Original Article

Modulatory Effect of Bone Marrow-derived Stem Cells Against Tamoxifen Induced Liver Injury in Albino Rats: A Histological, Immunohistochemical and Biochemical Study

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ABSTRACT

Introduction: Tamoxifen (TAM) is commonly used for breast cancer treatment. It is considered non-steroidal antiestrogen agent. Toxic hepatitis, significant hepatic steatosis, and cirrhosis have been reported to be caused by TAM. Stem cells (BM-MSCs) are used for regenerative medicine and therapy.

Aim of the Work: To know how BMMSCs can improve tamoxifen induced damage in the liver of female albino rats.

Materials and Methods: Ten female albino rats of average 200 gm were served as a donor for stem cells. Thirty female adult albino rats of 200-240 gm were randomly divided to three equal groups (10 animals each). Group I (Control group): provided ordinary diet. Group II (tamoxifen treated group): rats were orally given TAM with a dosage 20 mg per kg body weight daily for nine weeks consecutively. Group III (TAM & BM-MSCs group): animals were given TAM with a dosage 20 mg per kg body weight daily for nine weeks consecutively as in group II then received single intraperitoneal injection of 2×10^6 BM-MSCs suspended in PBS per rat then scarified after another four weeks. Biochemical studies were done, and liver sections were processed for histological, immunohistochemical and electron microscopic studies.

Results: Sections of the animals' liver taking TAM showed disturbance of the normal structure, showing cytoplasmic vacuolation, leukocytic infiltration, pyknotic nuclei, dilated cisternae of rough endoplasmic and dilated blood sinusoids also excessive collagen fibers, as well as increased expression of caspase 3. The levels of liver enzymes((AST), (ALT) and (ALP)) were elevated, On the other hand, animals' liver sections treated with TAM followed by BM-MSCs showed improvement of the disturbed structure.

Conclusion: BM-MSCs revealed ameliorative effect against TAM toxicity in rats.

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Key Words: BM-MSCs, hepatitis, pyknosis, tamoxifen.

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INTRODUCTION

Tamoxifen (TAM), a non-steroidal antiestrogenic drug, is commonly taken for the treatment and prevention of breast cancer dependent on hormone[1,2]. For men with breast cancer, it is the most effective hormonal therapy[3]. TAM-induced hepatotoxicity cases have been reported in humans, such as hepatitis, fat infiltration or steatosis, massive necrosis and cirrhosis[4,5]. TAM begins the process of lipid peroxidation cycle by extracting hydrogen from unsaturated fatty acids and creating carbon-centered lipid radicals; Then the carbon-centered lipid radicals bind molecular oxygen to form lipid peroxy radicals[6,7]. Tamoxifen intake decreases the liver expression of fatty acid synthase (FAS)[8] also uncouples and inhibits the mechanism of mitochondrial respiration[9].

This is further conjugated as putative reactive intermediates to form the sulphate esters, this documented by Nemoto *et al*.[10]. Tamoxifen, a selective estrogen receptor modulator (SERM), acts as estrogen receptor (ER) antagonist in breast tissue. It decreases breast cancer repetition and mortality in women with ER-positive breast cancer. It is also very effective in prevention of breast cancer in high-risk women. However, the use of TAM for prevention is very limited because of its side effect[10]. In addition, TAM typically has a variation of toxic effects such as hot flushes and more dangerous effects as endometrial hyperplasia and thromboembolic diseases. Other side effects include night sweats, gynecologic symptoms (vaginal dryness, vaginal discharge), anxiety, forgetfulness, changes in sleep. It is noted that estrogen receptor activation regulates food intake and body weight[11]. Diminished sexual functioning also recorded by Yeh *et al*.[12]. Mesenchymal stem cell is now commonly used as a regenerative medicine and foundation therapy. Transplantation of BM-MSCs has been widely used with strong results in various trials, such as cardiovascular, immunological and neurological diseases. Also, BM-MSCs are able to differentiate and self-renewal into multiple types of cells[13]. In this study we aim to study the toxic changes which might result from tamoxifen and the role of BM-MSCs transplantation in its modulation.

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MATERIALS AND METHODS

Chemicals

Tamoxifen was obtained from Sigma-Aldrich (St. Louis, Mo. USA), for its ingestion tamoxifen must dissolved in water and administered by gastric tube at a dosage of 20 mg per kg orally to the animals daily for nine weeks[14].

Animals

Ten female albino rats averaging 200 gm and thirty female adult albino rats weighing 200-240 gm were studied. The rats were kept with regular diet and tap water ad libitum under usual laboratory environment.

Culture and characterization of BM-MSCs

For getting the bone marrow, the proximal and the distal ends of both femurs and tibiae of six-week-old rats were trimmed and the bone marrow was flushed by inserting a needle attached to a syringe with 10 percent serum of foetal bovine with Dulbecco's modified Eagle's medium (DMEM) (obtained from Lonza Company, Switzerland).

Cells of the bone marrow were then collected and kept in culture medium that contained 1% penicillin–streptomycin (obtained from Lonza Company, Switzerland) then Cells were incubated in 5% humidified CO2 at 37°C and examined every day with inverted microscope (Axiovert 100; Carl-Zeiss, Jena, Germany).

Growth and division of these cells and detection of any infection was followed up. Upon development of large colonies, typically, after 10-12 days, (80-90 percent confluence); washing of these colonies with PBS and suspension for 5 minutes with 0.25 percent trypsin in 1mM EDTA at 37 °C was done. The cell suspension was centrifuged, and the pellet was combined with 0.1 ml trypan blue. Cell suspension was put on the top of a hemocytometer slide. The number of viable and nonviable cells was counted then viable cells were sub- cultured at 4 x10^3 cells/cm^2 and used for experiments after the third passage. Stem cells in the culture were adhesive to each other and fusiform shape. Streptavidin-biotin immune peroxidase technique used to detect CD29 and CD44 (purchased from Lab vision, New York, USA) as a marker of MSCs[16]. Stem cell preparation and examination were done, at Faculty of Medicine, Cairo University.

Experimental design

Ten albino rats were kept as a donor for stem cells, in which BM-MSCs obtained from their bone marrow.

Thirty female albino rats were randomly separated to three groups (10 animals each).

Group I (Control group): animals were provided ordinary diet and left without treatment.

Group II (Tamoxifen treated): animals were orally received TAM with a dosage of 20 mg/kg body weight daily for nine weeks.

Group III (TAM & BM-MSCs treated group): animals were orally received TAM with a dosage of 20 mg/kg body weight daily for nine weeks as group II then received single intraperitoneal injection of BM-MSCs (2× 10^6 cells suspended in 0.5 ml PBS) and left for another four weeks according to Maron-Gutierrez T.[15].

For nine weeks, rats were fed their respective diets. At the start of the experiment, and after nine weeks, the body weights of experimental animals were measured. At the end of the experiment they had been fasted for 10h but water was not reduced. At the appropriate time, animals were anesthetized using ether and perfused trans cardiac with cold 1% para form-aldehyde in 0.1 M PBS pH 7.4 (phosphate buffered saline) for 1 min, followed by cold 4% paraformaldehyde in 0.1 M PBS pH 7.4 for 10 min[17]. Blood samples taken from orbital venous plexus, undergo centrifugation for 20 min[18]. At the end of the experiment, Animals were sacrificed and liver was quickly taken and fixed in 10% neutral buffered formalin. After fixation, dehydration, clearing, and embedding in paraffin was done. Moreover, sections were cut with a thickness of 5 µm and mounted on clean glass slides. Other specimens at the same time, post fixed for ultrastructural examination in 2.5 per cent phosphate-buffered glutar-aldehyde.

Histological study

Histological Specimens were processed for light microscopic study, (using the hematoxylin and eosin (H&E)[19] Mallory's trichrome[20], Caspase 3 for immunohistochemical study[21] and electron microscopic study[22].

Morphometric study and Statistical analysis

Slides from each rat from 5 rats from each group with magnification×400 were studied for:

1. The diameter of blood sinusoid in (H&E).
2. Area of % collagen fibers in Mallory stained sections.
3. The intensity of caspase 3 immunoreactive cells were evaluated.

Data were analysed and compared by student's t-test. *P-value* for detection the significant changes in each parameter in the animals to compare with the control group.

Data were tabulated as mean ± SD, analyzed using statistical (SPSS) software (version 17.0 on an IBM compatible computer; SPSS Inc., Chicago, Illinois, USA). *P value* was calculated at 0.05, $P>0.05$ was non-significant, while $P\ value<0.05$ significant and $P\ value<0.001$ was highly significant in the Department of Anatomy, Faculty of Medicine, University of Menoufia, used to test these parameters in all studied parameters[23].

RESULTS

General Appearance

Animals during experimental period were well tolerated and no mortality detected.

Mean weight in grams in different groups

At the end of the experiment, there was a highly significant decrease (p<0.001) in the mean weight of (group II) when compared with group (I). Also, a significant increase (P<0.05) in the mean weight of (group III) as compared (group II), While group (III) showed no significant difference compared with the control group (Table 1, Histogram 1).

Biochemical results

Group (II) revealed highly significant increase (p<0.001) in serum level of ALT, AST and ALP in comparison to group (I), significant decrease (P<0.05) in the mean of ALT, AST and ALP of (group III) as compared with (group II). Also, group (III) showed no significant difference compared with the control group (Table 2, Histogram 2).

Histological results

By examination of the sections of the liver of control group (I) stained with H & E revealed that the classic hepatic lobules in the form of branching and anastomosing plates or cords of hepatocytes, these hepatocytes were radiating from the central vein and were separated by hepatic blood sinusoids (Figure 1) which are lined by endothelial cells and phagocytic Kupffer cells (Figure 2). The hepatocytes appeared with acidophilic cytoplasm and single central rounded vesicular nuclei whereas, some of the hepatocytes were binucleated (Figure 2). In tamoxifen treated rats (group II); histopathological changes were detected in the form of loss of normal architecture of hepatic lobules (Figure 3), ballooning degeneration of hepatocytes and vacuolation of their cytoplasm, other cells appear as ghosts' (Figure 4) others with dark pyknotic nuclei. Also, inflammatory cells infiltration, Kupffer cells hyperplasia and dilated congested central vein was seen (Figures 4,5). Administration of BM-MSCs in group (III) revealed remarkable improvement as the hepatic lobules appeared as branching and anastomosing cords of hepatocytes radiating from central vein separated by blood sinusoids (lined by flat endothelium and Von Kupffer cells). Hepatocytes showed acidophilic cytoplasm and single central rounded vesicular nuclei nearly like the control rats (Figures 6,7).

In Mallory trichrome stained sections, the liver parenchyma of the control group (I) supported with a stroma of very delicate meshwork of collagen fibers (Figure 8). Few collagen fibers surround the central veins (Figure 8). Tamoxifen treated group (II) showed large amount of collagen fibers which are deposited around the central vein and the blood sinusoids (Figure 9). In liver sections of co-treated tamoxifen with BM-MSCs group (III), revealed mild amount of collagenous fibers, more or less like control group (Figure 10).

Immunohistochemical results

Caspase -3 immunohistochemical staining of the liver of control rats (I) showed mild positive cytoplasmic immune reaction for caspase immunostaining (Figure 11). Tamoxifen treated group (II) revealed strong cytoplasmic intensity of hepatocytes for caspase immunostaining (Figures 12,13). Co-treated tamoxifen with BM-MSCs group (III)showed nearly the same appearance as control group (Figure 14).

Ultrathin sections of the liver of the control group (I) hepatocyte appeared with oval or rounded nuclei surrounded by smooth nuclear membrane with nucleoplasm showing fine granular component with euchromatin condensation and chromatin margination. The cytoplasm of the hepatocytes contains numerous mitochondria, rER, and free ribosomes (Figure 15). Tamoxifen treated group revealed hepatocytes with flat indented nucleus, swollen degenerated mitochondria and multiple lysosomes (Figures 16,17). Note the presence of many electron dense vesicles (Figure 16). other hepatocyte with irregular shaped nucleus with clumping of nuclear chromatin (Figure 18)., Others with small electron dense nuclei and multiple vacuolation of their cytoplasm (Figure 19)., Also there are multiple collagen fibers. TAM & MSCs treated group showed no ultrastructural difference from control group as hepatocytes appeared with oval or rounded nuclei surrounded by smooth nuclear membrane, their cytoplasm contains numerous mitochondria and rER (Figure 20).

Morphometric results

Data in (Table 3, Histogram 3) demonstrated that (group II) compared to group (I) show a highly significant (P<0.001) increase of intensity of caspase 3 immunoreaction of hepatocytes, While, (group III) exhibited significant decrease of intensity of caspase 3 immunoreactions in comparison with (group II). Also, group (III) showed no significant difference compared with the control group

In addition, (Table 4, Histogram 4), also showed that (group II) compared with group (I). exhibited highly significant increase (P<0.001) in collagen fibers around central vein, while, in (group III), they exhibited significant decrease of collagen fibers when compared with (group II). Also, group (III) showed no significant difference compared with the control group.

In addition, (Table 5, Histogram 5) also showed that (group II) exhibited highly significant increase (P<0.001) of diameter of blood sinusoid, compared with group(I). While, in (group III), they exhibited significant decrease of diameter of blood sinusoid in comparison with (group II) and no significant difference compared with the control group.

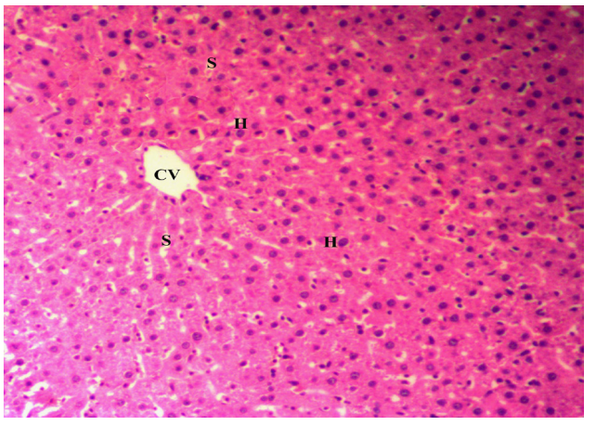


Fig. 1: Photomicrograph of the liver section of control group(I) showing regularly arranged hepatocytes (H) in the form of hepatic cords or plates around the central vein (CV), these cords separated by blood sinusoid (S) (H&E, 100)

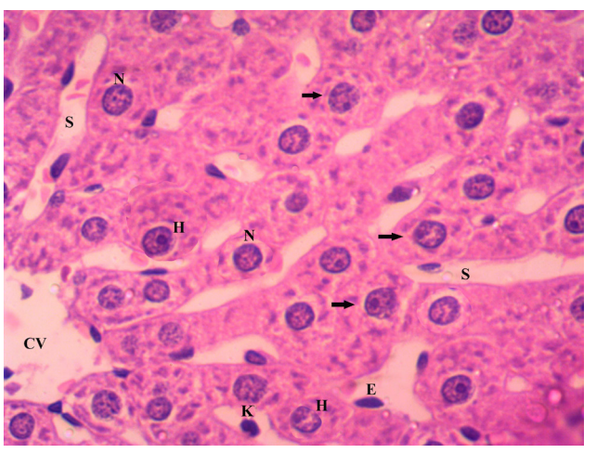


Fig. 2: Photomicrograph of the liver section of control group(I) showing regularly arranged hepatocytes (H) forming hepatic cords or plates around a central vein (CV), hepatocytes have central rounded vesicular nuclei(N) and acidophilic cytoplasm (↑) the cords are separated by hepatic sinusoid (S) lined with flat endothelium (E), Von Kupffer cells also appear in the sinusoid (K) (H&E, 400)

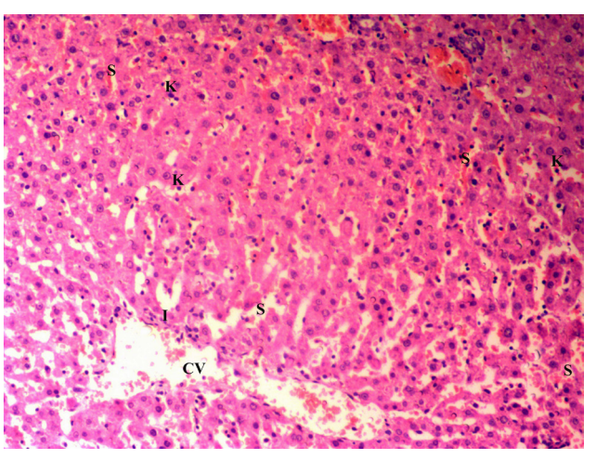


Fig. 3: photomicrograph of tamoxifen treated group (II) showing loss of normal hepatic architecture and distorted congested central vein (CV) surrounded by cellular infiltration (I) also there are congested blood sinusoid(S) and hyperplasia of Kupffer cells(K) (H&E, ×100).

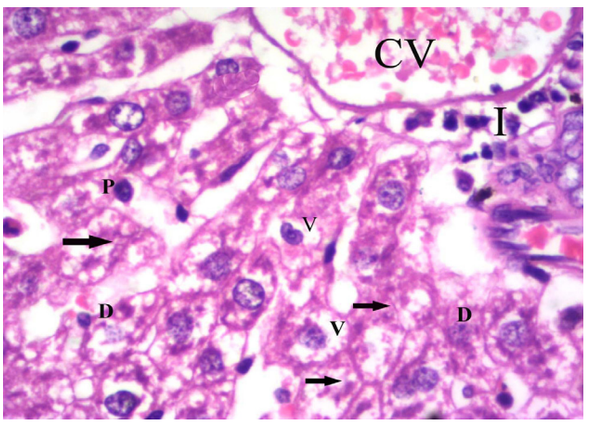


Fig. 4: photomicrograph of tamoxifen treated group (II) showing Highly disturbed irregular pattern of hepatic cords with dilated congested central vein (CV)surrounded by cellular infiltration(I). There are focal areas of ghost-like degenerated hepatocytes (D), Other hepatocytes show marked cytoplasmic vacuolation (V)and others with pyknotic nuclei(P). notice there is focal area of hepatocytes loss (↑) (H&E, ×400).

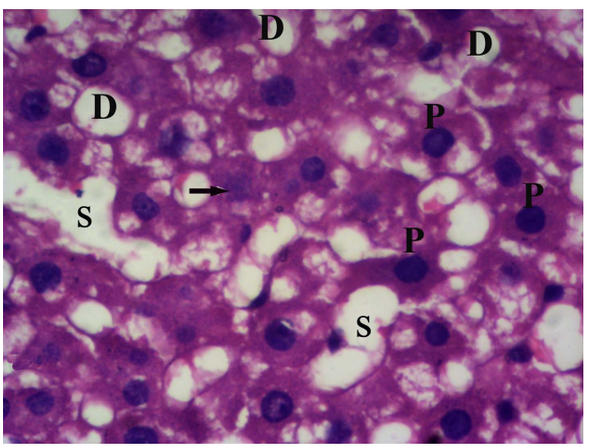


Fig. 5: Photomicrograph of liver sections of tamoxifen-treated group (II) showing ballooning degeneration of hepatocytes(D), other hepatocytes with pyknotic nuclei(P), others are degenerated (↑). Dilated disturbed blood sinusoids (S) also seen (H&E, ×400).

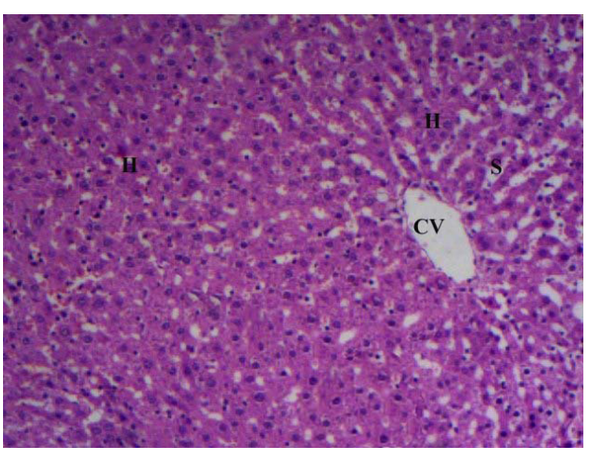


Fig. 6: Photomicrograph of the liver section of TAM & MSCs treated group (III) showing regularly arranged hepatocytes (H) forming hepatic cords around a central vein (CV), these cords are separated by blood sinusoid (S) more or less like control group (H&E, 100)

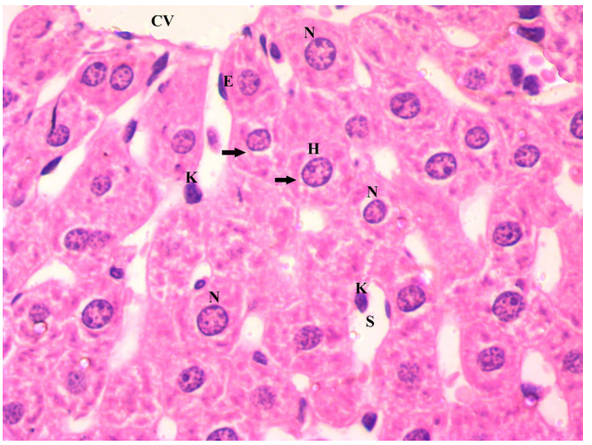


Fig. 7: Higher magnification of the previous picture showing regularly arranged hepatocytes (H) forming hepatic cords around a central vein (CV), These hepatocytes have central, rounded, vesicular nuclei(N) with acidophilic cytoplasm (↑) the cords are separated by blood sinusoid (S) lined by flat endothelium (E), Von Kupffer cells(K) also appear in the sinusoid more or less like control group. (H&E, 400)

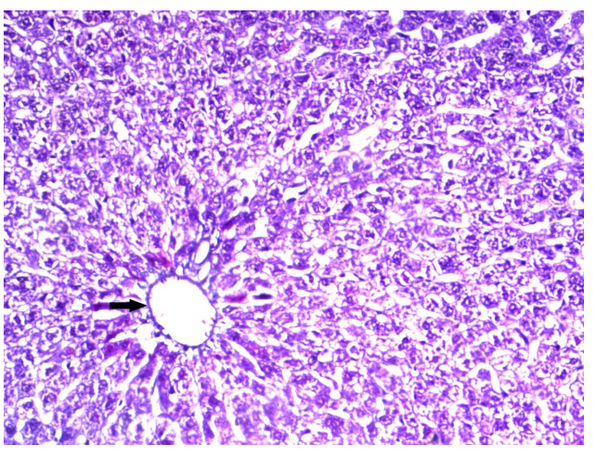


Fig. 8: Photomicrograph of Mallory trichrome stained liver section of control group(I) showing mild collagenous fibers around central vein(arrow) (MT, ×200)

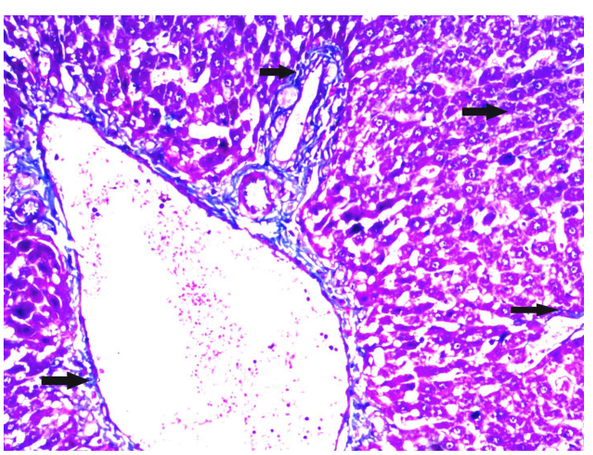


Fig. 9: Photomicrograph of Mallory trichrome stained liver section of tamoxifen treated group (II) showing excessive collagen fibers deposition around central vein, blood sinusoids and in between hepatocytes (arrows). (MT, ×200)

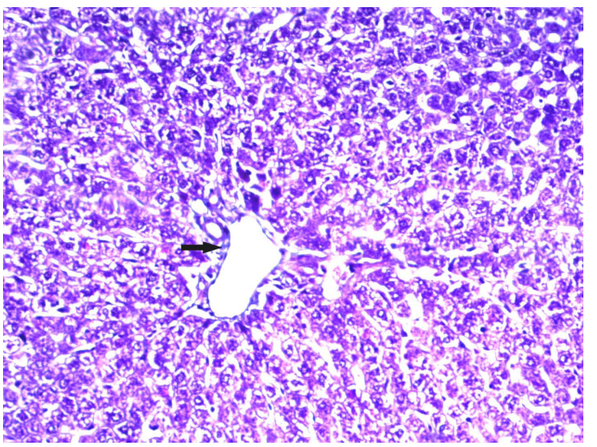


Fig. 10: Photomicrograph of Mallory trichrome stained liver section of TAM & MSCs treated group (III) showing mild collagenous fibers around central vein, more or less like control group(arrow) (MT, ×200)

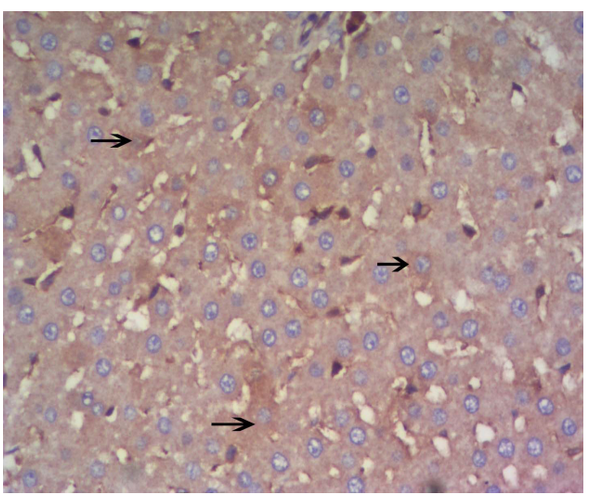


Fig. 11: Caspase 3 immunohistochemical staining of control group(I) showing weak cytoplasmic reaction(arrows) (caspase 3×200)

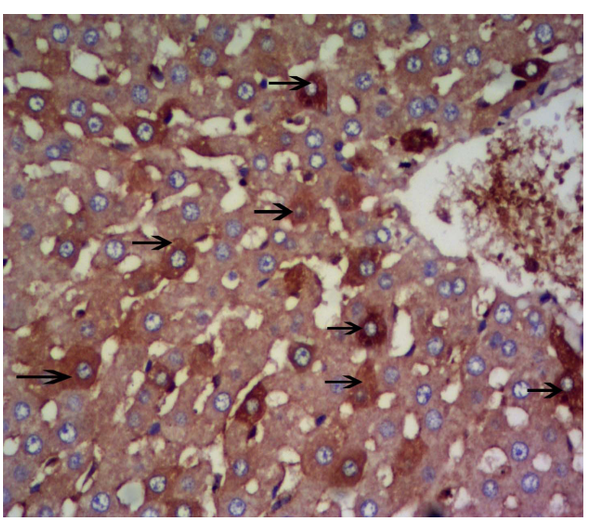


Fig. 12: Caspase 3 immunohistochemical staining of tamoxifen treated group (II) showing strong cytoplasmic reaction (arrows) (caspase 3×200)

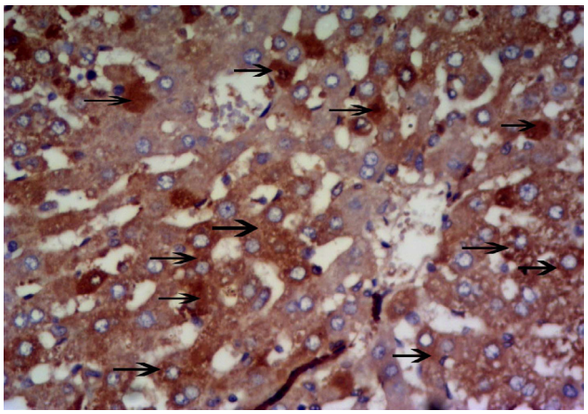


Fig. 13: Caspase 3 immunohistochemical staining of tamoxifen treated group (II) showing strong cytoplasmic reaction (arrows) (caspase 3×200)

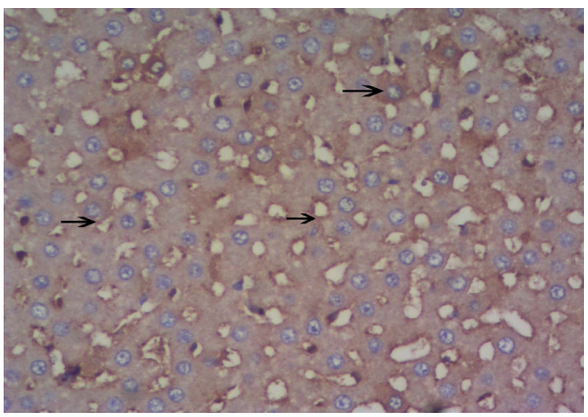


Fig. 14: Caspase 3 immunohistochemical staining of TAM & MSCs treated group (III) showing weak cytoplasmic reaction(arrows) (caspase 3×200)

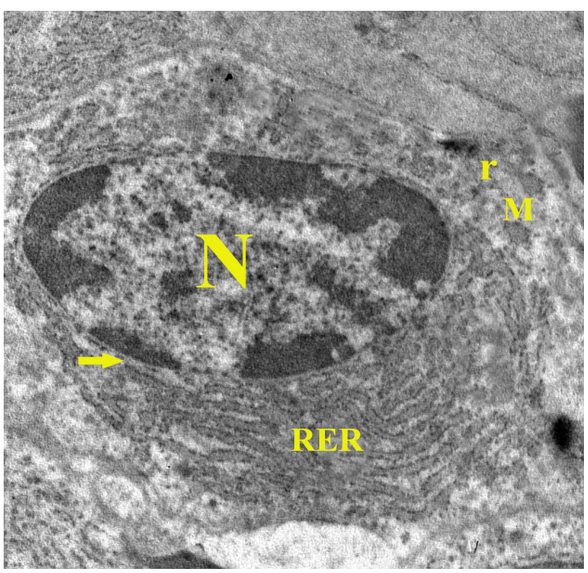


Fig. 15: Electron micrograph of the liver sections of control group (I) revealing hepatocyte with an euchromatic nucleus (N) and well-defined nuclear envelope (arrow). The cytoplasm contains multiple mitochondria(M), free ribosomes (r) and well defined (RER). (TEM x 15000)

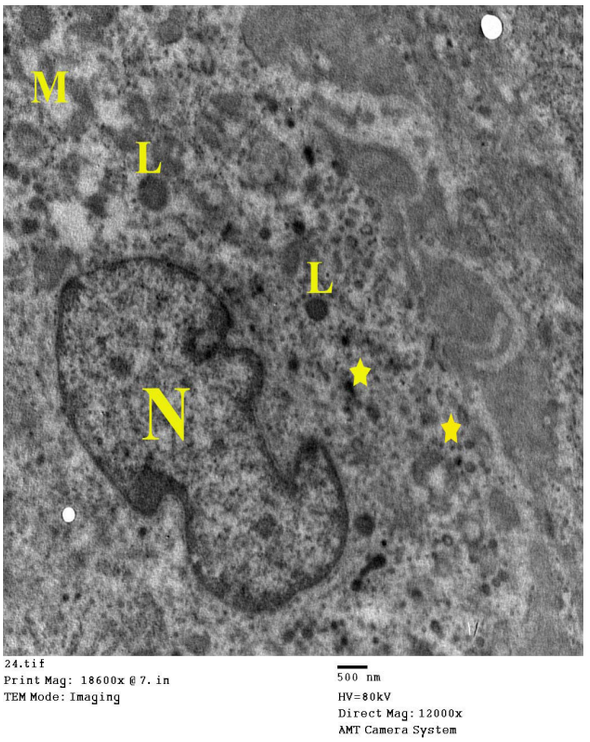


Fig. 16: Electron micrograph of the liver sections of tamoxifen treated group (II) showing hepatocyte with flat indented nucleus(N), degenerated mitochondria (M)and multiple lysosomes(L). Note the presence of many electron dense vesicles (star). (TEM x 12000)

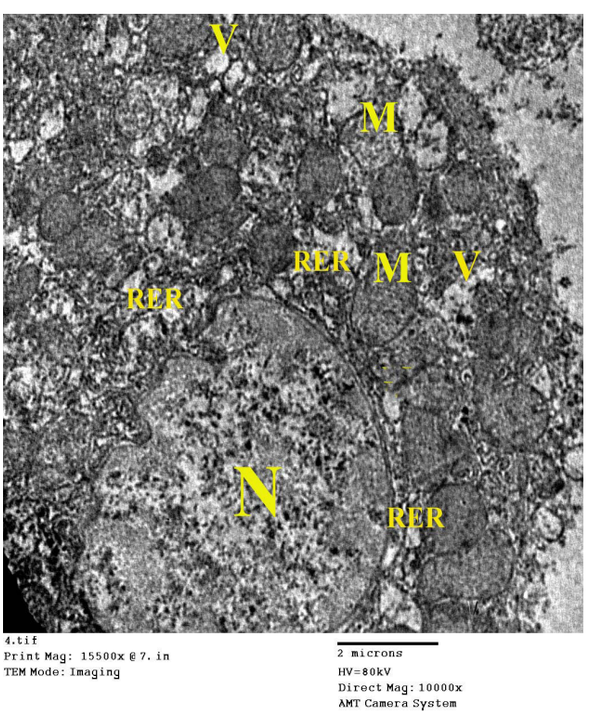


Fig. 17: Electron micrograph of the liver sections of tamoxifen treated group (II) showing hepatocyte with flat indented nucleus(N), large swollen mitochondria (M) and dilated cisternae of rough endoplasmic reticulum (RER). Notice there are multiple vacuoles(V). (TEM x 10000)

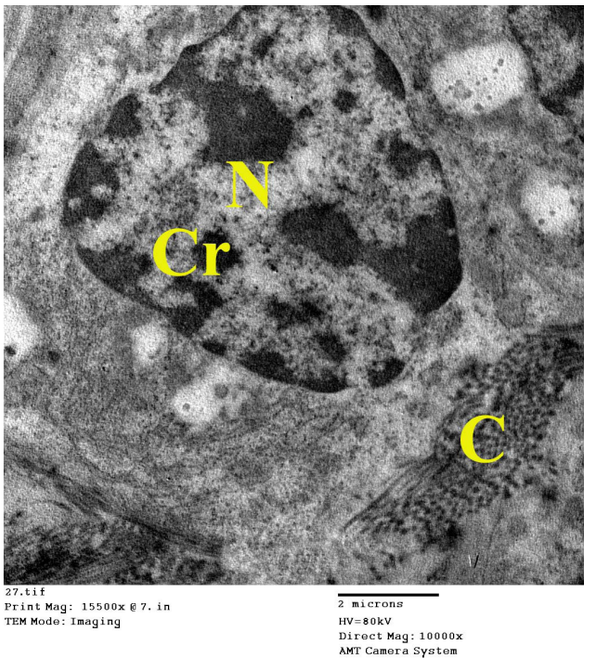


Fig. 18: Electron micrograph of the liver sections of tamoxifen treated group (II) showing hepatocyte with irregular shaped nucleus(N)with clumping of nuclear chromatin (CR), Notice there are multiple collagen fibers around the cell(C). (TEM x 10000)

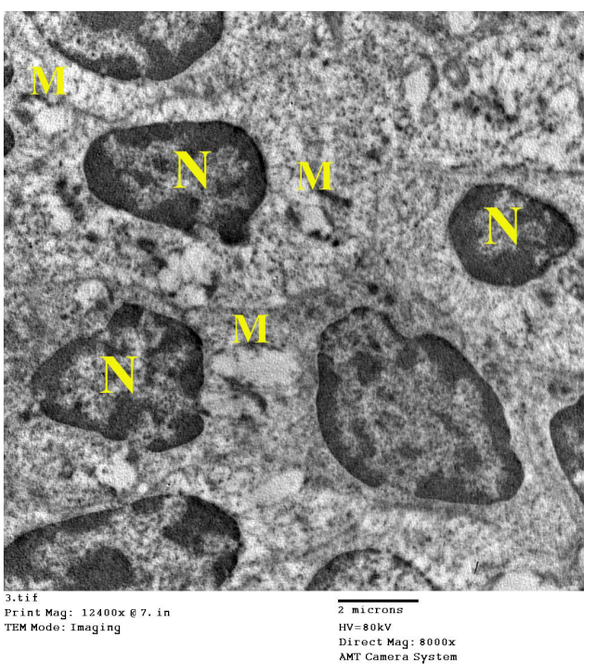


Fig. 19: Electron micrograph of the liver sections of tamoxifen treated group (II) showing multiple hepatocytes with electron dense nucleus (N) and dilated degenerated mitochondria (M). (TEM x 8000)

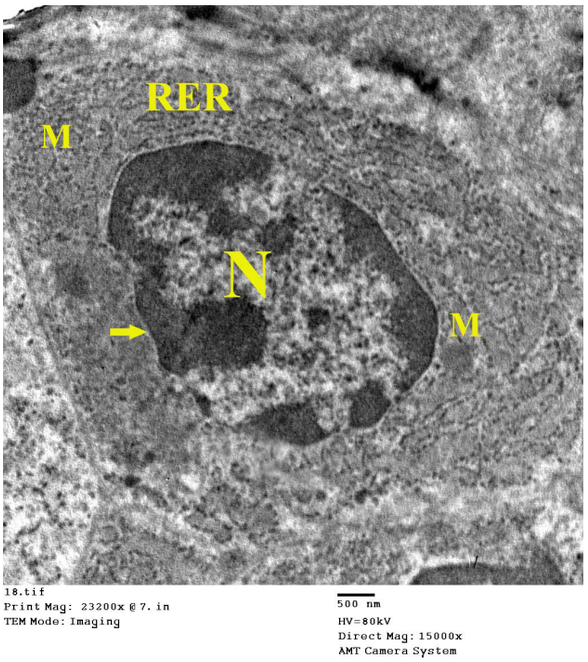


Fig. 20: Electron micrograph of the liver sections of TAM & BM-MSCs treated group (III) showing recovery of the structure as liver cells appear with rounded euchromatic nucleus (N) and well-defined nuclear envelope (arrow). The cytoplasm has mitochondria(M) and well-defined cisternae of rough endoplasmic reticulum (RER) appearing nearly similar to the control group. (TEM x 15000)

Table 1: Mean weight in grams in different groups

Mean weight	Control (group I) $\bar{x}$±SD	Group II (tamoxifen treated) $\bar{x}$±SD	Group III (TAM & MSCs treated) group $\bar{x}$±SD
Initial weight	222.8±7.811	229.8±9.21	230.2±6.71
Weight at End of study	301±7.598	246 ±8.54**	305±10.222♦

$P > 0.05$ non-significant
$P \leq 0.05$ significant ♦
$P \leq 0.01$ highly significant**

Table 2: Mean levels of liver enzymes (ALT, AST and ALP) in different groups

Groups	Control (group I) $\bar{x}$±SD	Group II (tamoxifen treated) $\bar{x}$±SD	Group III (TAM & MSCs treated) group $\bar{x}$±SD
ALT, U/l	61.6±11.1♦	248 ± 79.2c**	42 ± 4.8
AST, U/l	72 ± 13.6♦	250 ± 79.9**	59 ± 10.5
ALP, U/l	95 ± 37.3♦	420 ± 295.1**	74 ± 13.6

Table 3: Mean intensity of caspase-3 immunoreactive cells in different groups

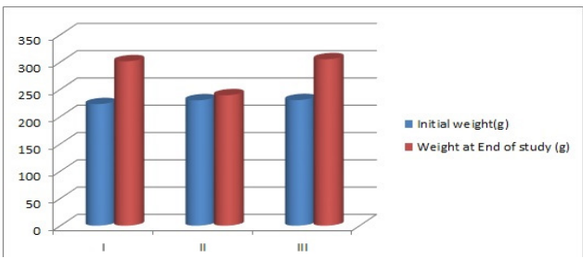
Groups	Control (group I) $\bar{x}$±SD	Group II (tamoxifen treated) $\bar{x}$±SD	Group III (TAM & MSCs treated) group $\bar{x}$±SD
Intensity of caspase-3 immunoreactive cells	7.51+1.15	48.8+3.80**	10+ 1.73♦

Table 4: Mean of the area% of collagen fibers in different groups

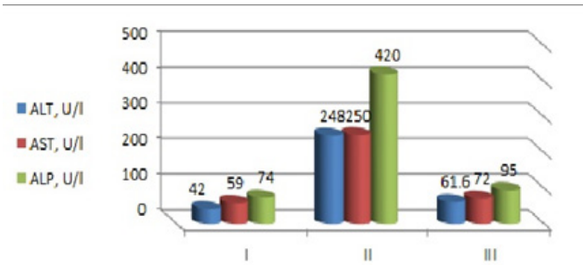
Groups	Control (group I) $\bar{x}$±SD	Group II (tamoxifen treated) $\bar{x}$±SD	Group III (TAM & MSCs treated) group $\bar{x}$±SD
area % of collagen fibers	14.2±2.51	73.2±3.36**	20.4±3.3♦

Table 5: Mean of the diameter of blood sinusoid in the groups

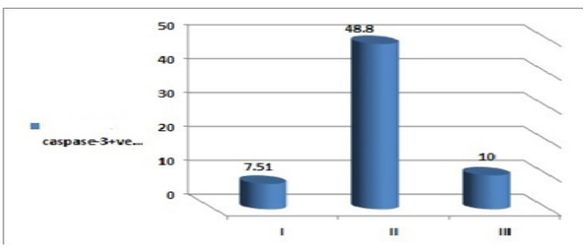
Groups	Control (group I) $\bar{x}$±SD	Group II (tamoxifen treated) $\bar{x}$±SD	Group III (TAM & MSCs treated) group $\bar{x}$±SD
Sinusoidal diameter, µm	3.88±0.23	5.24±0.14**	4.05±0.14♦



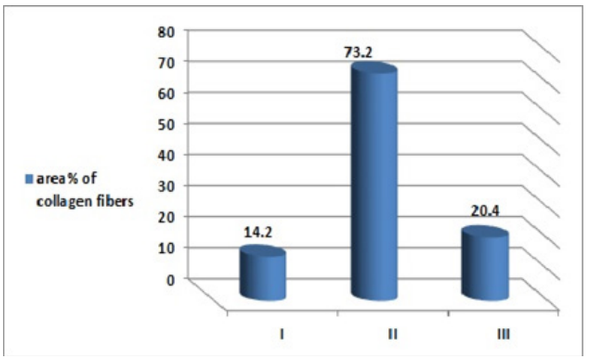
Histogram 1: Mean of initial weight and end study weight



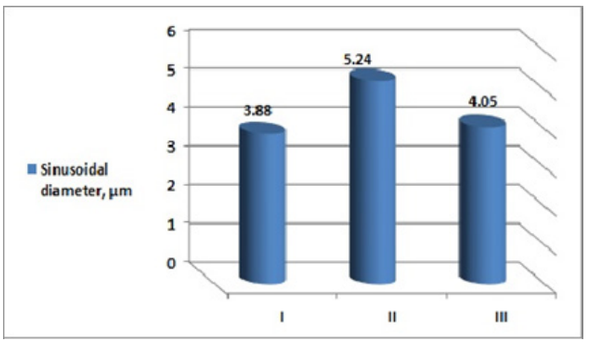
Histogram 2: Mean levels of liver enzymes (ALT, AST and ALP) in different groups



Histogram 3: Morphometric analysis of the intensity of caspase-3 immunoreactive cells in different groups



Histogram 4: Morphometric analysis of the area% of collagen fibers in different groups



Histogram 5: Morphometric analysis of the diameter of blood sinusoid in different groups

DISCUSSION

Tamoxifen is a chemo preventive and chemotherapeutic drug used for treatment of cancer breast. One of the most serious side effects, which limit the use of tamoxifen for long duration is hepatic toxicity or even hepatocarcinoma[24]. Tamoxifen undergoes metabolic activation reactions that increase production of reactive oxygen radicals leading to hepatic oxidative stress and liver damage, which are the causes of hepatotoxicity[25].

Jordan, 2003[1] stated that tamoxifen is a selective modulator of estrogen receptors (SERMs), acts as both estrogen receptor agonists and antagonists depending on the target tissue.

SERM (tamoxifen) inhibits the effects of estrogens on the growth factors, on breast cancer cells while looks like the effects of estrogens in the uterus[26].

It is well established that estradiol decreases food intake and body weight also improves lipid profile in the blood, may be via stimulation of Erα (estrogen receptors) [27]. These effects resulted from significant increase in expression of anorexigenic genes and decrease in expression of orexigenic genes[28]. It resembles the effects of estradiol on food intake in rats[29].

In the current study, changes in body weight in tamoxifen treated group were observed as opposed to control value this was in agreement with Wallen *et al*.[30].

The effects of tamoxifen are very significant, as they can affect tamoxifen clinical use, and body weight[31], As tamoxifen decreases food intake, reduces body fat and prevents increase in the body weight[30]. Another study was with an agreement with our results that tamoxifen suppresses the increase of weight in female Wistar-Kyoto (WKY) rats, suppression of food intake was not the only way[32]. Butera and Beikirch[33]. have explained that the mechanism involved was a decrease in the FAS and mRNA expression, which leads to accumulation of malonyl-CoA in the ventromedial nucleus of the hypothalamus.

Many animal studies have shown that estrogen reduces both the amount of food consumed, and body weight, in both gender of male and female rats and mice[34,35].

In this study, Significant elevation of serum ALT & AST and ALP, which indicates damage to hepatocytes and release of intracellular enzymes into the blood, was caused by tamoxifen administration for nine weeks this is supported by Gao *et al*[36].

These enzymes are cytoplasmic enzymes. An increase in their levels in the blood represents a defect in plasma membrane permeability and cell death, which is considered an indicators of hepatocytes necrosis[37].

The lipid peroxidation resulting from tamoxifen leads to a disruption of the lipid bilayer cell membrane and cell necrosis causing enzymes leakage into the blood[38].

Tamoxifen can induce disruption in the defense mechanism of hepatocytes against oxidative stress. This was confirmed by histological and ultrastructure examination.

In the present study, TAM-treated group revealed loss of normal architecture of hepatic lobules, Hepatocytes showed ballooning degeneration, there are focal areas of ghost-like cells, Other hepatocytes showed marked cytoplasmic vacuolation and others with pyknotic nuclei. Also, there are focal areas of hepatocytes loss, inflammatory cells infiltration, Kupffer cells hyperplasia, and dilated congested blood sinusoids in addition to marked deposition of collagen fibers around blood sinusoid, central vein and the hepatic lobules in agreement with Ibrahim *et al*.[39].

Tamoxifen causes disappearance of mitochondrial cristae, mitochondrial depolarization[40] and dysfunction that leads to deficiency in energy production,

imbalance of the ions and reactive oxygen species elevation and damage[41]. This leads to ATP depletion and failure of sodium – potassium pumps work. As a result, failure of the ion transport mechanism leading to accumulation of water inside the cells, this explains the ballooning degeneration of hepatocytes[42]. Furthermore, Tamoxifen induces damage of endoplasmic reticule and vacuolation of the cytoplasm which might be reaction to cell injury[43].

Noted that hepatic cell damage is caused by weak blood flow to the liver due to arterial blockage or coagulation of the hepatic artery, resulting in O_2 deficiency and release of lysosomal enzymes that are the most significant cause of liver insult Park *et al*.[44].

Explained that ROS encourage production of inflammation, infiltration of various inflammatory cells, Moreover production of inflammatory mediators Li *et al*.[43]

such as TNF-α and IL-6 which promotes the progression of steatosis to fibrosis, cirrhosis, and cancer.

Expansion of the blood vessels is due to the release of fatty acids which is the most important component of toxicity, activate certain prostaglandin forms that cause vasodilation and blood sinusoidal expansion[43].

The marked dilatation of hepatic sinusoids resulted from pressure of necrotic and inflammatory cells on portal vein tributaries may be a sign of portal hypertension[45].

In the present study, hepatic fibrosis and deposition of collagen fibers around vessels may be caused by increased levels of malondialdehyde (MDA) and decreased development of antioxidant enzymes (glutathione-S-transferase, glutathione peroxidase, superoxide dismutase, and catalase), increased thiobarbituric acid reactive material, and initiated lipid peroxidation process[5]. Development of hepatic fibrosis and necrosis is due to oxidative stress[46].

Previous studies have shown that TAM undergoes metabolic activation reactions in liver tissues with subsequent accumulation in different tissues of certain metabolites, such as 4-hydroxytamoxifen, 4-hydroxy-N-desmethyl-tamoxifen, and N-des (dimethyl) tamoxifen, which may have altered estrogen receptor affinity[47]. The dilated congested blood sinusoids with Kupffer hyperplasia found in the current work may be caused by its direct toxic effect on the blood sinusoids and lipofuscin phagocytosis released from adjacent necrotic hepatocytes[48].

Immunohistochemical staining has confirmed the observed histological results. Tamoxifen treated animals showed increased expression of caspase 3 in relation to control group. Tamoxifen increases the intra-mitochondrial ionized Ca2+ which stimulate mitochondrial nitric oxide synthase (mtNOS) activity, furthermore, increasing NO production and hindering mitochondrial respiration[49]. The produced NO act as with superoxide anion O_2 - and produce peroxy nitrite which increases lipid peroxidation leading to oxidative stress and cytochrome c release into cytoplasm activating the caspase-9, which in turn, activates caspase-3 leading to DNA fragmentation[50]. Also, Tamoxifen induces endoplasmic reticulum stress (ERS) induces various damage, leading to oxidative stress, inflammation and apoptosis[51,52]. TAM induced apoptosis involves cleavage of caspase 3. In the current study, TAM administration induced increase in hepatic MDA content, NO production as represented by increase in hepatic total nitrate/nitrite and strong positivity of immunohistochemical staining of caspase-3 activity. These results provide an evidence and

support to the previous studies that TAM hepatotoxicity seem to favor the mechanism mitochondrial injury and/or ERS, resulting in oxidative stress, inflammatory response and activation of apoptotic response leading to hepatocyte toxicity and death[50].

Ultrastructure changes of the liver confirmed light microscopic results in the form of flat indented nucleus, degenerated mitochondria and multiple lysosomes.

Also, many electron dense vesicles, dilated cisternae of rough endoplasmic reticulum and the nuclei with clumping of their chromatin are also seen. Nuclear changes in the form of irregular nuclear membrane, marginated chromatin was observed as previous studies[36]. Because of increased level of TNF-α as tamoxifen increases TNF-α and causes inflammation resembling that of alcoholic hepatitis[53].

Mitochondria degeneration noted in this study leads to fatty metabolic changes. Mitochondria contains enzymes necessary for the metabolism of triglycerides[54]. Tamoxifen also modulates the expression of the genes involved in the triglyceride homeostasis pathway leading to hepatocyte steatosis[55].

The multiple electron dense bodies may be degenerated mitochondria, the product of auto-phagocytosis accompanied by lysosomal processing. This is considered a major degrading Mitochondria pathway[56]. In agreement with the current findings Fatma *et al*.,[57] have found that peripheral chromatin clumping after administration of tamoxifen is due to adduct formation between it and hepatocyte DNA leading to DNA damage[58].

Earlier studies noted that the oxidation process resulting from TAM intoxication leads to release of iron ions. These ions become more active in the liver.

Free iron ions enter in the process of generation of hydroxyl radicals, which are the most active ROS, and they react readily with most cellular components[59]. In addition, Tamoxifen inhibits mitochondrial DNA synthesis and mitochondrial β oxidation which subsequently decreases removal of the fat from the liver, causing hepatic steatosis[60].

Examination of the hepatic sections of TAM & MSCs treated group (III) indicates restoration of the classical hepatic architecture along with substantial reduction in the region percentage of collagen fibres in the liver parts of this group also improvement of the ultrastructural image as compared to group II.

Prockop D.J *et al*.[61] detected that MSCs are able to restore the normal picture of the tissues through differentiation and self-renewal into the damaged cell phenotype and cytokine development and growth factors that help restore hepatic cells and regulate the local immune system, apoptosis, fibrosis, and angiogenesis. Furthermore, MSCs have anti apoptotic and pro-mitotic effects on hepatocytes[62].

Similarly, Berardis S *et al*.[62] added that, conditioned MSCs inhibit hepatocyte death and encourage liver regeneration in *vivo*. Additionally, Hu *et al*.[63]hypothesized that hepatic protective genes documented to be regenerated by MSC transplantation. Transplantation of MSCs causes oxidative stress suppression and apoptosis in rats. In addition, MSCs proved successful in reducing the fibrotic area with good results[64,65].

Da Silva *et al*.[66] detected that MSCs have the ability to select, migrate and settle in injured tissue. This mechanism called “homing” capacity. This explains the ability of stem cells to reach different tissues.

MSCs leaves the bloodstream through adhesion molecules VCAM-1 / VLA-4 and β1 integrin that enter the endothelium. MSCs have the ability to invade blood vessels through its plasma podia and reach target tissue after passing the endothelial barrier[62].

CONCLUSION

Tamoxifen treatment induces liver damage with histological, biochemical, immunohistochemical and ultrastructural changes. These changes are due to production of reactive oxygen species (ROS) which has the ability to destruct the cellular organelles. MSCs ameliorate the toxic effects of tamoxifen.

By differentiating of these cells into the phenotype of the damaged cells and generating cytokines and growth factors that promote hepatocyte repair.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير التعديلي للخلايا الجذعية المشتقة من نخاع العظم على سمية عقار تاموكسيفين لكبد إناث الجرذان البيضاء: دراسة نسيجية وكيميائية مناعية وكيميائية حيوية

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تاموكسيفين هو مضاد للإستروجين غير ستيرويدي يستخدم عادة لعلاج سرطان الثدي لدى النساء. ويسبب التهاب الكبد السام، والتشحم الكبدي الهائل ويؤدي إلى تليف الكبد. وتعتبر الخلايا الجذعية الوسيطة المشتقة من نخاع العظم هي مصدر فريد للخلايا القائمة على الخلايا العلاجيه والطب التجديدى.

الهدف من البحث: التحقق من التأثير الوقائى المحتمل للالخلايا الجذعية على الضرر الناجم عن عقار تاموكسيفين في كبد إناث الجرذان البيضاء.

المواد والطرق: تم تقديم عشرة إناث من الجرذان البيضاء بمتوسط ٢٠٠ جرام كمانحات للخلايا الجذعية التي تم الحصول عليها من نخاع العظام، تم استخدام ثلاثون من اناث الجرذان البيضاء البالغة، وقد قسمت عشوائيا الي ثلاث مجموعات متساوية، المجموعة الأولي (مجموعة ضابطة) أعطيت الغذاء المعتاد، المجموعة الثانية (المجموعة المعالجة بالتاموكسيفين): أعطيت الجرذان عن طريق الفم تاموكسيفين يوميًا لمدة تسعة أسابيع، المجموعة الثالثة (مجموعة تاموكسيفين والخلايا الجذعيه): اعطيت الجرذان التاموكسيفين بالجرعه والمده ثم تلقى حقنة واحدة من الخلايا الجذعيه وتم تركها لأربعة أسابيع أخرى، تم أخذ عينات دم من جميع المجموعات لتقييم مستوي الانزيمات، كما تم معالجة الكبد لدراسته هستولوجيا وبيوكيميائيا وهستوكيميائية مناعية وبالمجهر الالكتروني.

النتائج: أظهرت أقسام الكبد من الفئران المعالجة بالتاموكسيفين فقدًا في البنية الطبيعية، وتفريغًا هيوليًا، وتسلل الكريات البيض، ونواة متضخمة، وصهاريج متوسعة من الجيوب الأنفية الباطنة والجيوب الدموية المتوسعة، بالإضافة إلى زيادة ألياف الكولاجين، بالإضافة إلى زيادة صبغة كاسباس ٣.

من ناحية أخرى، أظهرت عينات الكبد من الفئران المعالجة بالتاموكسيفين تليها الخلايا الجذعيه تحسنًا في الصورة المضطربه.

الاستنتاج: كشفت الخلايا الجذعيه عن تأثير محسن ضد سمية التاموكسيفين في الجرذان.