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Biochemical studies on the effect of flaxseed and corn oils on cell membrane phospholipids in Ehrlich ascites carcinoma and solid tumor in mice

Zakaria El-khayat[1], Osama Ahmed Abas[2], Dalia Medhat[1*], Mohamed Elghreeb[3], Abdel Razik Farrag[4] and Noura Mostafa[2]

[1]Department of Medical Biochemistry, National Research Center, Cairo, Egypt
[2]Department of Zoology, Faculty of Science, Port- Said University
[3]Department of Chemistry, Faculty of Science, Port- Said University
[4]Department of Pathology, National Research Centre, Doki, Giza, Egypt

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ABSTRACT

Tumor growth is a process associated with an enhanced rate of membrane phospholipids de novo turnover and assembly. Erythrocytes are the initial objectives for circulating drugs. In addition, erythrocyte membrane structural compounds are similar to those in many cell types of the organism. In malignant cells, the ultra structural architecture of the cell membrane is altered, partially as a result of changes in the quantities of membrane components present. We aimed to study the effect of flaxseed and corn oils on erythrocyte membrane components in Ehrlich ascites carcinoma (EAC) and solid carcinoma in mice. Sixty four healthy albino female mice weighting 20-25 g were divided into 8 groups; corn oil, flaxseed oil, EAC, solid tumor, EAC treated with corn oil, EAC treated with flaxseed oil, solid tumor treated with corn oil and solid tumor treated with flaxseed oil. Results showed that liver functions, α-fetoprotein, erythrocyte membrane lipid profile and phospholipid fractions were improved in EAC and solid tumor groups treated with corn and flaxseed oils compared to untreated groups, which may be due to improvement of membrane components especially phospholipids, these results were confirmed by histopathological examination. We concluded that polyunsaturated fatty acids (PUFA) had a good effect in the treatment of EAC and solid tumor.

Key words: Erythrocyte membrane phospholipids - Flaxseed oil- Ehrlich ascites carcinoma-Solid tumor-High performance liquid chromatography (HPLC).

INTRODUCTION

Cancer is defined as a combination of diseases described by unlimited growth accomplished by abnormal cells propagation [1].

Experimental tumors have major concern in designing. One of the prevalent tumors is Ehrlich ascites carcinoma (EAC). EAC is defined as an undifferentiated carcinoma that has high power in transplantation, absence of regression, shorter life span, assured malignancy, originally hyperdiploid, quick proliferation and described by absence of TSTA (tumor-specific transplantation antigen) [2].

Recent findings support a growing body of evidence that flaxseeds, or its extracted oil exert anti-carcinogenic effects in some in vitro and in vivo experiments, and that flaxseed oil and related extracts also play an important dietary role in various biological activities in the body [3].

Corn oil is consists of 99% triacylglycerols with 59% polyunsaturated fatty acid (PUFA), 24% monounsaturated fatty acid (MUFA), and 13% saturated fatty acid (SFA). linoleic acid with lectin represents the PUFA content, unique group of proteins and glycoproteins attribute various health benefits possess anticancer properties through binding to receptors or membranes of cancer cells, causing cyotoxicity, cancer cells apoptosis and as a result tumor growth suppression [4]

Phospholipids are one of the most important structural elements of the cell membrane which consists of fatty acids. Fatty acids of cell membranes are determined by the types of fatty acids in the diet. The cell membrane formed of phospholipids derived from saturated fatty acids is less fluid and characterized by discrete structure than the one that involved essential fatty acids [5].

Aim of the work

We aimed to study the effect of flaxseed and corn oils on erythrocyte membrane components in both EAC and solid tumor in mice.

MATERIALS AND METHODS

Material

Chemicals

Phosphatidylcholine(PC), phosphatidylethanolamin(PE), phosphatidylserine (PS) and sphingomyelin(SM) from bovine sources as phospholipids standards were purchased from Sigma Chemicals Co. (Munih, Germany). Flaxseed and corn oils were purchased from local market.

Experimental animals

Adult female Swiss albino mice (20-25g) were obtained from the Animal House, National Cancer Research Institute (Kasr El-Ainy St, Cairo, Egypt).They were housed in stainless steel cages in a controlled environment (temperature 20±2 ◦C and 12D:12L) with standard laboratory diet and water *ad libitum* at the Animal House of the National Research Centre, Giza, Egypt.

Tumor transplantation

A cell line of EAC supplied through the courtesy of Dr. Gklien, was maintained in experimental female Swiss albino mice by intraperitoneal injection of 2.5×10^6 cells per mouse. After 5-7 days from EAC cell inoculation, tumor was observed. The solid tumor was done by inoculating 2.5×10^6 cells per mouse intramuscular with a fine needle in the hind limb of mice. After 10 to 13 days from EAC cell inoculation, solid tumor was being observed [6].

Flaxseed and corn oils administration

Flaxseed and corn oils were orally administrated in a dose of 1.2 ml oil / kg body weight daily for 14 days after 48 hours of EAC cell line induction [7].

Experimental design

Sixty four healthy Swiss albino mice were randomly assigned into eight experimental groups (8 mice /group) and classified as follows:

Group (I) healthy mice were orally administered corn oil.
Group (II) healthy mice were orally administered flaxseed oil.
Group (III) healthy mice injected once intraperitoneal with EAC cell line**.**
Group (IV) healthy mice injected once intramuscular with EAC cell line.
Group (V) mice bearing EAC and then administrated corn oil.
Group (VI) mice bearing EAC and then administrated flaxseed oil.
Group (VII) mice bearing solid tumor then administrated corn oil.
Group (VIII) mice bearing solid tumor then administrated flaxseed oil.

Tumor sizes were determined in all mice. The radii of the developing tumors were measured every 3rd day from day 8 to day 32, using vernier calipers and the tumor volume was estimated using the formula: $V = 4/3\ r1\ 2\ r2$, where $r1$ and $r2$ represent the radii from two different sites.

Blood sampling and biochemical analysis

After the experimental period, animals were kept fasting for 12 hours, blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes in heparinized tubes, centrifuged at 4000 rpm for 10 minutes. Plasma was immediately separated and kept at -20ºC for estimation of biochemical parameters and packed RBCs was used for determination of cell membrane components.

Determination of liver functions: ALT and AST were determined according to [8], albumin and total protein levels were estimated according to [9] and [10] respectively.

Determination of α-feto protein using ELISA kit according to [11].
The method used for erythrocyte ghost preparation is based on the hemolysis of RBCs in hypotonic solution for removal of hemoglobin according to [12].

Extraction of erythrocyte membrane lipids was carried out by chloroform /methanol method according to [13].

Erythrocyte membrane total lipids, cholesterol, total phospholipids and triglycerides were estimated by enzymatic colorimetric method using Centronik kit, Germany.

Fractionation of erythrocyte membrane phospholipids by High Performance Liquid Chromatography (HPLC) according to [14].

Histopathological examination
Liver were dissected out and fixed instantaneously in 10% formalin saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point 55-60 ^{o}C). Sections of 6µm thickness were prepared and stained with Haematoxylin and eosin [15].

Statistical analysis
Results were expressed as mean ± standard error. Data were analyzed by independent sample *t* test (SPSS) version 15 followed by (LSD) test to compare significance between groups. Difference was considered significant when *P* value ≤ 0.05.

RESULTS

Results obtained from this study indicated that administration of flaxseed and corn oils significantly reduced tumor volume and size in both mice bearing EAC and solid tumor groups compared to untreated groups **(Fig. 1, 2)**.

In this study it was observed that plasma levels of ALT, AST and erythrocyte membrane total lipids were significantly increased in both mice bearing EAC and solid tumor groups compared to control groups. While, there was a significant decrease in the levels of albumin and total protein in EAC and solid tumor groups, while, these values were significantly increased after administration of corn and flaxseed oils **(Fig. 3-7)**.

Administration of flaxseed and corn oils showed a significant decrease in erythrocyte membrane total phospholipids, total lipids, cholesterol and triglycerides **(Fig. 7-10)**.

In this study there was a significant increase of plasma AFP in both mice bearing EAC and solid tumor compared to control group **(Fig.11)**, while AFP significantly decreased in treated groups compared to mice bearing EAC and solid tumor groups.

In this study, it was observed that percent of changes of PE and PS contents were increased in both mice bearing EAC and solid tumor compared to control groups **(Table 1, Fig. 12)**. Also in this study there was increased in percent of change of PC from control group in both mice bearing EAC and solid tumor groups **(Table 2, Fig.13)**.

Histopathological data obtained from this study showed that the hepatic lobules are the structural units of the liver **(Fig.15)**. Each hepatic lobule is formed of cords of hepatocyte. The hepatocytes are polyhedral cells with round central nuclei and abundant cytoplasm. Hepatocytes are oriented in cords composed of a single row of cells separated from vascular sinusoids by endothelial cells. The wall of these sinusoids contains irregular cells with multiple processes and known as Von Kupffer. The sinusoids run radially, converging in the center of the hepatic lobule to form the central or centrolobular vein. The central vein has thin walls consisting only of endothelial cells supported by a sparse population of collagen fibers **(Fig. 16)**.

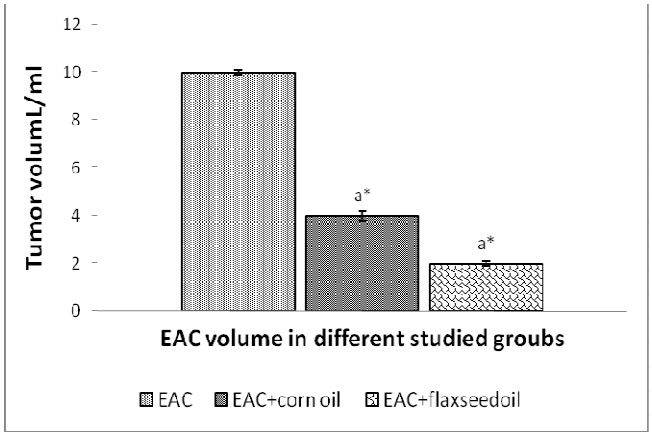

Fig. (1): EAC tumor volume in different studied groups

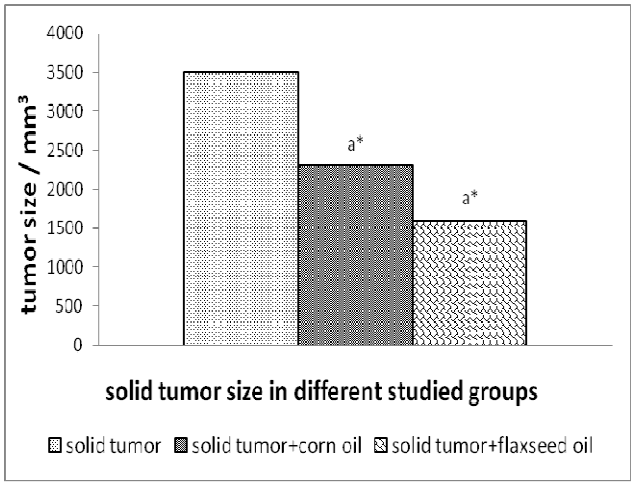

Fig. (2): Solid tumor size in different studied groups

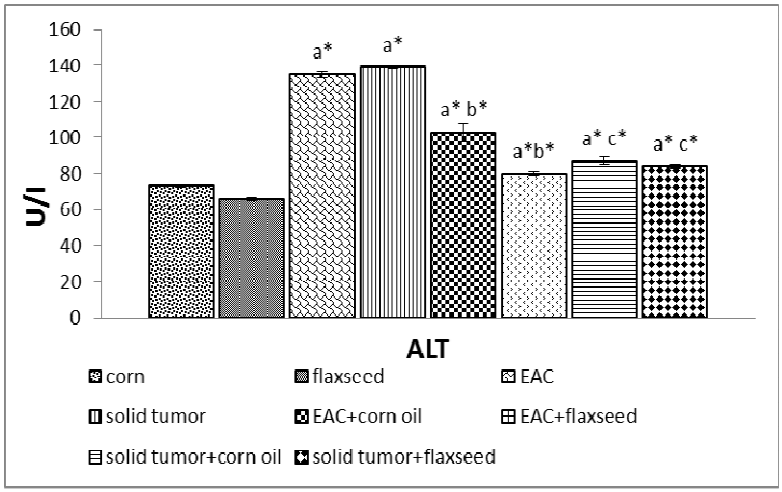

Fig. (3): ALT activity in different studied groups

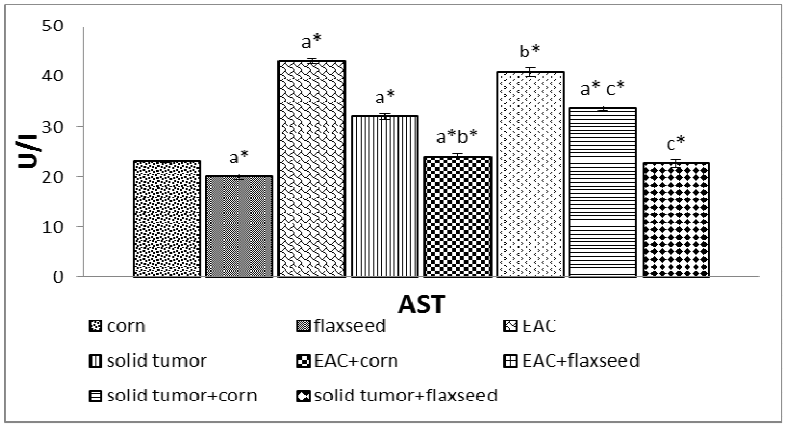

Fig. (4): AST activity in different studied groups

*Significant p value ≤ 0.05, a*significant change from corn oil group, b*:significant change from flaxseed oil group, c* :significant change from solid tumor group.*

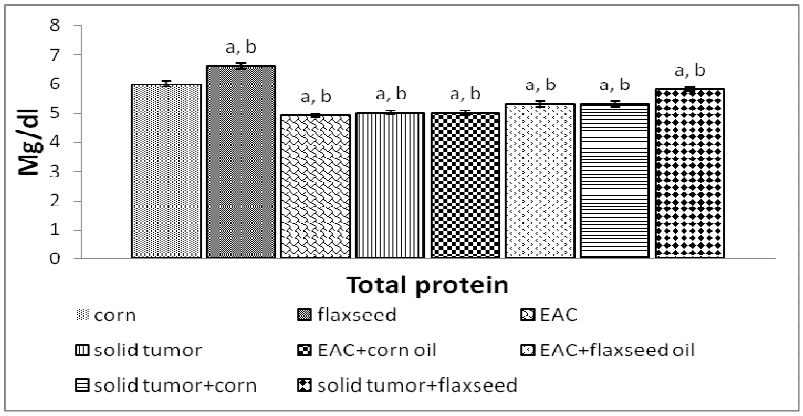

Fig. (5): Total protein level in different studied groups

*Significant p value ≤ 0.05, a*significant change from corn oil group, b*:significant change from flaxseed oil group, c* :significant change from solid tumor group.*

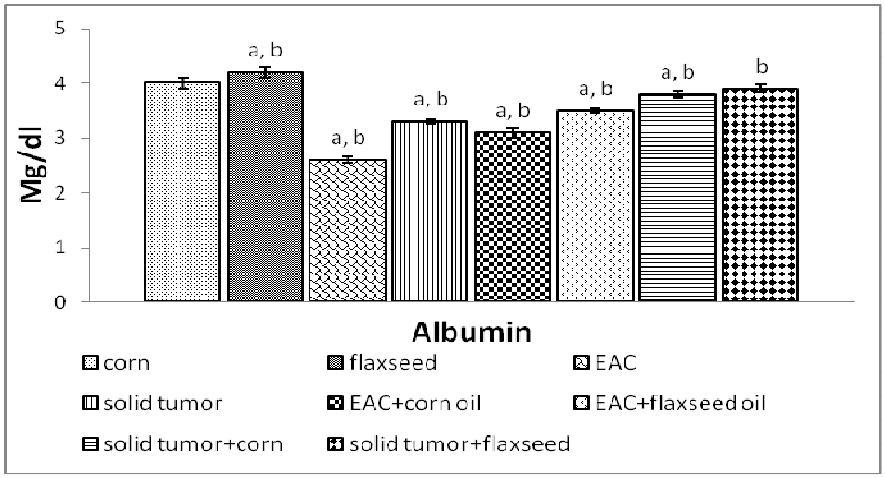

Fig. (6): Albumin in different studied groups

*Significant p value ≤ 0.05, a*significant change from corn oil group., b*:significant change from flaxseed oil group, c* :significant change from solid tumor group.*

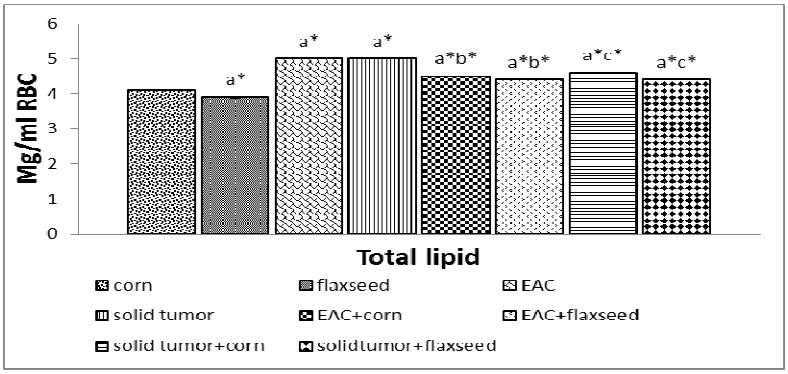

Fig. (7): Total lipids in different studied groups

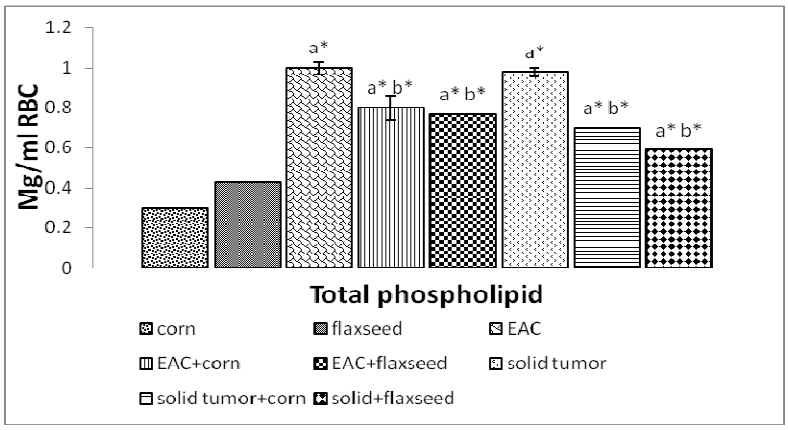

Fig. (8): Total phospholipids in different studied groups

*Significant p value ≤ 0.05, a*significant change from corn oil group., b*:significant change from flaxseed oil group, c* :significant change from solid tumor group.*

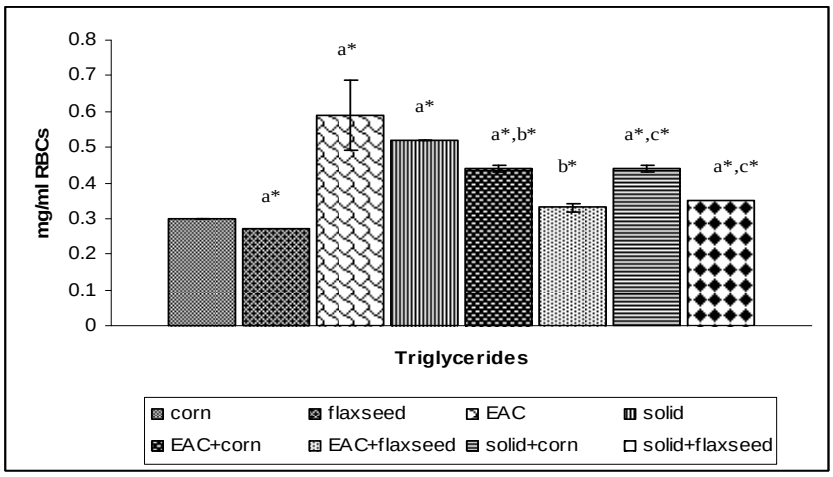

Fig. (9): Erythrocyte membrane triglycerides in different studied groups

*Significant p value ≤ 0.05, a*significant change from corn oil group, b*:significant change from flaxseed oil group, c* :significant change from solid tumor group.*

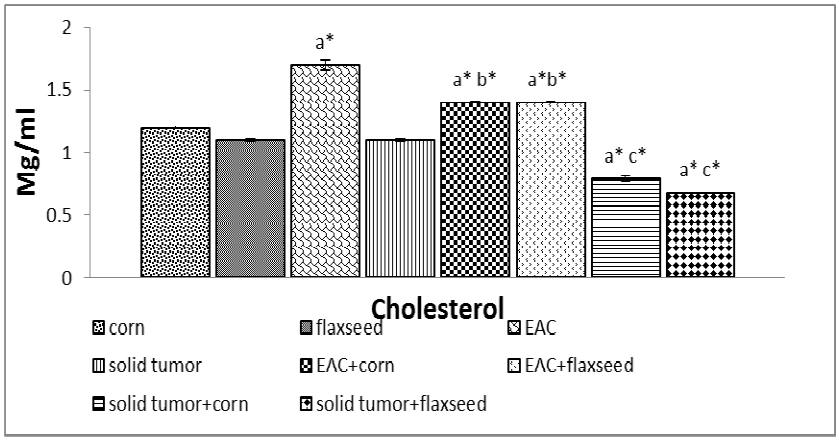

Fig. (10): Erythrocyte membrane cholesterol in different studied groups

*Significant p value ≤ 0.05, a*significant change from corn oil group, b*:significant change from flaxseed oil group, c* :significant change from solid tumor group.*

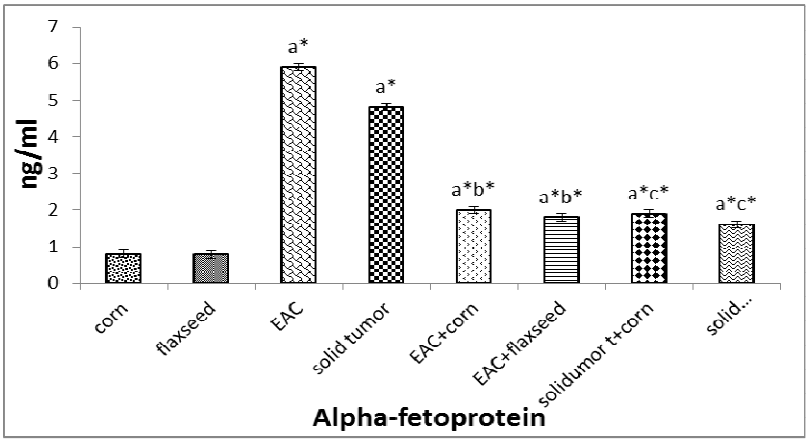

Fig. (11): α- feto protein levels in different studied groups

*Significant p value ≤ 0.05, a*significant change from corn oil group, b*:significant change from flaxseed oil group, c* :significant change from solid tumor group.*

Table (1): Percentage of change of erythrocyte membrane phospholipid fractions in EAC groups

Erythrocyte membrane phospholipid fractions	EAC group	EAC+corn oil	EAC+flaxssed oil
PE	47.9% [a]	18% [a] -35% [b]	10.5% [a] -41.8% [b]
PS	18% [a]	2% [a] -9% [b]	7.1% [a] -12% [b]
PC	29% [a]	5% [a] -25.5% [b]	6% [a] -25% [b]
SM	17% [a]	15.2% [a] -4.8% [b]	19% [a] -5.8% [b]

%[a] :percent of change from control group, %[b]:percent of change from EAC group.

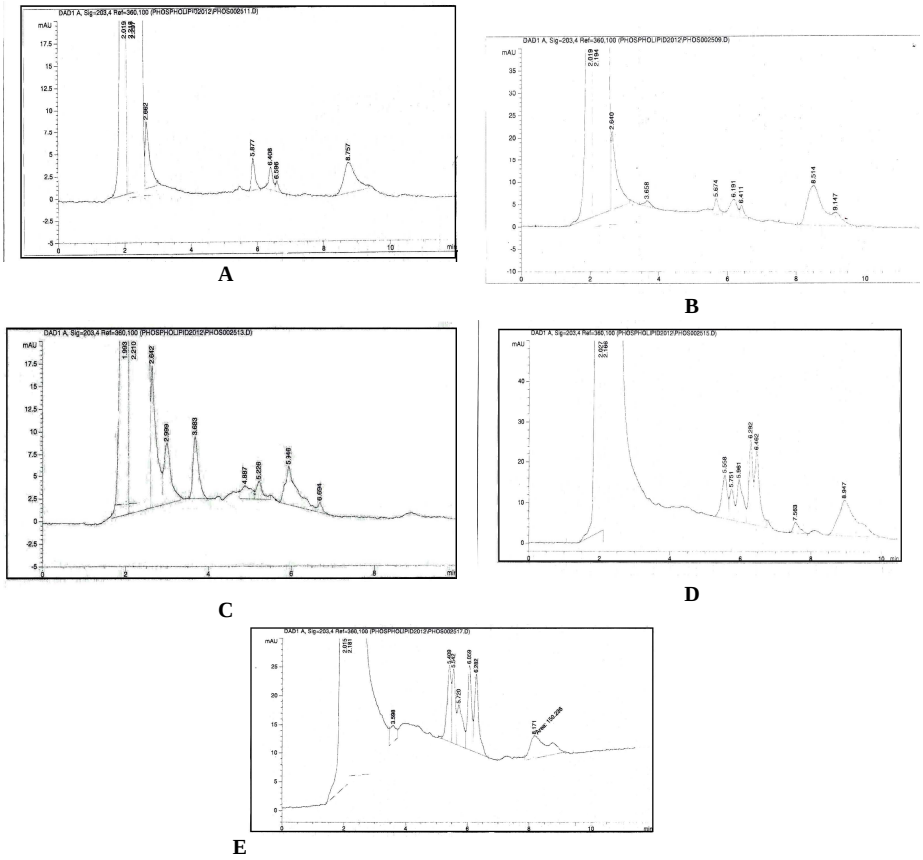

Fig.(12): Chromatogram showing phospholipids factions in A:corn oil control group, B:flaxseed oil group, C:EAC group, D:EAC treated with corn oil group, E:EAC treated with flaxseed oil group

Table (2): Percentage of change of erythrocyte membrane phospholipid fractions in solid tumor groups

Erythrocyte membrane phospholipid fractions	Solid tumor group	Solid tumor +corn oil group	Solid tumor +flaxseed oil group
PE	32.8% [a]	15.9% [a] -21.6% [b]	28.9% [a] -12.8% [b]
PS	15.9% [a]	11.3% [a] -3.9% [b]	9.8% [a] -12.7% [b]
PC	27.8% [a]	16.5% [a] -8.8% [b]	12% [a] -14% [b]
SM	17.6% [a]	16.4% [a] -14% [b]	9.4% [a] -7% [b]

%[a]: percent of change from the control group, %[b]: percent of change from the solid tumor group.

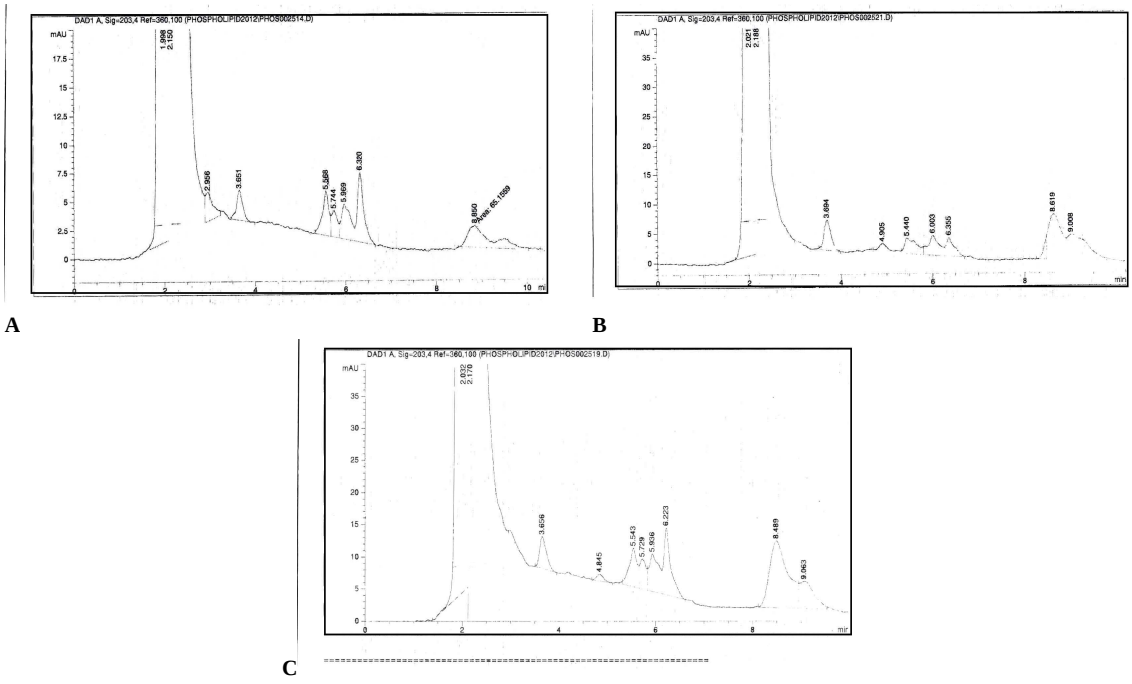

Fig. (13): Chromatogram showing phospholipid fractions in A: solid tumor group, B: solid tumor group treated with corn oil, C: solid tumor group treated with flaxseed oil

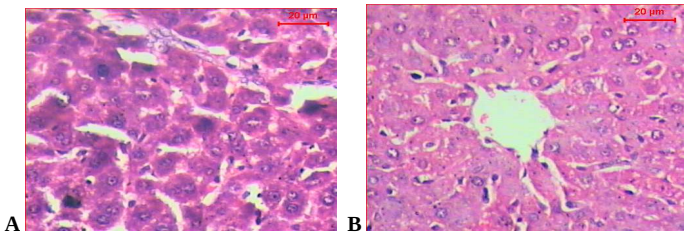

Fig. (14): Sections of liver of A: control mouse administered with Flax seed oil shows normal structure of the hepatic lobule. Notice hyperchromasia of few hepatocytes nuclei (arrow), C: control mouse administrated with corn oil shows normal structure of the hepatic lobule (H & E; scale bare 20 µm)

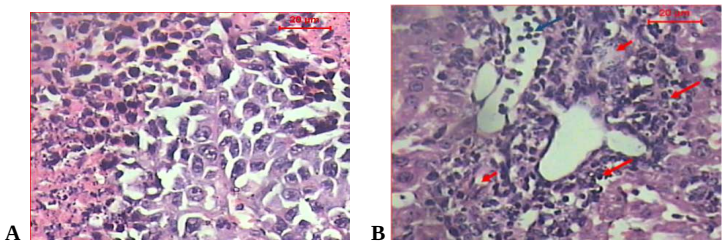

Fig. (15): Sections of liver of A): solid tumour shows tumour cells associated with the inflammatory infiltration and necrotic hepatocyte fibers, B): mouse with EAC shows tumour cells, inflammatory infiltration and foci of necrotic hepatocytes (arrowhead), (H & E; scale bare 20 µm)

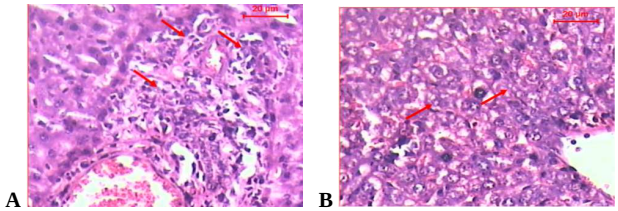

Figure (16): Sections of liver of mouse with A): EAC treated with corn oil shows normal structure of some hepatocytres while a large area of small tumor cells associated with inflammation was seen in the portal area , B): mouse with EAC and treated with flaxseed oil shows the hepatic lobule that appears more or less like normal. Notice vacuolation in some hepatocytes and congested sinusoids (arrows).(H & E; scale bare 20 µm)

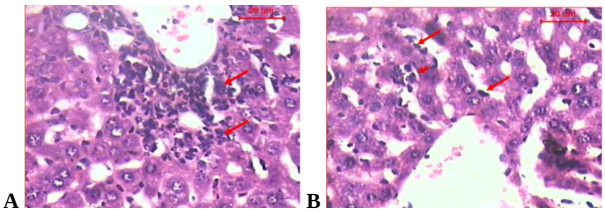

Figure (17): Sections of liver of mouse of A): Ehrlich solid tumor and treated with flaxseed oil shows normal structure of the hepatic lobule. Notice few of tumour cells associated with inflammatory infiltration (arrows) that surrounded the central vein, B): Ehrlich solid tumor and treated with corn oil shows normal structure of the hepatic lobule. Notice the activated kuppfer cells (arrow) and small vacuoles in the cytoplasm. Focus of tumour cells is present (arrowhead) (H & E; scale bare 20 µm)

DISCUSSION

The inhibitory effect of flaxseeds and corn oils on cellular proliferation might have played a role in the inhibition of the carcinoma and caused a reduction in their volumes. This reduction of the tumor volumes indicates that flaxseed and corn oils affected not only the initiation but also the progression of tumors.

Oleic acid (OA) increase the growth of non-malignant cells while, it promotes apoptosis in cancerous cells [16]. Several researchers have also indicated that both α-linolenic and oleic acid had a repression effect on prostate carcinoma cells proliferation [17]. In addition Tan, suggested that mono and poly-unsaturated fatty acids combination in corn oil prevents liver injury induced by iron and alcohol [18].

Ehrlich carcinoma caused abnormalities in liver function by increasing the activity of serum enzymes compared to normal control group, which were in accordance to our results [19].

Administration of flaxseed and corn oils caused significant improvement in hepatic enzyme levels in both mice bearing EAC and solid tumor which may be due to the high content of omega-3 fatty acids with their activity in sweeping reactive oxygen species and protecting membrane of hepatocytes through inhibiting level of peroxidation of lipids [19].

Alpha-fetoprotein as the most important parameter of mammalian fetal serum that is vastly used in clinical diagnosis is a significant marker for detection of liver tumors [20].

Results of this study showed that administration of flaxseed and corn oils significantly reduced levels of AFP. This was in agreement with [3] whose findings support a growing body of evidence that flaxseeds or its extracted oil exert anti-carcinogenic effects in some in vitro and in vivo experiments, and that flaxseeds oil and related extracts also play an important dietary role in various biological activities in the body.

Our histopathological examination results were in accordance with results of [21] who found that tumor growth of prostate cancer was downregulated resulting in increased rat's survival rate when treated with ω-3 polyunsaturated fatty acids.

Erythrocytes served as an object of this study. Our data showed significant increase in erythrocyte membrane lipids in both mice bearing EAC and solid tumor compared to control groups. Administration of flaxseed and corn oils showed a significant decrease in erythrocyte membrane total phospholipids, total lipids, cholesterol and triglycerides. These finding were in agreement with [22] and [23] who indicated that hyperlipidemia altered composition of plasma lipoproteins, elevate lipids content as well as increasing plasma low density lipoprotein (LDL) and a decreasing plasma high density lipoprotein (HDL) which were shown to be accomplished in animals with several types of tumors.

Administration of flaxseed and corn oils significantly reduced erythrocyte membrane lipids, Studies *in vivo* revealed that transcription of lipogenesis genes in hepatocytes were downregulated by polyunsaturated fatty acids (n-3 or n-6) [24].

Phospholipids are an integral part of a cell membrane and determine its structure. Accordingly, different biological conditions are associated with differences in membrane phospholipids composition particularly during cancer transformation [25].

In this study, it was observed that percent of change of PE, PS and PC contents were increased in both mice bearing EAC and solid tumor groups compared to the control groups. These results were in agreement with [25] who found that most cases of colorectal cancer involve an increase in the concentration of all phospholipid types at the cell membrane, including: phosphatidylinositol (PI), PS, PE and PC. Increasing the concentration of phospholipids in the cell membrane was associated with human colon cancer cells and murine mammary tumor cells. Moreover, this increase has been proposed to be the result of augmentation of cell membrane assembly as a result of acceleration in neoplasm cell replication.

In this study administration of flaxseed and corn oils caused decrease in the percent of change of erythrocyte membrane phospholipid fractions compared to mice bearing EAC and solid tumor groups, which may be due to the improvement of cell membrane permeability and fluidity that allows anticancer bioactive materials to cross into cell membrane [26].

In addition, carcinogenesis is affected by polyunsaturated fatty acids through arachidonic acid suppression which in turn leading to alteration in the response of cancer cell immunity properties and modulate inflammation, apoptosis, cell proliferation, angiogenesis and metastasis characteristics [27].

Polyunsaturated fatty acids collaborate in the ordinary performance of a cell, especially by contributing to intracellular cell signaling. Also, PUFAs represent nutritional components of a human diet and can indirectly affect tumorigenesis. For example, ω-3 fatty acids modify co-stimulatory molecules and energizing markers, as well as calcium signaling and protein kinase C translocation at the cell membrane of immune cells [28].

Lund indicated that the incorporation of ω-3 fatty acids in the membrane of other cell types has been shown to change membrane permeability, fluidity and binding of hormones and growth factors [29].

In the same line [30] found that PUFAs can be incorporated into the phospholipids of inflammatory cell membranes.

CONCLUSION

We concluded that, Flaxseed and corn oils have a good effect on inhibiting tumor growth in Ehrlich ascites carcinoma and solid tumor.

REFERENCES

[1] A. Mandal, N.K. Islam, J. Scott, B. Okafor and P.K. Mandal. *African Americans and Cancer*, **2014**, 4(7), 1:4.
[2] M. Ozaslan, I.D. Karagoz, I. HalilKilic and M.E. Guldur, *Afr. J. Biotechnol*., **2011**, 10 (13), 2375-2378.
[3] E. Basch, S. Bent, J. Collins, et al, *J Soc Integr Oncol*., **2007**, 5, 92-105.
[4] E.G. De Mejía, V.I. Prisecar, *Crit Rev Food Sci Nutr*., **2005**, 45(6):425-45.
[5] B. White, *Am Fam Physician*., **2009**, 15:80(4): 345-350.
[6] M. El-Gawish, *Egypt. J. Biomed. Sci*., **2003**, 11: 106-121.
[7] G.C.D. Taylor, A. Noto, D.M. Stringer, S. Froese, L.L. Malcolmson, *J. Am. Coll. Nutr*., **2010**, 29(1):72-80.
[8] S. Reitman, and S. Frankel, Amer. *J.Clin.path*., **1957**, 28:56.
[9] B.T. Doumas, W.A. Watson and H.G. Biggs, *Clin. Chim. Acta*., **1971**, 31:87-96.
[10] H. Passing, and W.A. Balok, *J clin biochem*., **1993**, 21:709-720.
[11] S.E. Bates, *Ann Intern Med*., **1991**, 115:623-8.
[12] J.T. Dodge, C. Mitchell, D.J. Hanahan, *From the department of Biochemistry, University of Washington, Seattle, Washington*., **1962**.
[13] J. De Gier, L.L. Van Deenen, Br *J Haem*., **1964**, 10:246.
[14] S.U. Rehman, *J Chromatography*., **1991**, 567:29-37.
[15] A.R. Drury, E.A. Wallington, *University Press. London* 140., **1980**.
[16] L. Zeng, K.M. Biernacka, J.M. Holly, C. Jarrett, A.A. Morrison and Morgan, *Endocr Relat Cancer*., **2010**, 17: 539-551.
[17] J. Liu, K. Shimizu and R. Kondo, *Chem Biodivers*., **2009**, 6: 503-512.
[18] T. Tan, D.H. Crawford, L. Jaskowski, T.L. Murphy, V.N. Subramaniam, L.M. Fletcher, *Hepatology*., **2011**, 54: 932-933.
[19] U.Z. Said, N.H. Ahmed, A.M. Medhat and M.M. Mustafa, *Journal of cancer research and Experimental oncology*., **2014**, 20:28.
[20] N.L. Lazarevich, *Biochemistry (Mosc)*., **2000**, 65(1):117-33.
[21] Z.Y. Chen and N.W. Istfan, *journal of nutritional biochemistry*., **2000**, 301–308.
[22] H.L. Crenin, and A.K. Narayan, *Z.Krebsforsch*., **1971**, 75, 93.
[23] S. Brenneman, E. Douglas, N. Mathur and A. Arthur, *Europ. J. Cancer*., **1975**, 225-230.

[24] M.B. Anderson, M.A. DWL , Lipids in Health and Disease., **2009**, 8:33 doi:10.1186/1476-511X-8-33.
[25] B. Szachowicz-Petelska, I. Dobrzyńska, S. Sulkowski and Z.A. Figaszewski, *Colorectal Cancer Biology – From Genes to Tumor, Dr. Rajunor Ettarh (Ed.).*, **2012**, 978-953-51-0062-1.
[26] J. E. Manson, S.S. Bassuk, I.M. Lee, N.R.Cook, M.A. Albert, D. Gordon, E. Zaharris,
[27] S.C. Larsson, M. Kumlin, M. Ingelman-Sundberg, A. Wolk, *Am J Clin Nutr*., **2004**, 79: 935–45.
[28] D.A. Hughes and A.C. Pinder, *American Journal of Clinical Nutrition*., **2000**, 1, 357S-360S, ISSN 0002-9165.
[29] E.K. Lund, L.J. Harvey, S. Ladha, D.C. Clark and I.T. Johnson, *Annals of Nutrition and Metabolism*., **1999**, 290-300, ISSN 0250-6807.
[30] S. Kew, E.S. Gibbons, F. Thies, G.P. Mc Neill, P.T. Quinlan, P.C. Calder, *Br J Nutr*., **2003**, 90(6):1071–1080.