

<b>Original Article</b>	<b>Protective effects of black seed and vitamin C on tamoxifen induced liver changes in adult female albino rat; biochemical and microscopic study</b> <i>Rania N. Sherif, Huda M. Eltahry, Sara E Abu bakr</i> <i>Department of Anatomy and Embryology, Faculty of Medicine, Mansoura University, Mansoura, Egypt</i>
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### ABSTRACT

**INTRODUCTION:** Tamoxifen is one of the ideal drugs in treatment of breast cancer however it has genotoxic and cytotoxic activity on the liver via producing reactive oxygen species.

**AIM:** To investigate the injurious effect of tamoxifen on liver of the adult female albino rats and to find out the possible protective effect of Black Seed and/or Vitamin C against this hepatic injury.

**METHODS:** Thirty adult female albino rats were randomly divided into 5 groups; control, tamoxifen, tamoxifen + blackseed, tamoxifen +vitamin C and tamoxifen +blackseed and vitamin C treated groups. On the 22<sup>nd</sup> day after tamoxifen administration the rats were sacrificed; the livers were processed for measurement of MDA & SOD and for light and electron microscopic examination.

**RESULTS:** Co-administration of black seed and/or vitamin C with tamoxifen caused significant decrease in the elevated serum GPT and GOT and the liver tissue MDA and increase in the decreased liver tissue SOD when compared with tamoxifen group. By Light microscopic examination, tamoxifen has been found to cause several liver changes in the form of hepatocyte degeneration and necrosis, inflammatory cell infiltration, dilated sinusoids with hypertrophy of Kupffer cells, collagen deposition and nuclear changes as chromatin clumping and trizomy. Electron microscopic examination of tamoxifen group revealed nuclear changes, dilated vesicular rER, secondary lysosomes, swollen mitochondria and lipid droplets. Administration of black seed and/or vitamin C with tamoxifen reduced the hepatic pathological changes.

**CONCLUSION:** Combination of both black seed and vitamin C might have synergistic hepato-protective effect.

**Key Words:** Tamoxifen, blackseed, vitamin C, hepatocyte.

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### INTRODUCTION

Tamoxifen acts by blocking the of estrogen action through binding with the estrogen receptors in breast carcinomas. The risk of breast cancer has been greatly reduced in high risk

women following tamoxifen treatment (*Dray et al., 2000, Moerkens et al., 2014*).

Tamoxifen has been demonstrated to be carcinogenic to the liver in rats (*Yang et al., 2013*). The genotoxic and cytotoxic properties of

tamoxifen might be due to it produces reactive oxygen species (ROS) that destructs the cellular organelles and induce modifications of DNA by the process of oxidative (Yilmaz *et al.*, 2014).

Black Seed (*Nigella Sativa*) is one of the herbaceous plant with properties that makes it a beneficial therapy for several complications including liver diseases. Black seed has an antioxidant and anti-inflammatory activities and can prevent lipid peroxidation thus reduce hepatotoxicity results from several insults (Mabrouk *et al.*, 2002).

Vitamin C has antioxidant properties, it can scavenge the reactive oxygen radicals and so prevents their destructive effect on many important biological molecules such as lipids, DNA and proteins (Konopacka, 2004).

Many researchers reported that nutrition has an important role in protection against of chronic diseases (López-Varela *et al.*, 2002). Adequate dietary antioxidant supplementation can be effective in minimizing the oxidative stress. Combinations of any antioxidants may amplify their positive antioxidant effect via synergism between their action (Shargorodsky *et al.*, 2010)

## **AIM OF THE WORK**

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The present study was designed to investigate the tamoxifen effect on liver of the adult female albino rats and to find out the possible protective effect of Black seed and / or Vitamin C against this effect.

## **MATERIAL AND METHODS**

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### ***Animals Model:***

Thirty female adult albino rats, weighting 150-200gm were purchased from experimental research center of Mansoura (MERC), Egypt. Rats were housed in stainless steel made mesh cages under relative humidity and adjusted temperature (23°C±3), with free access to food and water ad libitum access and fixed light/dark cycle 12:12-hours. All the experiments were

performed according to the regulations and rules lay down by the committee of animals' experimentation in University of Mansoura.

### ***Experimental Design:***

The rats were isolated and divided in random way into 5 groups; six rats in each group:

1) Control group: received 10 ml distilled water orally using stomach tube daily for 21 days.

2) Tamoxifen treated group: received tamoxifen (Sigma-Aldrich (USA) orally (20 mg/kg per day in 10 ml distilled water) for 21 days

3) Tamoxifen + black seed treated group: received daily black seed extract orally (50 mg/kg/day in distilled water) 15 minutes before tamoxifen administration for 21 days. Black seed extract was purchased from El-Captain Company for Natural Herbal and Oil Extracts.

4) Tamoxifen + vitamin C treated group: received daily vitamin C (Sigma-Aldrich (USA) orally (1mg/kg/day in 10 ml distilled water) 15 minutes before tamoxifen administration for 21 days

5) Tamoxifen+ black seed+Vitamin C treated group: received both black seed extract and vitamin C in the same previous doses 15 minutes before tamoxifen administration daily for 21 days.

### ***Specimen collection:***

On the 22<sup>nd</sup> day, blood samples were obtained from the tail vein. Then, the rats were sacrificed by dislocation of cervical vertebrae; the livers were dissected and divided. Parts of the liver were processed for measurement of MDA & SOD in tissue homogenate. Other parts were prepared for examination by light and electron microscopes.

### ***Biochemical assay:***

The serum levels of liver enzymes SGPT and SGOT were measured by colorimetric method using the kits used in commercial diagnostic tests

manufactured by Randox Co., United Kingdom.

#### ***Lipid peroxidation and oxidative markers assessment:***

The lipid peroxidation and oxidative stress were assessed through colorimetric measurement of the liver tissue levels of malondialdehyde (MDA) and superoxide dismutase enzyme (SOD).

#### ***Light microscopic examination***

The liver specimens were fixed and processed for paraffin sections, cut at thickness 5-6  $\mu\text{m}$  for staining with H&E, PAS and Masson trichrome stains.

#### ***Transmission electron microscopic examination***

The specimens of the liver were fixed by using 2.5% glutaraldehyde in phosphate buffer for duration of 24 hours then post fixed with osmium tetroxide in a fume cupboard for duration of 24 hours at temperature 4  $^{\circ}\text{C}$  followed by rinsing in cacodylate buffer for two changes. The specimens were dehydrated then cleared in propylene oxide. The specimens were placed in epoxy resin then inserted in the Beam capsule using propylene oxide. Ultrathin sections were stained and examined by the transmission electron microscope in the Unit of Electron Microscope in Tanta University using Zeiss EM 100 S transmission electron microscope at 60 KV.

#### ***Statistical analysis:***

Data were collected, organized in tables then encoded for analysis using the computer program SPSS (Statistical package for social science) version 17.0. Descriptive statistics were calculated and gathered in the form of mean and  $\pm$  Standard deviation (SD). Comparison between different groups was tested using analysis of variance (ANOVA) followed by post-hoc tukey test for comparisons between each 2 different groups. *P* value  $<0.05$  was considered statistically significant.

## **RESULT**

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### ***1. Assessment of liver functions:***

Tamoxifen administration significantly elevated the levels of sGPT and sGOT when compared with level of control group. The serum level of GPT and GOT of tamoxifen group were  $48.00 \pm 3.03 \text{ U/L}$  and  $148.33 \pm 1.63 \text{ U/L}$  respectively. Co-administration of blackseed or vitamin C with tamoxifen caused significant lowering of the serum level of GPT and GOT as compared with tamoxifen group. Vitamin C effect in lowering the elevated liver enzymes was more than that of black seed. Daily co-administration of both black seed and vitamin C with tamoxifen has restored liver enzymes toward the normal with highly significant decrease in the elevated serum GPT and GOT as compared to tamoxifen group. In this group, the level of GPT and GOT in the serum were  $12.83 \pm 1.17 \text{ U/L}$  and  $91.3 \pm 24.27 \text{ U/L}$ , respectively (Table 1).

### ***2. Assessment of MDA & SOD in liver tissue:***

Tamoxifen administration caused significant elevation in MDA and significant lowering of SOD in the liver in comparison with control group.

Co-administration of black seed or vitamin C and tamoxifen produced a significant elevation of the level of MDA in liver tissue and significant lowering of the level of SOD in liver tissue as compared with tamoxifen group. Co-administration of both black seed and vitamin C with tamoxifen caused high significant lowering in the liver tissue level of MDA and high significant elevation of the level of SOD in comparison with tamoxifen value (Table 1).

### ***3-Liver histopathology:***

The liver tissue sections of the control group revealed the classical hepatic lobules with cords of hepatocytes arranged as flat, anastomosing plates radiates from the central vein. The hepatic sinusoids were seen separating the hepatocytes plates. The hepatocytes had eosinophilic cytoplasm with one or two vesicular central nuclei with prominent nucleoli and peripherally dispersed chromatin (Fig.1). The masson

trichrome stained liver slides showed normal pattern of collagen fibers deposited around central vein and portal tract (Fig. 2). Hepatocytes of liver of control group showed positive PAS mucopolysaccharide granules; higher mucopolysaccharide content were observed in the peripheral zonal cells than the cells in the central zones (Fig. 3).

By Transmission electron microscope, the liver sections of the control group showed oval or rounded hepatocytes nuclei with nucleoplasm showing a fine granular component with euchromatin condensation and chromatin margination. The cytoplasm of the hepatocytes contains numerous mitochondria, rER, lipid droplets and glycogen granules (Figs. 16, 17).

In tamoxifen group there was perivenular inflammatory cell infiltration and necrosis. The hepatic sinusoids appeared congested, dilated with hypertrophy of Kupffer cells. Some hepatocytes showed swollen cytoplasm with feathery (hydropic) degeneration, while others showed marked vacuolation and fatty degeneration (macro and microvesicular steatosis). Hepatocytes nuclei appeared variable in size and shape as some of them were swollen and large while others appeared small. Peripheral chromatin clumping and trizomy were evident among some hepatocytes (Fig. 4). Minimal increase in collagen fibers content in the portal tracts and around central veins was observed by masson trichrome stain (Fig.5) and there was marked decrease in PAS +ve stain (Fig. 6).

By electron microscope, some hepatocytes nuclei showed peripheral clumping of chromatin (Fig. 18), bubbling of the nuclear membrane (Fig. 19) while others had indentation of nuclear membrane by lipid globules (Fig. 20). The hepatocytes cytoplasm showed fragmented and dilated vesicular rER and secondary lysosomes (Figs. 18, 19). Mitochondria showed abnormality as they appeared swollen with degenerated cristae and irregular membrane (Figs. 18, 21).

Administration of black seed with tamoxifen showed some protective effects on the liver. There was mild residual hydropic and fatty degeneration of the hepatocytes, periportal inflammation, congested sinusoids and hypertrophy of kupffer cells. Some nuclear changes were still present as

peripheral chromatin clumping and trizomy of some nuclei (Figs.7). Normal pattern of collagen fibers deposition around blood vessels as central vein and in the portal tract was observed by masson trichrome stain (Figs. 8) and there was moderate increase in PAS +ve stain compared with tamoxifen group (Fig.9).

Administration of black seed daily with tamoxifen showed improvement in the ultrastructural changes in the form of diminution of cytoplasmic dissolution, disappearance of the fat droplets. However, nuclear envelope still shows some irregularity. The hepatocyte cytoplasm exhibited few vacuoles, dilated swollen mitochondria, secondary lysosomes and dilated rER (Figs. 22-24).

Co-administration of vitamin C with tamoxifen revealed manifestations of mild hepatic damage as the hepatocytes cytoplasm revealed slight fatty changes with minimal vacuoles. Mild residual infiltration of mononuclear inflammatory cells and hypertrophied Kupffer cells was also observed. Some nuclear changes were still present as peripheral chromatin clumping and trizomy of some nuclei (Fig. 10). Normal pattern of collagen deposition around veins in the center of the lobules and in the portal tract was observed by masson trichrome stain (Fig. 11). Also there was strong +ve PAS stain of mucopolysaccharides content in the hepatocytes (Fig. 12). The nuclei had normal chromatin distribution with some irregularity and indentation of the nuclear envelope (Fig. 25). The hepatocytes contained numerous mitochondria of variable shapes and sizes and electron-dense matrix, rER almost retained their normal appearance. Some fat droplets were noticed compressing the nucleus (Fig. 26).

Daily co-administration of both black seed and vitamin C with tamoxifen has prevented liver injury as compared with tamoxifen treated group. However, there were some nuclear changes as peripheral chromatin clumping and lymphocytic infiltration (Fig.13). No increase in collagen fibers (Fig. 14) and marked increase in mucopolysaccharide content with strong PAS reaction of hepatocytes could be seen (Fig. 15). The treatment of rats daily with black seed and vitamin C with tamoxifen showed marked improvement in the ultrastructural

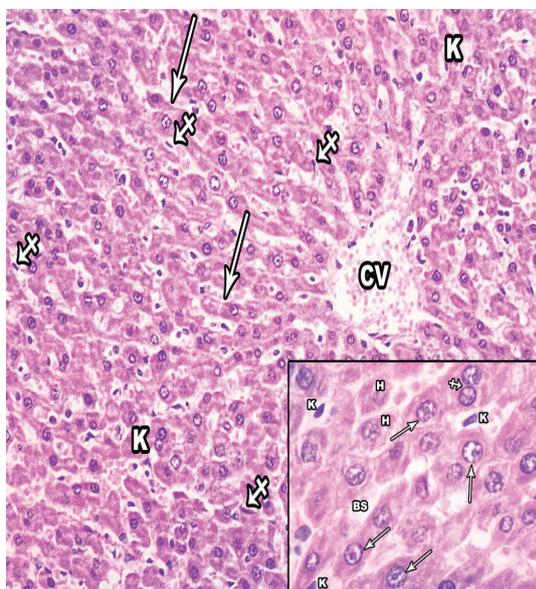
changes. Secondary lysosomes and numerous electron dense of uniform size and shape mitochondria have been observed (Fig. 27).

There was some irregularity of the nuclear membrane (Fig. 28).

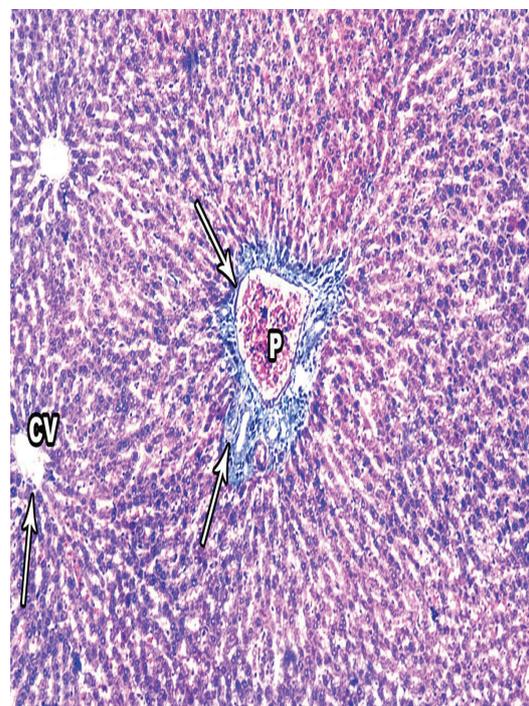
**Table 1:** Mean  $\pm$  standard deviation of GPT, GOT blood level and SOD and MDA levels in liver tissue in different groups.

Groups	SGPT U/L	SGOT U/L	MDA (nmol/g. tissue)	SOD U/gm tissue
	ANOVA <i>p</i> value <0.001	ANOVA <i>p</i> value <0.001	ANOVA <i>p</i> value <0.001	ANOVA <i>p</i> value <0.001
Control	13.67 $\pm$ 1.21	77.83 $\pm$ 1.6	1.25 $\pm$ 0.05	24.08 $\pm$ 0.26
Tamoxifen	48 $\pm$ 3.03*	148.33 $\pm$ 1.63*	15.48 $\pm$ 0.3*	6.92 $\pm$ 0.15*
Black seed	31.17 $\pm$ 1.6*#	102.5 $\pm$ 1.38*#	6.62 $\pm$ 0.28*#	12.9 $\pm$ 0.41*#
Vitamin C	24.5 $\pm$ 0.55*#\\$	97.5 $\pm$ 3.78*#	5.57 $\pm$ 0.19*#\\$	16.22 $\pm$ 0.49*#\\$
Black seed + vitamin C	12.83 $\pm$ 1.17#\\$ $\infty$	82 $\pm$ 7.87#\\$ $\infty$	1.4 $\pm$ 0.3#\\$ $\infty$	19.68 $\pm$ 0.35*#\\$ $\infty$

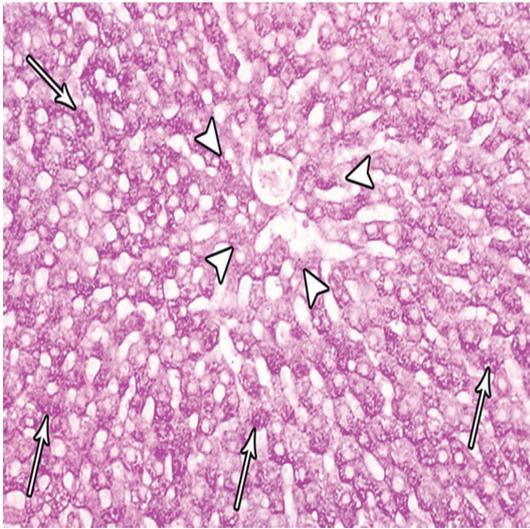
\*Significant difference compared with control group  
 #Significant difference compared with tamoxifen group  
 \\$Significant difference compared with black seed group  
 $\infty$ Significant difference compared with vitamin C group



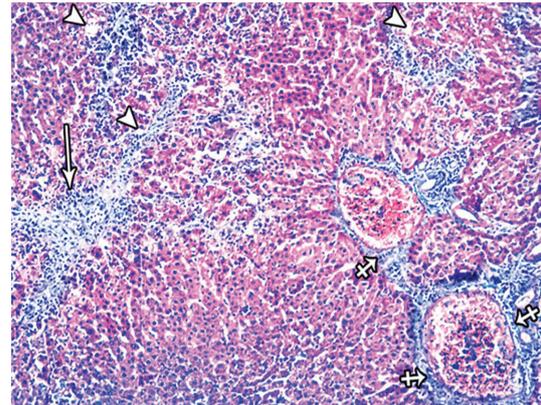
**Fig. 1:** a photomicrograph of a section of the liver from control group showing hepatic plates radiates from central vein (C) and separated by sinusoids (arrows) lined by endothelial cells (crossed arrows) and kupffer cells (K). Inset: normal eosinophilic hepatocytes with open face nuclei that have normal euochromatin (arrows), binucleated hepatocytes (crossed arrow), blood sinusoids (BS) and kupffer cells (K).  
 Hx & E x100 inset x 400



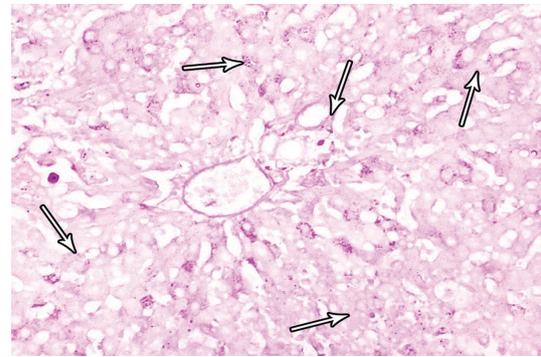
**Fig. 2:** a photomicrograph of a section of the liver from control group showing normal pattern of collagen fibres in portal tract (P) and around central vein (CV) (arrows).  
 Masson trichrome x40



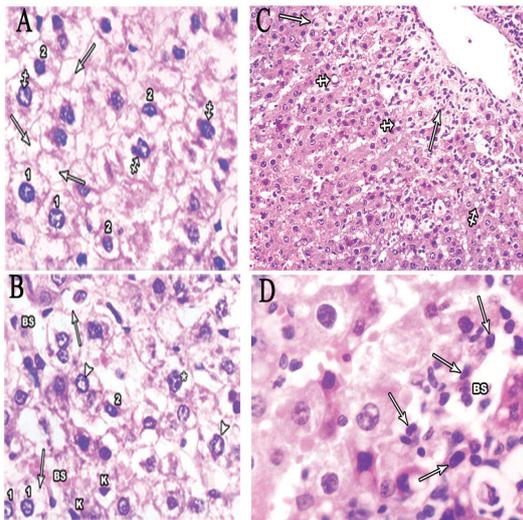
**Fig. 3:** a photomicrograph of a section of the liver from control group showing strong positive PAS stain more in the peripheral zone (arrows) than central zone (arrow heads).



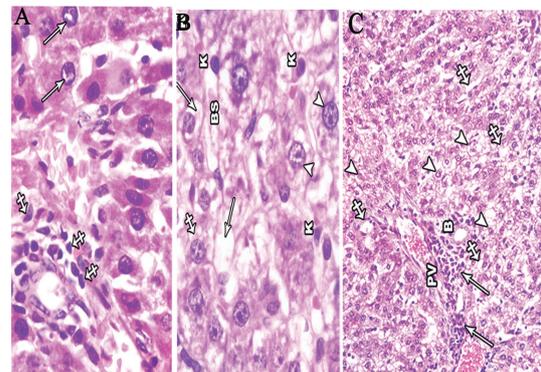
**Fig. 5:** a photomicrograph of a section of the liver from tamoxifen treated group showing perivenular fibrosis (arrow) that spreads around the hepatocytes (arrow heads) and portal tracts (crossed arrows).  
Masson trichrome x 40



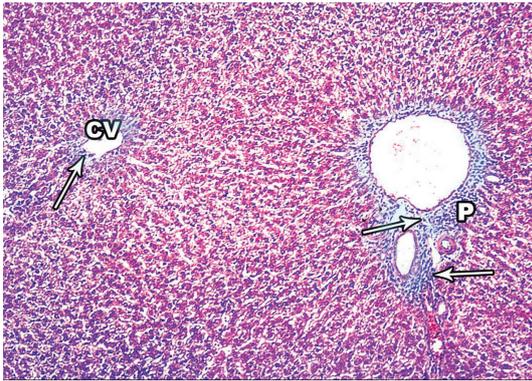
**Fig. 6:** a photomicrograph of a section of the liver from tamoxifen treated group showing weak PAS + stain in the hepatocytes (arrows).  
PAS x 100



**Fig. 4:** a photomicrograph of a section of the liver from tamoxifen treated group showing A: ballooning of the hepatocytes with vacuolated cytoplasm (arrows), many nuclei with abnormal appearance (1,2) and chromatin margination (crossed arrows), B; hepatocytes with hydropic degeneration (arrows), variable size and shaped nuclei (1, 2) with chromatin margination (arrow heads), nuclear trizomy (\*), hypertrophied kupffer cells (K) and dilated congested sinusoids (BS), C; perivenular necrosis with lymphocytic infiltration (arrows) and micro and macrovesicular steatosis (crossed arrows), D; dilated sinusoids (BS) and mononuclear inflammatory cellular infiltration (arrows).  
Hx & E A, B, D x400 C x 100

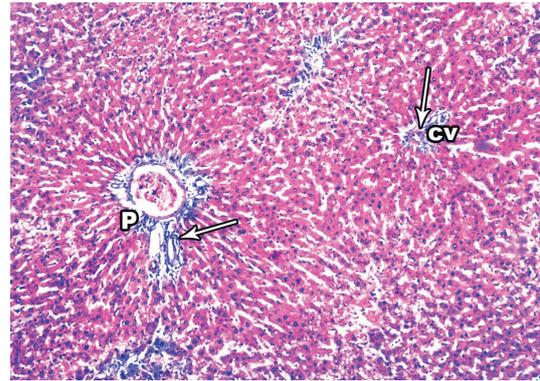


**Fig. 7:** a photomicrograph of a section of the liver from tamoxifen +black seed treated group showing A: lymphocytic infiltration around portal tract (crossed arrows), hepatocytes nuclei with chromatin margination (arrows), B; hepatocytes with hydropic degeneration (arrow heads), nuclear trizomy (crossed arrows), hypertrophied kupffer cells (K) and dilated congested sinusoids (BS), C; inflammatory infiltration (arrows), microvesicular steatosis (crossed arrows) and hydropic degeneration (arrow heads).  
Hx & E A, B, x400, C x 100



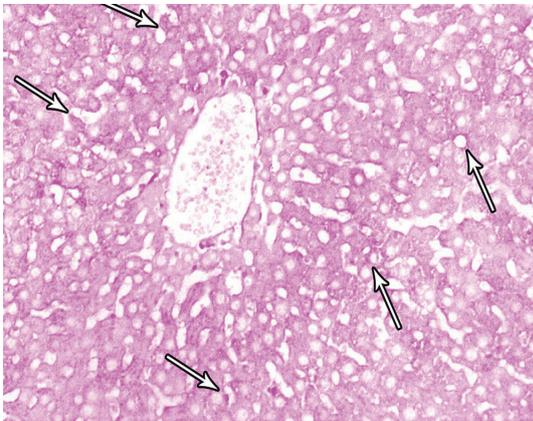
**Fig. 8:** a photomicrograph of a section of the liver from tamoxifen +black seed treated group showing normal amount of collagen fibres in portal tract (P) and around central vein (cv) (arrows).

Masson trichrome x 40



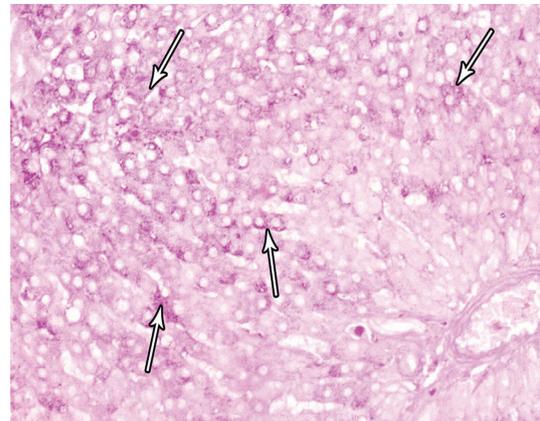
**Fig. 11:** a photomicrograph of a section of the liver from tamoxifen +vitamin C treated group showing normal amount of collagen fibres in portal tract (P) and around central vein (cv) (arrows).

Masson trichrome x 40



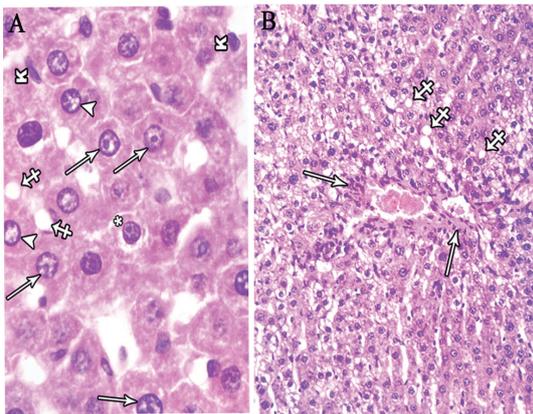
**Fig. 9:** a photomicrograph of a section of the liver from tamoxifen + black seed treated group showing moderate PAS + stain in the hepatocytes (arrows).

PAS x 100

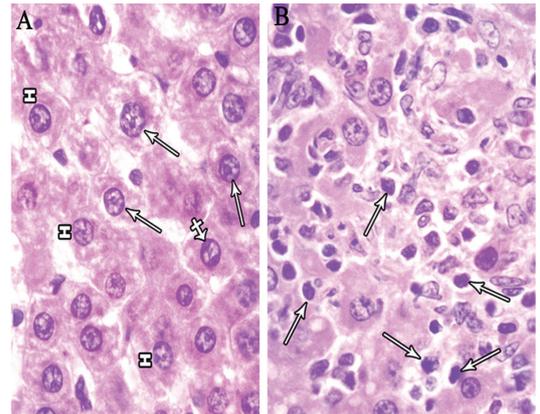


**Fig. 12:** a photomicrograph of a section of the liver from tamoxifen + vitamin C treated group showing moderate PAS + stain in the hepatocytes (arrows) more in peripheral zone.

PAS x 100

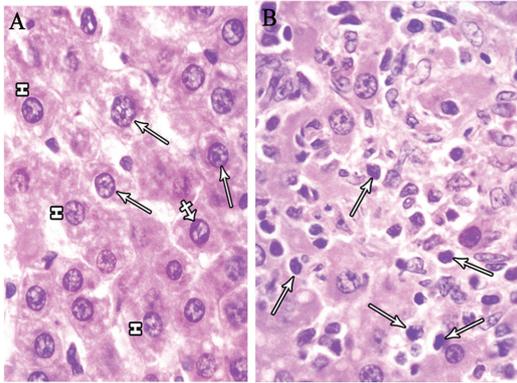


**Fig. 10:** a photomicrograph of a section of the liver from tamoxifen +vitamin C treated group showing A: normal hepatocytes with normal euochromatic nuclei (arrows), nuclei with chromatin margination (arrow heads) and trizomy (\*) and hypertrophied kupffer cells (K), B; lymphocytic infiltration (arrows) and macrovesicular steatosis (crossed arrows). Hx & E A x400, B x 100



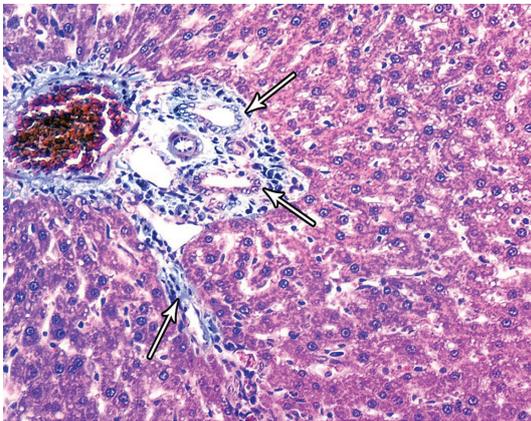
**Fig. 13:** a photomicrograph of a section of the liver from tamoxifen +black seed and vitamin C treated group showing A: normal hepatocytes (H) with normal euochromatic nuclei (arrows) and few nuclei with peripheral clumped chromatin (crossed arrow) B; lymphocytic infiltration (arrows).

Hx & E A, B x400



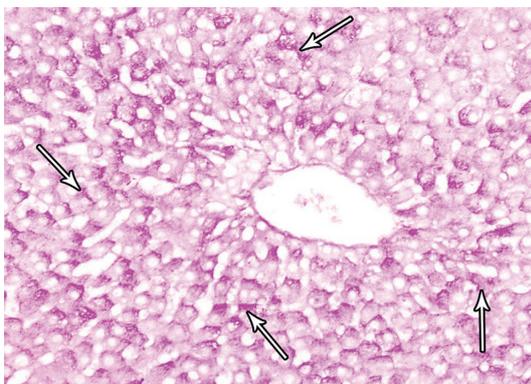
**Fig. 13:** a photomicrograph of a section of the liver from tamoxifen +black seed and vitamin C treated group showing A: normal hepatocytes (H) with normal euochromatic nuclei (arrows) and few nuclei with peripheral clumped chromatin (crossed arrow) B; lymphocytic infiltration (arrows).

Hx & E A, B x400



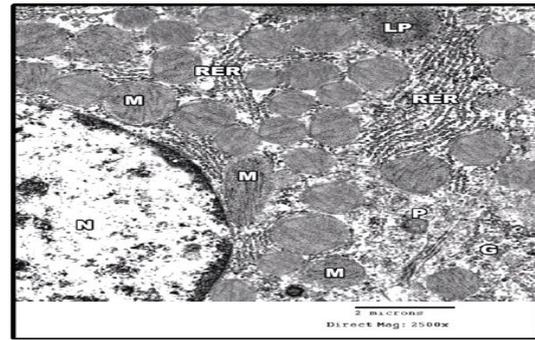
**Fig. 14:** a photomicrograph of a section of the liver from tamoxifen+black seed and vitamin C treated group showing normal amount of collagen fibres in portal tract (P) (arrows).

Masson trichrome x 40



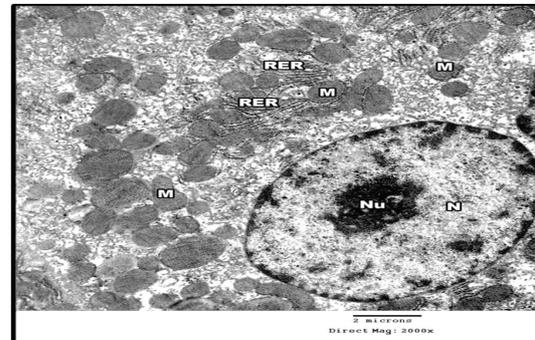
**Fig. 15:** a photomicrograph of a section of the liver from tamoxifen +black seed and vitamin C treated group showing strong PAS + stain in the hepatocytes (arrows).

PAS x 100



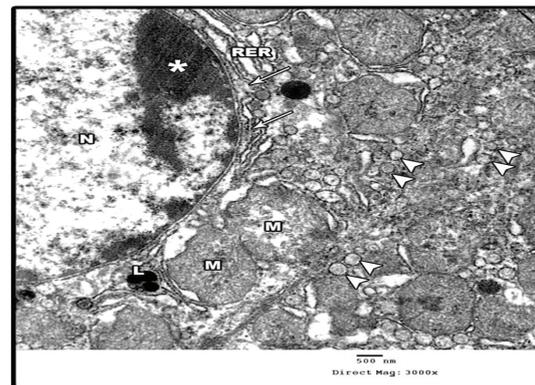
**Fig. 16:** a photomicrograph of a section of the liver from control group showing numerous mitochondria with regular cristae and intact membrane (M), rough endoplasmic reticulum arranged in parallel stacks rich in surface ribosomes (RER). Euochromatic nucleus with regular nuclear envelope (N), glycogen inclusions (G), lipid droplets (LP) could be seen.

(TEM x 2500)



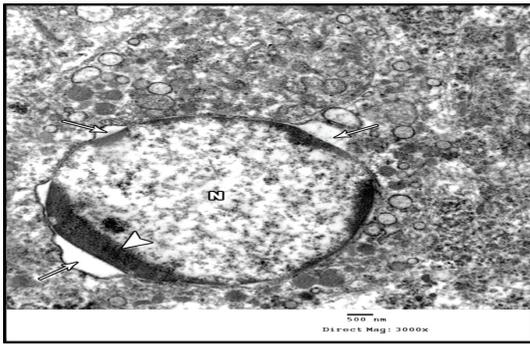
**Fig. 17:** a photomicrograph of a section of the liver from control group showing euochromatic nucleus (N) with central nucleolus (Nu) and normal peripherally dispersed chromatin, mitochondria (M), rough endoplasmic reticulum (RER).

(TEM x 2000)

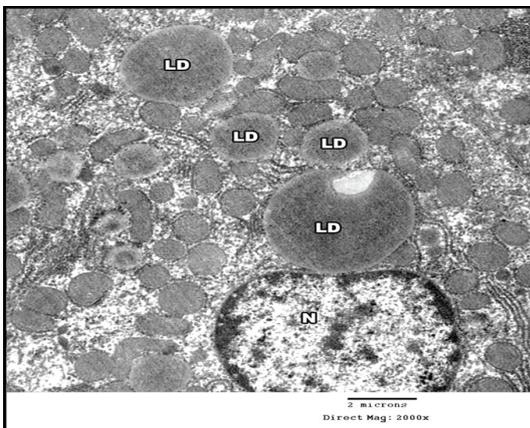


**Fig. 18:** a photomicrograph of a section of the liver from tamoxifen treated group showing abnormal nucleus (N) with peripheral clumped chromatin (\*), degenerated mitochondria with irregular membrane (M), swollen mitochondria with irregular membrane (M), secondary lysosomes (L), dilated rough endoplasmic reticulum (RER), with some breaks (arrow) and vesicular dilated rER (arrow heads).

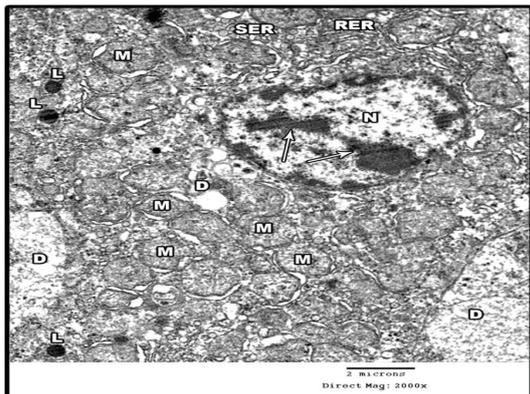
(TEM x 3000)



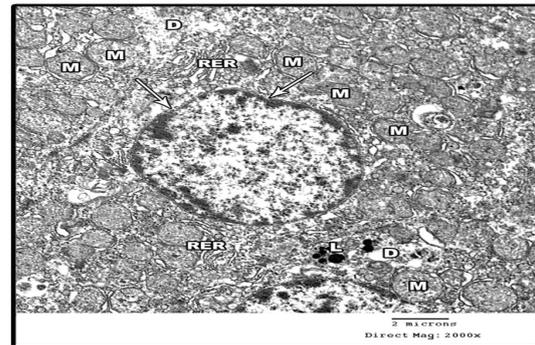
**Fig. 19:** a photomicrograph of a section of the liver from tamoxifen treated group showing abnormal nucleus (N) with bubbling of nuclear membrane (arrows) and peripheral clumped chromatin (arrow head).  
(TEM x 3000)



**Fig. 20:** a photomicrograph of a section of the liver from tamoxifen treated group showing lipid globules (LD) indented the nucleus (N).  
(TEM x 2000)

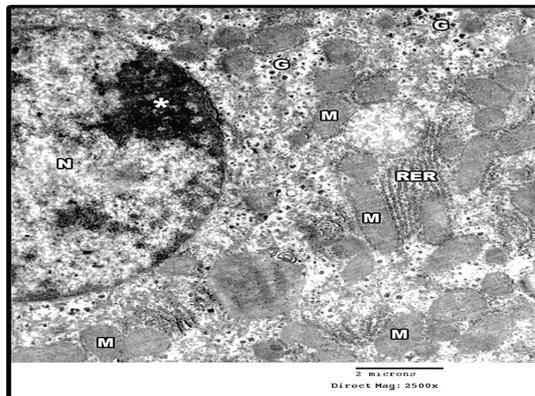


**Fig. 21:** a photomicrograph of a section of the liver from tamoxifen treated group showing cytoplasmic dissolution (D), swollen mitochondria with irregular membrane (M), secondary lysosomes (L), proliferating smooth endoplasmic reticulum (SER), dilated rough endoplasmic reticulum (RER), nucleus with irregular membrane (N) and abnormal clumped chromatin (arrows).  
(TEM x 2000)



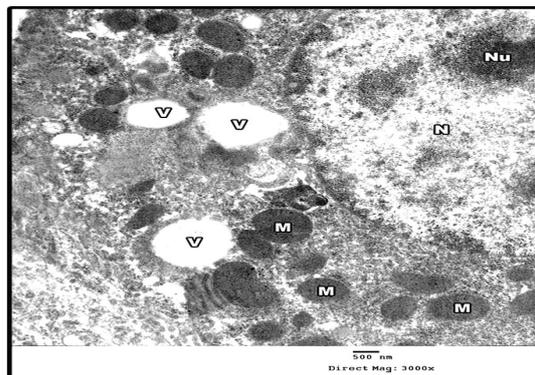
**Fig. 22:** a photomicrograph of a section of the liver from tamoxifen +black seed treated group showing nucleus (N) with some irregularity in the membrane (arrow), cytoplasmic dissolution (D), swollen degenerated mitochondria (M), and secondary lysosomes (L). Some RER are normal while others show vesicular dilatation.

(TEM x 2000)



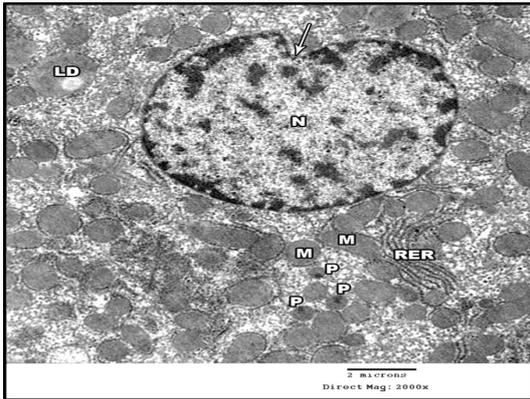
**Fig. 23:** a photomicrograph of a section of the liver from tamoxifen +black seed treated group showing nucleus (N) with regular membrane and peripheral clumped chromatin (\*), normal mitochondria (M), normal rough endoplasmic reticulum (RER) and abundant alpha glycogen rosettes (G).

(TEM x 2500)

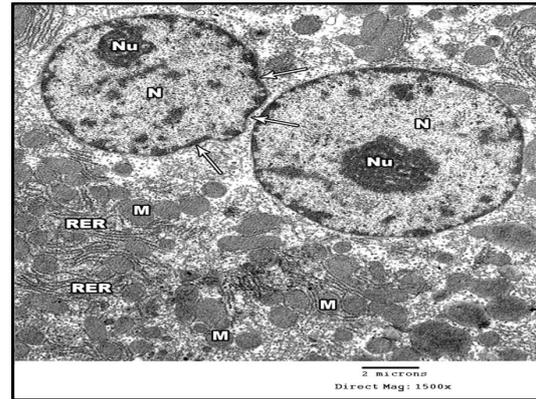


**Fig. 24:** a photomicrograph of a section of the liver from tamoxifen +black seed treated group showing nucleus (N) with peripheral nucleolus (Nu), cytoplasmic vacuoles (V) and normal mitochondria (M).

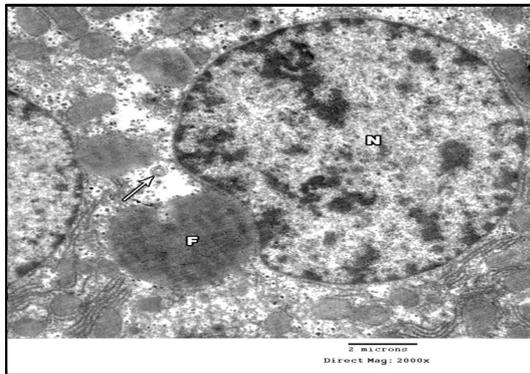
(TEM x 3000)



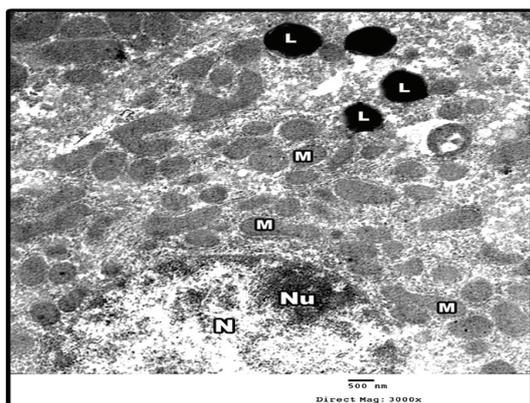
**Fig. 25:** a photomicrograph of a section of the liver from tamoxifen + vitamin C treated group showing nucleus (N) with normal chromatin distribution and indented membrane (arrow), normal mitochondria (M), normal rough endoplasmic reticulum (RER) and peroxisomes (P). (TEM x 2000)



**Fig. 28:** a photomicrograph of a section of the liver from tamoxifen + black seed and vitamin C treated group showing binucleated hepatocyte, nucleus (N) with irregular membrane (arrow), numerous mitochondria (M) and normal rough endoplasmic reticulum (RER). (TEM x 1500)



**Fig. 26:** a photomicrograph of a section of the liver from tamoxifen + vitamin C treated group showing large fat globule (F) compressing the nucleus (N) and myelin figure (arrow). (TEM x 2000)



**Fig. 27:** a photomicrograph of a liver section from tamoxifen+black seed and vitamin C treated group showing euchromatic nucleus (N) with normal chromatin distribution and peripheral nucleolus (Nu), normal mitochondria (M), and secondary lysosomes (L). (TEM x 3000)

## DISCUSSION

This study was performed to explore the possible protective role of black seed and/or vitamin C on liver injury caused by tamoxifen in female rats. Tamoxifen has been extensively used as a chemopreventive and chemotherapeutic agent for treatment of cancer breast (*Goss and Stresses-Weipple, 2004*). One of the most serious side effects, which limited the use of tamoxifen for long duration is the hepatic injury or even hepatocarcinoma (*Yang et al., 2013*). The current study was done on female rats since breast cancer is more common in female. Great attention now focused on side effects of this drug, mainly idiosyncratic hepatotoxicity due to its high affinity to hepatic tissue (*Kumarappan et al., 2011*). Tamoxifen undergoes metabolic activation reactions which raised production of reactive oxygen radicals leading to hepatic oxidative stress and liver damage (*Yilmaz et al., 2014*).

In the current study, tamoxifen administration for three weeks caused significant elevation of serum GOT & GPT which reflects damage to hepatocytes and release of intracellular enzymes into the blood (*El-Beshbishy, 2005*). The current results were supported with past studies who reported sever liver damage with elevation of serum GPT and GOT following treatment of rats with tamoxifen for different durations (*Al-Jassabi et al., 2011, Suddek, 2014a,b, Jena et al., 2015, Gao et al., 2016*).

Tamoxifen increased lipid peroxidation with significant increase in the level of malondialdehyde (MDA) and decrease in SOD level in liver tissue in agreement with (Hesham et al., 2010, Al-Jassabi et al., 2011, Kumarappan et al., 2011, Jena et al., 2015). Pathological damage of the liver can lead to disturbances in the circulation and oxygenation which subsequently causes lipid peroxidation and subsequent elevation in the concentration of MDA. SOD scavenges superoxide anions generated in the mitochondrial and cytosolic compartments of the cell (Rolo et al., 2003). Decreased activity of hepatic SOD may be due to its inactivation by oxidative stress, as oxidative stress decreases hepatic glutathione activity which leads to accumulation of H<sub>2</sub>O<sub>2</sub> in the liver which in turn can produce SOD inactivation (Seven et al., 2004). Tamoxifen induced disruption of the defense mechanism against oxidative stress of the liver cells was confirmed by histological and ultrastructure examination.

Production of ROS is induced by administration of tamoxifen that can destruct the cellular organelles and cause modifications of DNA, lipids and proteins via oxidation and thus can result in serious cellular injury to the liver (Rolo et al., 2012).

In the current work, tamoxifen caused many degenerative changes, necrosis, infiltration of inflammatory cells and changes in the ultrastructure structures of the liver in agreement with (Smith et al., 2000, Fatma et al., 2010, Muhammed and Husein, 2012, Ibrahim et al., 2014). In controversy, no hepatic pathological changes have been reported following tamoxifen administration (Kasahara et al., 2002).

Tamoxifen induces mitochondrial disappearance of cristae, mitochondrial depolarization (Gao et al., 2016), and dysfunction that leads to deficiency in energy, imbalance of the ions, and reactive oxygen species elevation and damage (Andreassen et al., 2000). Tamoxifen induced damage of endoplasmic reticulae and vacuolation of the cytoplasm which might be reaction to cell injury (Robbin's and Cotran, 2010).

The obscure of glycogen rosettes within the cytoplasm of most hepatocytes observed in the

current study could be attributed to the destruction of the membrane of the endoplasmic reticulum and enzymes necessary for glycogen synthesis (Farghaly, 2006).

Nuclear changes in the form of irregular nuclear membrane, marginated chromatin were observed as previous studies of (Fatma et al., 2010, Gao et al., 2016).

The observed vacuolation in the current study may be caused by extension of the outer mitochondrial membrane and expansion of mitochondrial inter-membrane space (Higgins et al., 2003) or it may be due to disturbance of the ions of the cell that leads to water and sodium retention and consequent swelling of the cells (Wiedemann et al., 2002).

Tamoxifen increases the possibility of fatty liver in patients having cancer breast (Liu et al., 2006) and increases the risk of having of non-alcoholic steatohepatitis especially in women having obesity (Ashraf et al., 2009, Akhondi-Meybodi et al., 2011). In the current work, some fatty changes were observed in hepatocytes after treatment of rats with tamoxifen. These fatty changes may be related to the damage in rER and impaired of the synthesis of protein and lipoprotein involved in transport of triglycerides of the liver to extrahepatic tissue (Zhao et al., 2014). Mitochondria damage observed in the present work leads to fatty changes as it contains enzymes necessary for the metabolism of triglycerides (Pan et al., 2016). Tamoxifen also modulates the expression of the genes involved in the triglyceride homeostasis pathway leading to hepatocyte steatosis (Cole et al., 2010, Zhao et al., 2014). Furthermore, it can regulate fatty acid oxidation (Begrache et al., 2011, Patel and Sanyal, 2013). Tamoxifen also inhibits mitochondrial DNA synthesis and mitochondrial  $\beta$  oxidation which subsequently decreases removal of the fat from the liver, causing hepatic steatosis (Lee et al., 2010).

In agreement with the current findings Fatma and colleagues (2010) have demonstrated peripheral chromatin clumping after daily administration of tamoxifen due to adduct formation between it and hepatocyte DNA leading to DNA damage (Cardoso et al., 2003). In the current work tamoxifen caused nuclear

trizomy that may be due to its genotoxic activity (Kasahara *et al.*, 2003).

The inflammation observed in this study might be due to increased level of TNF- $\alpha$  as tamoxifen increases TNF- $\alpha$  and causes inflammation resembling that of alcoholic hepatitis (Wullaert *et al.*, 2005, Suddek, 2014a).

The dilated congested blood sinusoids with hypertrophied kupffer cells observed in the current work might be caused by its direct toxic effect of on the blood sinusoids and phagocytosis of lipofusin released from adjacent necrotic hepatocyte (Klatskin and Ocean, 1993).

The diminution in mucopolysaccharides content of hepatocytes observed in the present work might be a consequent to hydropic and fatty degeneration due damage of the cytoplasmic organelles and the associated enzymes. The minimal increase in collagen fibers observed in the current study might be due to oxidative stress as it has a role in activation of the cells involved in hepatic fibrosis (Fatma *et al.*, 2010).

Daily administration of black seed extract with tamoxifen has proved some protection against tamoxifen liver injury. This protection was evident by significant changes in biochemical, histopathological and ultrastructure parameters of tamoxifen intoxicated rats in agreement with (El-Beshbishy *et al.* 2010) although it failed to normalize the ultrastructure changes induced by tamoxifen represented by dilated rough endoplasmic reticulum and mitochondria. The Black seed extract has protective effect against lipid peroxidation (Hesham *et al.*, 2010) and improve the histopathological changes induced by CCl<sub>4</sub> in liver (Al-Ghamdi, 2003, Turkdogan *et al.*, 2003).

Black seed prevented the tamoxifen-induced hepatotoxicity by minimizing the lipid peroxidation (MDA) and stimulating the antioxidant defense system activity (SOD) supported by (Hesham *et al.*, 2010) as the fixed oil of *Nigella Sativa* can inhibit nonenzymatic lipid peroxidation occurred in liposomes. Thymoquinone (one of *Nigella Sativa* components) might preserve the intracellular glutathione (El-Dakhkhny *et al.*, 2000). *Nigella Sativa* has antioxidant property as its components

can scavenge the free radical (Burits and Bucar, 2000). Also its anti-inflammatory activity plays a key role in its hepatoprotective effect as proved by (Hesham *et al.*, 2010) as it inhibited the elevation in TNF- $\alpha$  level when it was given before tamoxifen.

Vitamin C is a well-known antioxidant. It is protective to the body from injury caused by free radicals (Banerjee *et al.*, 2009). In the current study vitamin C supplementation with tamoxifen, produced significant decrease in the elevated liver enzymes and protected against changes in the level of MDA and SOD. This is supported by the work of Bashandy and Alwasel, (2011), who reported that vitamin C can normalize the levels of blood hydroperoxide, liver enzymes and MDA in liver of CCl<sub>4</sub> intoxicated rats. Vitamin C minimized the hepatotoxicity effect induced by tamoxifen, by reduction of the oxidative destruction through decreasing the alteration of the antioxidant defense and lipid peroxidation. Vitamin C also can donate electrons to free radicals and overcome their reactivity (El-Gendy *et al.*, 2010). Ascorbate prevents depletion of the liver glutathione in chemical-induced hepatotoxicity in mice (Cuddihy *et al.*, 2008). In accordance to (Friday *et al.*, 2012) vitamin C may inhibit the chain reactions of chemical agent-generated free radicals or scavenged the reactive free radicals before they reached their hepatic targets.

The supplementation of vitamin C led to marked improvement of the degenerative changes in tissues caused by toxic agents (Okolie and Iroanya, 2003). Coinciding with the current findings, a previous study reported that vitamin C can reverse some tamoxifen induce hepatic pathological changes in rats (Sharma *et al.*, 2003).

Vitamin C strongly inhibited the fatty change supported by previous work that proved that vitamin C, inhibited the development of steatosis induced by choline-deficient diet due to its antioxidant activity (Claudia *et al.*, 2003).

Regarding improvement in tamoxifen induced nuclear changes; vitamin C can protect from oxidative changes of the DNA induced by different agents. Some studies indicated that

vitamin C regulates the expression of some of the genes controlling the cellular apoptosis and DNA repairing processes (Konopacka, 2004).

In the present work the combination of both black seed extract and vitamin C has greatly prevented liver damage induced by tamoxifen as they prevented lipid peroxidation induced liver damage and resulted in significant increase in oxidant defense enzymes. These results were confirmed by marked improvement in both histopathological and ultrastructural examination of the liver tissue and hepatocytes.

The combination of both vitamin C and black seed might have synergistic hepatoprotective effect as their combination had nephroprotective effect against gentamicin induced renal injury. These two antioxidants might have the capacity to act in synergism as nephroprotective agents (Saleem et al., 2012).

In conclusion, tamoxifen administration induces liver damage that can be prevented by treatment with black seed extract and/ or vitamin C due to their antioxidant properties. Combination of both black seed and vitamin C might have synergistic hepato-protective effect.

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## التأثيرات الوقائية للحبة السوداء و فيتامين سى على تغييرات الكبد المستحثة الناتجة عن تعاطى عقار التاموكسيفين في انثى الجرذ الابيض. دراسة كيميائية ومجهريّة

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### ملخص البحث

**المقدمة:** يعد عقار التاموكسيفين هو الدواء المفضل في علاج سرطان الثدي إلا أنه له انشطه سمية على الكبد عن طريق إنتاج أنواع الاكسجين التفاعلية.

**الهدف:** دراسة تأثير عقار تاموكسيفين على كبد الجرذان الإناث البالغات البيضاء ومعرفة التأثير الوقائي المحتمل من الحبة السوداء و فيتامين سى ضد هذه الاصابة الكبدية.

**مواد وطرق البحث:** قسمت ثلاثون من الجرذان الإناث البالغة عشوائيا إلى 5 مجموعات، المجموعه الضابطه، مجموعه عقار التاموكسيفين، مجموعه الحبه السوداء، مجموعه فيتامين سى و مجموعه فيتامين سى و الحبه السوداء. تم التضحية بالجرذان في اليوم ال22 من بدء تعاطى التاموكسيفين. تم معالجة الكبد لقياس MDA و SOD والفحص المجهرى بالميكروسكوب الضوئى والإلكترونى.

**النتائج:** أحدث العلاج بالحبة السوداء و / أو فيتامين سى مع عقار تاموكسيفين انخفاضا كبيرا في مستويات GOT و GPT فى الدم و MDA وزياده SOD فى الكبد مقارنة مع مجموعة تاموكسيفين. عن طريق الفحص المجهرى الضوئى، فقد وجد ان عقار تاموكسيفين أحدث العديد من التغييرات في الكبد مثل تسلل خلايا التهابات، وموت ونخر الخلايا الكبدية ، و توسع الجيوب مع تضخم في خلايا كوبفر، ترسب الكولاجين والتغيرات النووية مثل تجمع الكروماتين و trizomy. كما كشف الفحص المجهرى الإلكتروني لمجموعة عقار التاموكسيفين عن تغييرات نووية، توسع فى TER، ليزوزومات ثانويه وتورم فى الميتوكوندريا وقطرات من الدهون.

**الخلاصة:** مزيج من الحبة السوداء و فيتامين سى معا يكون لها تأثير واقائى متأزر ضد أمراض الكبد.