82.

[44] Asirvatham R, Ajm C. Sustainment of Antioxidant Enzymes by Drosera peltata in Ehrlich's Ascites Carcinoma (EAC) Bearing Mice. Instıtute of Health Sciences Journal. 2022 Dec 30;7(3):209-14.

[45] Sayed Mohamed Ali O, Abdel-Rahman Eldesoky N, Muhammad Salahuddin A, Mohamed Abdel-Aziz Eisa N. Atorvastatin ameliorates cisplatin induced oxidative stress in ehrlich ascites solid tumor-bearing mice: a prospective case control study. Al-Azhar Medical Journal. 2018 Jan 1;47(1):97-107.

[46] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked?. Free radical biology and medicine. 2010 Dec 1;49(11):1603-16.

[47] Schneider CD, Oliveira AR. Radicais livres de oxigênio e exercício: mecanismos de formação e adaptação ao treinamento físico. Revista Brasileira de Medicina do Esporte. 2004;10:308-13.

[48] de Vasconcelos TB, Cardoso AR, Josino JB, Macena RH, Bastos VP. Radicais livres e antioxidantes: proteção ou perigo?. Journal of Health Sciences. 2014;16(3).

[49] Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clinical chemistry. 1997 Jul 1;43(7):1209-14.

[50] Yagi K. Lipid peroxides and human diseases. Chemistry and physics of lipids. 1987 Nov 1;45(2-4):337-51.

[51] Gönenç A, Özkan Y, Torun M, Şimşek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. Journal of clinical pharmacy and therapeutics. 2001 Apr;26(2):141-4.

[52] El-Din NK. Protective role of sanumgerman against γ-irradiation–induced oxidative stress in Ehrlich carcinoma-bearing mice. Nutrition research. 2004 Apr 1;24(4):271-91.

[53] Noaman E, El-Din NK, Bibars MA, Abou Mossallam AA, Ghoneum M. Antioxidant potential by arabinoxylan rice bran, MGN-3/biobran, represents a mechanism for its oncostatic effect against murine solid Ehrlich carcinoma. Cancer letters. 2008 Sep 18;268(2):348-59.

[54] Al Za'abi M, Ali H, Ali BH. Effect of flaxseed on systemic inflammation and oxidative stress in diabetic rats with or without chronic kidney disease. PLoS One. 2021 Oct 19;16(10):e0258800.

[55] Abd Allah RS, Ahmmed NH, Walaa A, Heibashy DM. The Curative Role of Moringa Leaf Extract Nanomaterial and Low Doses of Gamma Irradiation on Ehrlich Carcinoma Bearing Mice.

[56] Abd Allah RS, Heibashy DM, El-Nahrawy WA, Ahmmed NH. Therapeutic Role of Low Doses of Gamma Irradiation on Ehrlich Carcinoma Bearing Mice. Egyptian Academic Journal of Biological Sciences, B. Zoology. 2024 Jun 24;16(1):209-20.

[57] Saravanan D, Bhargavi B, Balla S, Kumar SV, Venkatachalam T, Pillai KK, Parimala MJ, Padmavathy J. In-vitro and In-vivo Evaluation of Cytotoxic Activity of Rutin Berberine Loaded Polycaprolactone Nanoparticles against EAC Cell Line. Journal of Neonatal Surgery. 2025;14(17s).

[58] Abd Elrazik NA, El-Mesery M, El-Karef A, Eissa LA, El Gayar AM. Sesamol upregulates death receptors and acts as a chemosensitizer in solid ehrlich carcinoma model in mice. Nutrition and Cancer. 2022 Jan 2;74(1):250-64.

[59] Nishida A, Andoh A. The role of inflammation in cancer: mechanisms of tumor initiation, progression, and metastasis. Cells. 2025 Mar 25;14(7):488.

[60] Allin KH, Nordestgaard BG, Flyger H, Bojesen SE. Elevated pre-treatment levels of plasma C-reactive protein are associated with poor prognosis after breast cancer: a cohort study. Breast Cancer Research. 2011 Jun;13:1-3.

[61] El-Mesery ME, Al-Gayyar MM, Salem HA, Darweish MM, El-Mowafy AM. Chemopreventive and renal protective effects for docosahexaenoic acid (DHA): implications of CRP and lipid peroxides. Cell Division. 2009 Dec;4:1-7.

[62] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. New England journal of medicine. 1999 Feb 11;340(6):448-54.

[63] Chandrashekara S. C-reactive protein: An inflammatory marker with specific role in physiology, pathology, and diagnosis. Internet Journal of Rheumatology and Clinical Immunology. 2014 Oct 30;2(S1).

[64] Ansar W, Ghosh S. C-reactive protein and the biology of disease. Immunologic research. 2013 May;56:131-42.

[65] Askarpour M, Karimi M, Hadi A, Ghaedi E, Symonds ME, Miraghajani M, Javadian P. Effect of flaxseed supplementation on markers of inflammation and endothelial function: a systematic review and meta-analysis. Cytokine. 2020 Feb 1;126:154922.

[66] Rahimlou M, Jahromi NB, Hasanyani N, Ahmadi AR. Effects of flaxseed interventions on circulating inflammatory biomarkers: a systematic review and meta-analysis of randomized controlled trials. Advances in Nutrition. 2019 Nov 1;10(6):1108-19.

[67] Morshedzadeh N, Rahimlou M, Shahrokh S, Karimi S, Mirmiran P, Zali MR. The effects of flaxseed supplementation on metabolic syndrome parameters, insulin resistance and inflammation in ulcerative colitis patients: An open-labeled randomized controlled trial. Phytotherapy Research. 2021 Jul;35(7):3781-91.

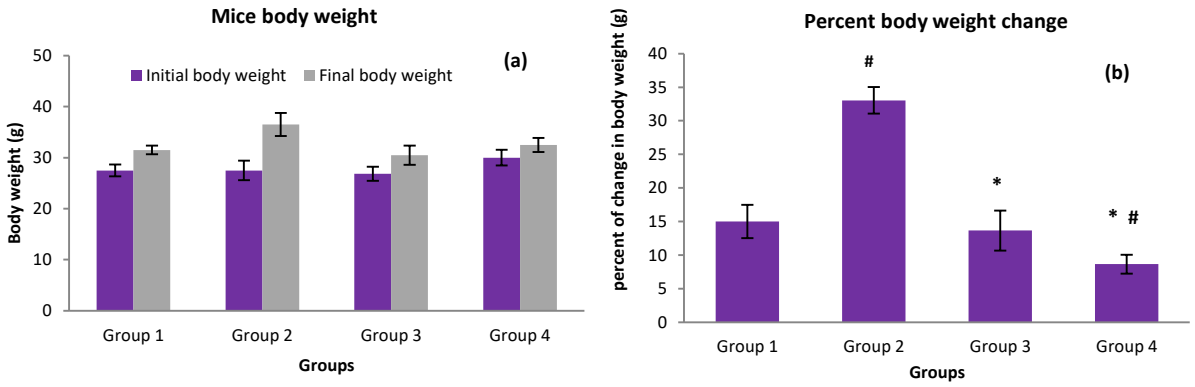

Figure (1) Mean ± SE of mice (a) initial and final body weights (b) body weight change % of all mice groups

Where: $^{\#}p \leq 0.05$ is significant with normal control group; **$^{*}p \leq 0.05$** is significant with tumor-control group; **Group1**: normal control group; **Group2:** tumor-control group; **Group3:** Flaxseed treated tumor-bearing group; **Group4:** Flaxseed protected tumor-bearing group

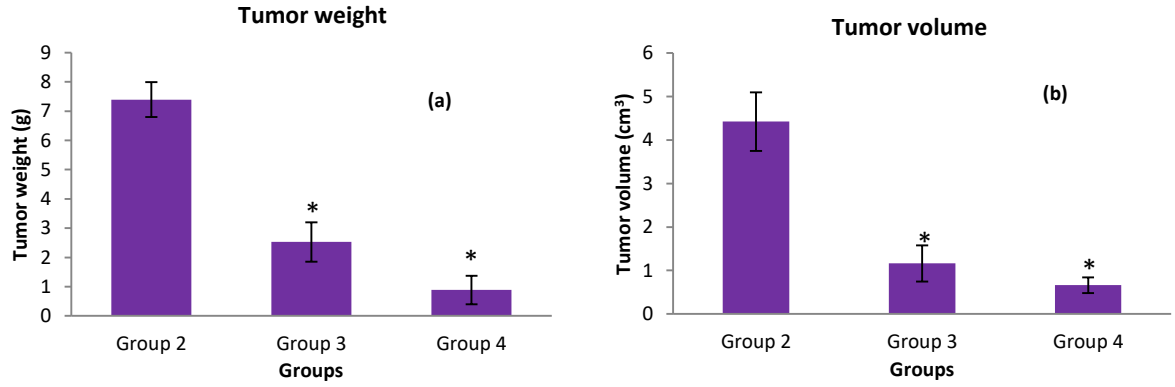

Figure (2) Mean ± SE of mice (a) tumor volume (b) tumor weight of protected and treated groups with tumor-control group

Where: $^{*}p \leq 0.05$ is significant with tumor-control group; **Group2**: tumor-control group; **Group3:** Flaxseed treated tumor-bearing group; **Group4**: Flaxseed protected tumor-bearing group.

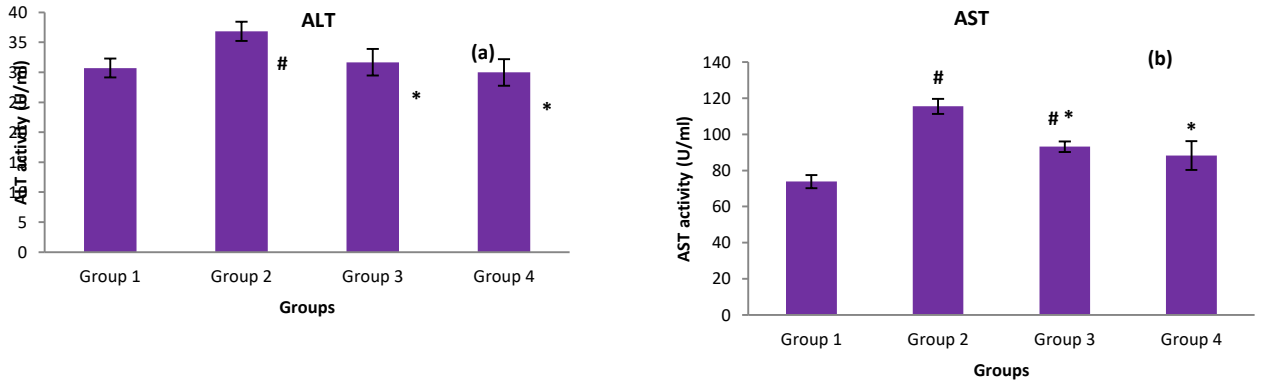

Figure (3) Mean ± SE of mice serum (a) ALT activity (b) AST activities of all mice groups
Where: $^{\#}$**p≤0.05** is significant with normal control group; ***p≤0.05** is significant with tumor-control group; **Group1**: normal control group; **Group2:** tumor-control group; **Group3:** Flaxseed treated tumor-bearing group; **Group4:** Flaxseed protected tumor-bearing group

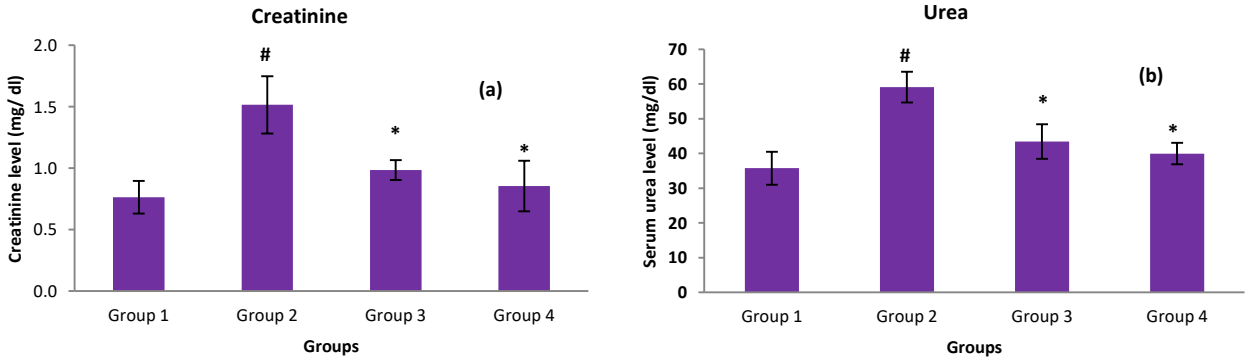

Figure (4) Mean ± SE of mice serum (a) Creatinine (b) Urea levels of all mice groups

Where: $^{\#}$**p≤0.05** is significant with normal control group; ***p≤0.05** is significant with tumor-control group; **Group1**: normal control group; **Group2:** tumor-control group; **Group3:** Flaxseed treated tumor-bearing group; **Group4:** Flaxseed protected tumor-bearing group

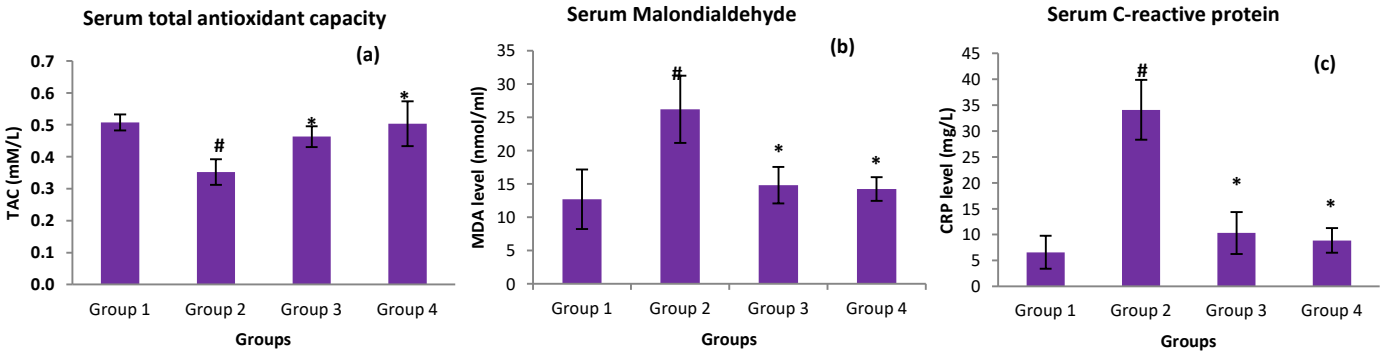

Figure (5) Mean ± SE of mice serum (a) Total antioxidant capacity (b) Malondialdehyde (c) CRP levels of all mice groups

Where: $^{\#}$**p≤0.05** is significant with normal control group; ***p≤0.05** is significant with tumor-control group; **Group1**: normal control group; **Group2:** tumor-control group; **Group3:** Flaxseed treated tumor-bearing group; **Group4:** Flaxseed protected tumor-bearing group .